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CITRUS PRODUCTS

Chemical Composition and Chemical Technology

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DEDICATED

to the memory
of my first beloved teacher
MY FATHER

and to my last teacher
and dear friend
Professor
E. GOLDBERG

PREFACE

The world is becoming increasingly more cognizant of the importance of fruits and especially of citrus fruits. The balanced ratios and the specific blend of acidity and sweetness impart to citrus fruits a delightful savor; enhanced by their abundant content of vitamins and other nutritive values, they constitute an excellent food.

As in the case of many other fruits, the cultivation of citrus is confined to limited areas and the fresh fruit must be shipped for long distances, with all the dangers which the transportation of such perishables involves. Principally for this reason the conversion of citrus fruits into various products, easily preserved and not readily subject to deterioration, has drawn the attention of a number of chemists and food technologists.

Although numerous articles on citrus cultivation and preservation are scattered throughout the scientific and technical literature and some data have been published in such comprehensive treatises as *Principles of Fruit Preservation,* by T. N. Morris, *Commercial Fruit and Vegetable Products,* by W. V. Cruess, and others, only two books have been especially devoted to citrus products: *Citrus Products,* by J. B. McNair (Chicago, 1926), now very much out-of-date, and the excellent Italian work of Carlo Rodanó, *Industria e Commercio dei Derivati Agrumari* (Milano, 1930). Since then, however, much new data and material have been accumulated, numerous new methods have been developed, and the entire industry has attained world-wide interest. The author has, therefore, thought it appropriate to attempt to give in this book compact information on the chemical composition (Part I) and chemical technology (Part II) of citrus products, and to present the practical results of twenty-five years of factory experience in the utilization of citrus for its by-products.

It has not been intended to approach the subject historically: in the chemical section only the most recent opinions and structural formulas have been given; in the technical section new developments have been described and particular attention has been paid to those

details which have been considered important for persons engaged in the industry. Although the material of some chapters, especially those describing the chemical and biochemical aspects of the components involved, may be found in textbooks, the author has nevertheless deemed it necessary to include them in this work, insofar as they relate to citrus fruits, and thus provide a complete picture for students of this branch of industry.

While the chemistry of the various components of citrus fruits is described briefly in the first part of the book, the chemistry of the changes which take place during and after processing is dealt with generally in the second part, where the various methods employed in manufacture are discussed. The mechanisms of these chemical changes are in many cases not very well known. It has been necessary, therefore, to draw the attention of the reader to the relative stability of the various resulting or degradation products as far as these are known. Although it was intended to cover the entire field as far as possible, especially in the technological part, standard procedures used in the canning industry have been mentioned only briefly.

Grateful acknowledgment is made to Dr. Ernst David Bergmann (Daniel Sieff Research Institute at Rehovoth), to Dr. Z. I. Kertesz (Cornell University, Ithaca, New York), and to Dr. F. Stern (Jaf-Ora Ltd., Rehovoth) for their valuable criticism and advice. My thanks are also due to Mr. D. Marcus for reading the manuscript and proofs and to Mrs. Gerda Rothschild who painstakingly copied the manuscript and assisted in preparing the numerous references with much devotion and care. The index was prepared by Dr. Sylvia Frank. The majority of the photographs for which the source has not been acknowledged in the text were taken at the Jaf-Ora Ltd. citrus-producing plant at Rehovoth.

Tel-Aviv, Israel
December, 1948

J. B. S. BRAVERMAN

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INTRODUCTION

CHAPTER I

HISTORY, BOTANY, CULTIVATION, MARKETING, AND DISEASES OF CITRUS FRUITS

A. HISTORY

1. Background and Recent Progress of Citrus Culture

Citrus is the collective generic term embracing a number of species and varieties of fruits, known the world over for their bright colors and characteristic perfume. The splendor of the evergreen foliage of the trees and the extraordinary fragrance and beauty of their flowers have attracted the curiosity of travelers since the days of antiquity and have been an inspiration to poets for centuries. Voyagers on ships passing along the coast of Florida or Palestine in April or early May, when the citrus trees are in full bloom, have noted their fragrance many miles at sea.

Citrus fruits are appreciated not only for their beautiful appearance and the penetrating odor of the peel but also for their excellent quality as a food. The thick spongy pith covering the fruit protects the tender inner pulp from insect attack and injury and preserves it in a sanitary condition.

It is not surprising, then, that during more recent times, with advanced horticultural methods more generally known, the cultivation probably of no other fruit has spread so rapidly through countries having appropriate climatic and soil conditions.

2. Historical Note on Development of the Citrus Industry

Although the cultivation of *Citrus* has been known to man since the first records of civilization, the actual country or origin of the genus has not yet been ascertained by historians. The earliest evidence seems to be the finding of seeds, identified as those of the citron, in the excavation of the ruins of old Nippur in southern Babylonia. It has now been definitely established that citrus fruits have been grown for many centuries in China and had reached a considerably advanced stage of cultivation before they became known

to Europeans. The citron, the first member of the genus known to European civilization, was mentioned by Theophrastus (about 300 B.C.). The citron, or rather its variety, the etrog, is mentioned in the Bible (Lev. 23:40): "Ye shall take, on the first day, fruits of the tree *hadar,* of palm branches, boughs of the thickest trees, and willows that cross the length of rapid waters and rejoice before the Lord your God."

Tolkowsky (1938), in his excellent book *Hesperides,* states in this connection: "The very earliest documentary evidence of the citron in Jewish sources is found in the representation of this fruit on coins struck by Simon the Maccabee in the fourth year of the 'Redemption

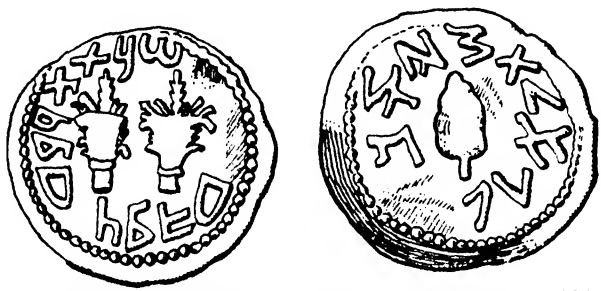


Fig. 1. Jewish coin issued by Simon the Maccabee in 136 B.C. bearing the emblem of an etrog (right) and two "lulavim" (left).

of Zion,' that is, 136 B.C." (Fig. 1). Since then, the Jews have been using the etrog in the religious ritual of the annual Feast of Tabernacles. From Palestine, the citron has apparently spread to Italy, Greece, and other countries of the Mediterranean region, the climate of which is mild and specially adapted to citrus fruits.

Only after many centuries, through the rise of Islam and the expansion of the Arab Empire, were other members of the citrus family introduced into Europe. The Arabs brought the sour orange and the lemon from India to Persia and Palestine sometime during the tenth century A.D., and later into northern Africa, Sicily, Sardinia, and Spain. Subsequently the Crusaders, who were excellent traders, extended the distribution of these and other varieties over the famous Genoese route. The Portuguese thereafter contributed much to the spread and popularization of orange growing by introducing superior varieties. In some parts of Europe the citrus trees were protected against injury from cold by being grown in greenhouses known as 'orangeries," a designation which has persisted in many European

languages. The sweet orange was the last species to be introduced into Europe, approximately in 1400 A.D.—only seventeen centuries after the introduction of the citron (Fig. 2).

Fig. 2. Title page of an old treatise on Hesperides by J. Commelyn, Amsterdam, 1676.

No citrus species were indigenous to the New World; they were apparently brought to America by Columbus in 1493. They were first spread in Florida by Spaniards and eventually were introduced into California through religious missions. In 1676 Captain Shaddock, commander of an East Indian ship, first brought to Barbados in

the West Indies the seeds of the pomelo, thereafter called the "shaddock."

In 1788 Captain Hunter, who came to Australia with the early settlers, first introduced and planted the orange in New South Wales.

3. Distribution and Main Centers of Production

Within the past century the cultivation and production of citrus fruits have increased so tremendously that it is difficult to describe briefly the present state of so extensive an industry. Moreover, the situation is continually changing owing to a rapid increase in production. Table I, compiled from various sources, presents production in terms of tons of fruit instead of in the usual figures of exportable cases of about 30 kg. each. Conversion into tons has been effected as a convenience in comparing related statistical data and to facilitate production computations, which are usually based upon tonnage.

TABLE I

Estimated Production (Tons) of Citrus in Principal Countries

Country	Year	Oranges and mandarins	Grapefruit	Lemons	Limes
United States	1944	3,742,000	1,730,125	420,000	3,500
Spain	1944	650,000	600	44,400	—
Brazil	1942	1,182,000	3,000	—	—
China	1937	750,000	—	—	—
Japan	1937	450,000	—	—	—
Italy	1944	396,000	—	406,000	—
Palestine	1939	429,000	66,000	4,600	—
Egypt	1944/45	210,000	—	—	52,000
Union of South Africa	1944	200,000	24,000	6,000	—
Mexico	1937	105,000	1,800	—	4,180,500
Paraguay	1938	90,000	—	—	—
Algeria	1944	150,000	—	3,000	—
Australia	1940	72,000	—	12,000	—
Cuba	1937	60,000	6,750	—	—
Greece	1938	54,000	—	13,200	—
Syria	1937	39,000	—	9,000	—
Puerto Rico	1937	30,000	19,500	—	—
British West Indies	1937	18,000	5,500	—	718,000
Tunis	1937	9,000	—	600	—
Cyprus	1937/38	14,500	—	1,080	—
Dominican Republic	1937	—	—	—	30,000
Turkey	1938	49,500	—	—	—
Argentina	1944	400,000	2,600	5,150	—

Table I (above) shows the principal countries that can serve as an important source of raw material to the citrus-products manufacturer: for *oranges,* the United States, Spain, Brazil, Italy, Palestine, and South Africa (China and Japan produce mainly mandarins); for *grapefruit,* the United States, Palestine, and Puerto Rico;

for *lemons,* the United States, Italy, and to some extent Spain; for *limes,* Mexico and the British West Indies.

The oldest center of the manufacture of citrus products was, of course, for many years Italy. Subsequently, the West Indies became an important area. The United States (where the first plant for this industry was established at National City, California, in 1899), has gradually developed into the foremost center for the utilization of citrus fruit. In recent years, Palestine has achieved a rank second in importance for the manufacture of citrus products; the first plant was established in 1921. World War II gave a great impetus to the development of this industry in both the United States and Palestine.

B. BOTANY

1. The Genera *Citrus, Fortunella,* and *Poncirus*

Botanically, all commonly cultivated citrus fruits are classed under three genera—*Citrus, Fortunella,* and *Poncirus*—of the orange subfamily Aurantioideae in the plant family Rutaceae.

To have an adequate picture of the classification of the various species of *Citrus* mentioned in this book, an abbreviated taxonomic chart compiled according to W. T. Swingle, who recently published his latest systematic account of the orange subfamily,[1] is presented here (Table II). It is noteworthy that the previous full account of the botany of this subfamily was published 124 years ago by Augustin de Candolle.[2]

Of the six genera belonging to the true *Citrus* subtribal group, only three—*Fortunella, Poncirus,* and *Citrus*—are of interest for the commercial citrus industry.

Fortunella is typified by *F. margarita* (Lour.) Swingle (or *Citrus margarita* Lour.), commonly called the oval kumquat, and by *F. japonica* (Thunb.) Swingle (or *Citrus japonica* Thunb.), commonly called the round kumquat. These species are widely distributed in southern China. They closely resemble *Citrus* in general appearance but have very small fruits with acid pulp and sweet edible skin.

Poncirus is typified by *P. trifoliata* (Linn.) Raf. (or *Citrus trifoliata* Linn.), commonly called the trifoliate orange, widely cultivated in Europe, North America, and Japan, mainly as a rootstock for other citrus varieties and as an ornamental plant.

[1] Swingle, W. T., "The Botany of Citrus" [in H. J. Webber and L. D. Batchelor, "The Citrus Industry," I, Univ. California Press (1943).]

[2] de Candolle, A. P., "Prodromus Systematis Naturalis Regni Vegetabilis," 1, 535–540 (1824).

TABLE II
Taxonomic Chart Showing the Cassification of the Principle Species of *Citrus*, *Fortunella*, and *Poncirus* (after W. T. Swingle)

Geraniales
(the natural order comprising some 21 families)

Geraniineae
(suborder comprising 12 families)

Rutaceae
(family with 7 subfamilies)

Aurantioideae
(orange subfamily with 2 tribes)

Clauseneae
(very remote citroid fruit trees consisting of 3 subtribes, 5 genera, 79 species, and 20 varieties)

Citreae
(with 3 subtribes)

Triphasiinae
(minor citroid fruit trees comprising 8 genera, 46 species, and 3 varieties)

Citrinae
(substitute of citrus fruit trees comprising 13 genera, 65 species, 15 varieties)

Balsamocitrinae
(hard-shelled citroid fruit trees comprising 7 genera, and 13 species)

Subtribal groups:

A
Primitive citrus fruit trees: 5 genera, and 14 species

B
Near-citrus fruit trees: 2 genera, 22 species, and 4 varieties

C
True citrus fruit trees: 6 genera, 29 species, and 11 varieties

Genera:

Fortunella (4 species, 1 variety)
- *F. margarita* (Lour.) Swingle (oval kumquat)
- *F. japonica* (Thunb.) Swingle (round kumquat)

Eremocitrus

Poncirus (1 variety)
- *P. trifoliata* (Linn.) Raf. (trifoliate orange)

Clymenia

Microcitrus (16 species, 8 varieties)

Citrus (6 spp., 1 var.)
- *C. limonum* Risso (lemon)
- *C. aurantifolia* (Christm.) Swingle (lime)
- *C. aurantium* Linn. (sour orange)
- *C. sinensis* (Linn.) Osbeck (sweet orange)
- *C. medica* Linn. (citron)
- *C. nobolis* Andrews (tangerine)
- *C. maxima* Merr. (grapefruit)
- *C. bergamia* Risso (bergamot)
- *C. grandis* (Linn.) Osbeck (pomelo)

The species of the genus *Citrus* constituting the numerous cultivated varieties are of particular interest and will, therefore, be described separately in some detail (Fig. 3). It must, however, be borne in mind that, although the modern commercial varieties have undoubtedly descended more or less directly from the original species, numerous mutations and chance hybridization have produced such profound changes that the affinities are often no longer recognizable.

Fig. 3. Different varieties of citrus fruits. Left to right: upper row, unusually large citron, etrog, shaddock, pomelo; middle row, grapefruit, Marsh seedless grapefruit, Valencia orange, blood orange, sour orange, Shamouti (Jaffa) orange, large Jaffa orange; lower row, rough lemon, Eureka lemon, bergamot, Villa Franca lemon, hybrid of lemon and citron, mandarin, tangerine, large tangerine.

Swingle has proposed to differentiate superficially similar species by the glucoside characteristic of the tissue of the particular species. This is possible because specific glucosides have been identified in various citrus fruits, i.e., *hesperidin* in lemon, *aurantamarin* in orange, *naringin* in grapefruit, and *tangeretin* in mandarin (Chapter III, page 96).

2. Some Individual Species of the Genus *Citrus*

The Sour or Bitter Orange (*Citrus aurantium* Linn.) —commonly known as Seville orange in Spain, bigaradier in France, melangolo in Italy, and khushkhash in Palestine—is native to southeastern Asia (Cochin China) and is widely distributed in the Mediterranean region. The tree is mainly used as a rootstock because of its great resistance to the gummosis disease of citrus. The fruit, being too acid, is not edible as such. It is, however, extensively used in the manu-

facture of marmalades: the famous "Dundee" marmalade is made in Great Britain from sour oranges imported mainly from Spain. The peel of the bitter orange is used in liqueurs (curaçao), and the essential oils of the peel as well as those of the flowers (neroli oil) and the green twigs (petit-grain oils) are very much prized in perfumery.

The Bergamot or Bergamot Orange (*Citrus bergamia* Risso) is confined almost entirely to the province of Calabria in southern Italy. The fruits of the bergamot are spherical in shape and have a sour inedible pulp. They are utilized exclusively for manufacturing:

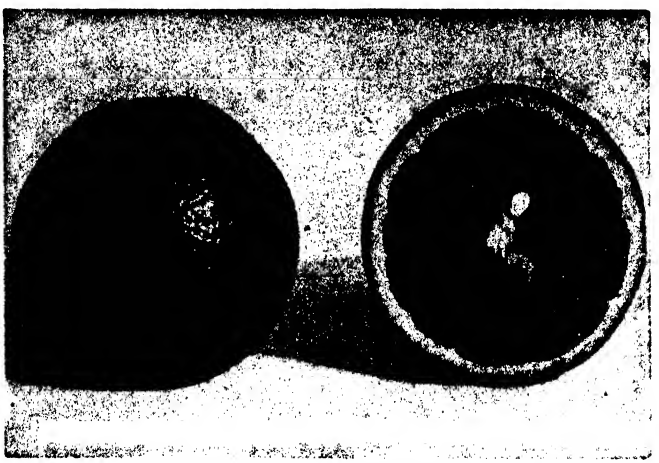


Fig. 4. Ruby blood orange (external view and cross section).

from the peel, an essential oil used in perfumery; from conversion of the juice, citrate of lime; and from the waste peel, a stock feed.

The Sweet Orange [*Citrus sinensis* (Linn.) Osbeck] occupies a dominant position in the citrus industry, for no other fruit is so universally liked and consumed. It is a native of China and is now cultivated in many tropical and subtropical regions where climatic and soil conditions are favorable.

The numerous varieties of sweet oranges can be classified into the following three large groups: those with normal fruits—round (such as Spanish oranges or Valencias) or oval in shape (such as the Jaffas or Mediterranean oranges); those with abnormal or navel fruits (Washington navel); and those with red or red-streaked pulp (blood oranges) (Fig. 4).

From the standpoint of marketing and of the manufacture of citrus products, orange varieties should be differentiated according to their picking season: (*1*) varieties maturing in winter, their shipping season occurring between October and June—for instance, the Washington navel in California, the pineapple orange in Florida, and the Jaffas in Palestine; and (*2*) varieties maturing in spring, their season extending through summer, such as the Valencia and Lue Gim Gong oranges.

Most varieties of sweet oranges, except blood oranges, are utilized for citrus products; their juices especially have become very popular in pasteurized, frozen, or concentrated forms.

The Mandarin and Tangerine Oranges [*Citrus reticulata* Blanco or *C. nobilis* Andrews (non Lour.)] are a group of loose-skinned oranges widely cultivated in Japan, China, the United States, Australia, and the Mediterranean region. They comprise several very popular varieties such as Dancy and Beauty tangerines, King and Satsuma oranges, and Clementines, of excellent quality and characteristic flavor—all cultivated only for marketing as fresh fruit. The names mandarin and tangerine are used more or less interchangeably, although tangerine has become the market designation for the deep orange or scarlet varieties.

These fruits are seldom used for by-products, although mandarin-peel oil is produced to some extent in Italy, and the segments are usually canned in syrup by the Japanese. A fair demand has recently been created for mandarin juice, which is now successfully canned in the United States.

The Grapefruit (*Citrus paradisi* Macfadyen or *C. maxima* Merr.). This species, of comparatively recent development, is probably a mutation or sport from the shaddock (pomelo). Its oblate fruits, borne mainly in clusters, are light yellow and have a refreshing, mildly bitter flavor. Grapefruit now occupies an important place in the citrus industry and is extensively cultivated in the United States (California, Florida, Texas, and Arizona), as well as in Palestine, South Africa, Brazil, and the West Indies. A very large proportion of the fruit grown in the United States is canned, either as segments (hearts) or as juice. The peel of the grapefruit contains little essential oil compared with that of other citrus species. Grapefruit is a very excellent source for the manufacture of pectin. Probably no other fruit has experienced such a surprisingly quick and interesting evolution of new varieties. The commonly cultivated

varieties are the Duncan, Walters, and McCarty—all seedy types, now usually superseded by seedless types such as Marsh seedless. Recently, new varieties have been introduced: the Foster, a bud mutation of the Walters, having pink-fleshed, seedy fruits with a red blush on the rind, and the Thompson, a pink-fleshed bud mutation of the Marsh. The latter is almost seedless and shows no color on the exterior. The newly discovered varieties, the Ruby and the Webb, have, in addition to the properties of the Thompson, a red-flushed rind.

Fig. 5. Pomelo.

The pigment of these red-colored varieties of grapefruit is apparently confined to the tissues of the peel, the segments, and the pulp and is not dissolved in the juice, for, if carefully extracted, the juice shows little or no red color.

The Pomelo or Shaddock [*Citrus grandis* (Linn.) Osbeck], introduced to the West Indies by Captain Shaddock in the latter half of the seventeenth century, very much resembles the grapefruit, which is of much later development. Its fruits—some oblate or globose, flattened, and neckless, and others elongated, pyriform, and necked—are much larger than the grapefruit and have a much thicker peel (Fig. 5). This fruit is an excellent source for the manufacture

of pectin. A native of Polynesia, it has reached its greatest commercial development in southern China, Indo-China, Siam, and Malaysia.

The Lemon [*Citrus limon* (Linn.) Burmann, *C. limonia* Osbeck, or *C. limonum* Risso] is a native of northern Burma and southern China. It was first commercially cultivated in Italy and Sicily where it was brought from Palestine during the thirteenth century. For a long time Italy was practically the sole center of lemon cultivation. This fruit is now successfully grown mainly in semi-arid districts in Spain, the United States, and Palestine. In California, the standard

Fig. 6. Lemon.

varieties are Eureka, Lisbon, and Villafranca. The current American practice is to pick these varieties while still green and to keep them for some time in cool dry storage, after which they are cured by the newly developed methods using ethylene gas. These methods are describe in detail later. The fruit of the lemon is usually smaller than that of the orange; it is of a lemon-yellow color, of obovate to elliptical or oblong shape, and frequently necked or somewhat collared; the areolar area protrudes as a pointed nipple (Fig. 6). Its surface is usually slightly rugose.

Although not directly edible as a fruit, the lemon has probably a greater variety of uses than any other citrus fruit: it is extensively used (especially in Russia) with tea, and serves all over the world for many culinary purposes. It is utilized for making marmalades,

soft drinks, citric acid, pectin, lemon oil, etc. The rough lemon variety is extensively employed in the citrus industry as a rootstock.

The Lime [*Citrus aurantifolia* (Christm.) Swingle] is one of the tenderest of the citrus species and is, therefore, easily subject to frost injury. Like the lemon it bears highly flavored acid fruits with a juice

Fig. 7. Etrog.

acidity averaging as high as 7.7% and a low sugar content (0.3%). The essential oil of the peel as well as the juice has a strong specific odor. Limes are used in the same way as lemons, and in beverages are preferred to lemons by some consumers. The fruit, on the average, is much smaller than that of the lemon. They are extensively cultivated in Mexico, the United States, and the West Indies, especially on the islands of Dominica, St. Lucia, Montserrat, Jamaica, and Trinidad. Of late, the cultivation of limes in the West Indies has received a seri-

ous setback owing to the ravages of the anthracnose disease (*Gloeosporium limetticolum*) and to damage by hurricanes.

A separate group in this species are the sweet limes, grown mainly in Tahiti and Egypt. They do not command any important export trade due to their insipid taste and are used mainly for local consumption.

The Citron (*Citrus medica* Linn.) was the first of the citrus fruits to become known to Europeans, most probably having been brought to Europe from Palestine. It is now cultivated commercially in Palestine, Italy, Greece, and Corsica (France). As the tree is very sensitive to cold, it can be successfully grown only in warm regions. The fruit is ellipsoidal in shape and light orange-yellow to cadmium-yellow in color, and has a rough and bumpy surface. Its peel is very thick and its solid, sweet, and acid-free pulp has practically no juice.

The main use of the fruit is for the production of candied peel or for preservation in brine (see later).

One variety of the citron, the etrog (Adam's or paradise apple) (Fig. 7), known from the Bible as "the fruit of goodly trees," is used by the Jews in the ritual ceremonies during the Feast of Tabernacles. The etrog is grown mainly in Palestine and on the island of Corfu.

C. CULTIVATION AND MARKETING

1. Cultivating Methods. Seasons of Harvesting

Citrus plantations require ample irrigation and are very susceptible to frost injury. In selecting a location for citrus cultivation, the foregoing two points must be borne carefully in mind. Citrus trees thrive best on well-drained soils, sufficiently protected from strong winds by means of suitable windbreaks.

Citrus trees are propagated by one of the following methods: budding, grafting, seeds, cuttings, or layering. Budding is by far the most popular procedure because budded trees bear at an earlier age, their crop is more uniform, and, in addition, mal-di-gomma (gummosis) and other root and trunk diseases can be avoided by using resistant stocks. The most commonly employed stocks are the sour orange and the rough lemon. The selection of the proper stock depends entirely on its suitability to the soil and on climatic and other conditions of the region, as well as on its resistance to diseases.

In older methods, citrus trees were planted very close to each other. Although such plantations bear much more fruit per acre, the

current practice is to plant the trees far apart (5 to 7 by 7 m) to permit cultivation of the soil by machinery and to facilitate the picking operations and transportation of the fruit.

In many citrus-growing regions, the trees must sometimes be protected from frost by firing piles of wood or by using oil heaters.

Like all other horticultural crops, citrus trees and fruit are exposed to many plant diseases and pests. To combat these, a considerable part of the annual cost of production is allocated to spraying, dusting, fumigation, and other means of pest and disease control.

Fig. 8. Grapefruit tree in Palestine.

The main seasons for crop movement are, in general, the winter and early spring months, although some varieties, such as Valencia, are harvested from June to October. A proper selection of varieties, maturing at different times of the year, would create a constant supply of fresh citrus fruits.

2. Packing and Marketing Methods

When properly matured, citrus fruits are carefully picked by experienced workers, for only by capable handling can the quantity of culled fruit be minimized. The fruit is picked by means of specially designed clippers (Fig. 9), put into picking bags, and carefully emptied into field boxes. The field boxes are then hauled in wagons to the packing house, where the fruit is properly selected, graded, sized, wrapped in tissue paper, and packed in wooden crates. In some

countries (mainly in the United States) the fruit is washed and dried prior to grading. Soaking in disinfecting solutions, such as borax, guards against decay from green and blue molds and stem-end rot; final polishing with the addition of a very thin layer of paraffin wax or a similar substance minimizes wilting through evaporation during storage and transportation. Fruits thus treated preserve their fine glossy appearance for many months after picking.

In most citrus-producing regions, packing is still done by hand, the individual grower picking and packing his fruit in his own pack-

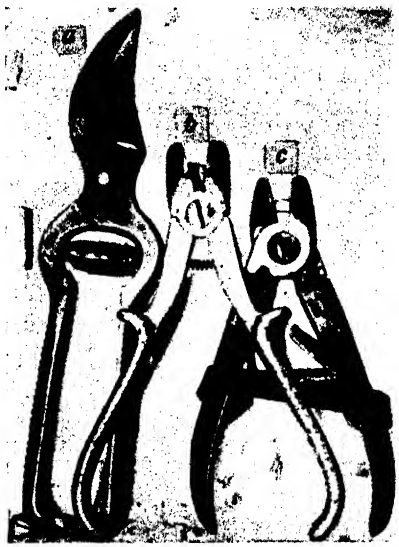


Fig. 9. Collection of clippers used in picking citrus fruits: *a*, sample of an unsuitable clipper; *b* and *c*, good specimens with rounded edges.

ing house—in many cases a dilapidated shed (Fig. 10); often the whole operation is effected in the open air. Such a procedure leads, of course, to irregular packs and lack of uniformity. The handling of citrus fruit in the United States has, during the last forty years, undergone a very important and far-reaching change. Fruit from large areas is now brought to a central modern packing house, well lighted and ventilated and equipped with up-to-date machinery and devices for handling the fruit properly and carefully (Fig. 11). Such operations as washing, drying, sizing, grading and marking, nailing the boxes, and stenciling are performed by mechanical means extensively developed during the last three decades. The fruit passes

from one operation to another by belt conveyors which are carefully padded to prevent injury and to safeguard the fruit at every stage.

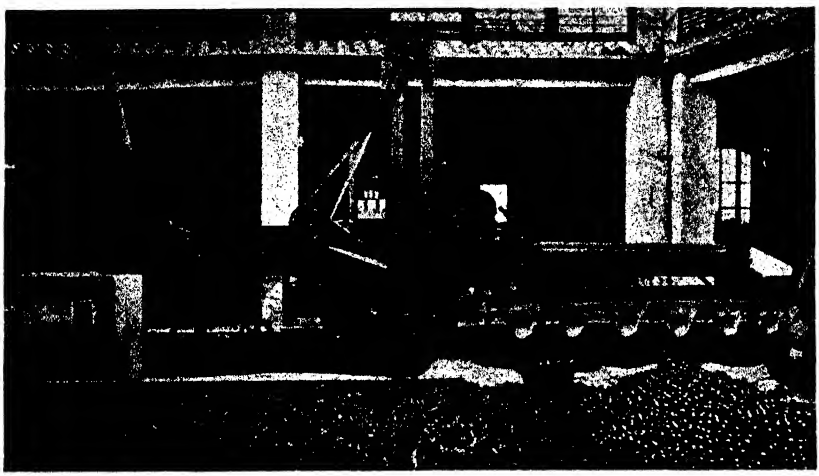


Fig. 10. Primitive packing shed in Spain.

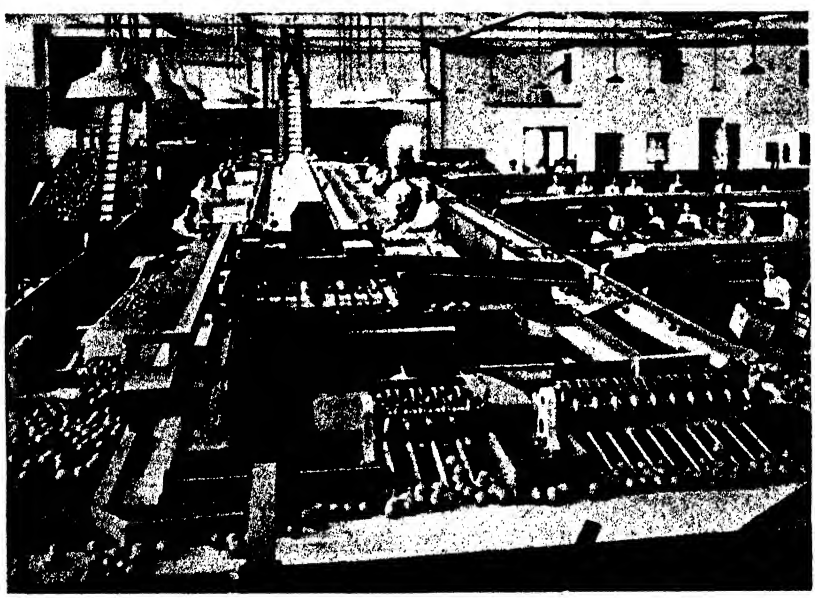


Fig. 11. Modern packing house in the United States.

Such packing houses are equipped also with rooms, when needed, for curing or hastening the coloring of fruits. This process is discussed later in some detail. In many cases the fruit is precooled in specially

designed cold storerooms, because practically all citrus fruits in the United States are shipped in refrigerator cars.

These excellent achievements in the processing of citrus fruit and especially in the techniques of marketing have been attained in the United States mainly through the cooperative associations of the growers. In California, over 80% of the growers belong to the California Fruit Growers' Exchange.

3. Selection of the Fruit—the Culls

Prior to grading, the so-called "culls" are eliminated by careful selection. Most culls are not waste fruit—i.e., diseased, rotten, or crushed—for such fruit, if any, must be discarded. Culls are sound fruit unfit for export or for shipping to distant markets because of irregular shape, oversize, undersize, frost damage, heat damage, clipper or nail cuts caused by careless picking, thorn or twig pricks, bruises, wind scars, marks caused by thrips, excessive scale infestation, or mechanical injury. "Windfalls" should not, of course, be included in the category of culls. Culls are considered very good fruit for manufacturing purposes despite the fact that they are not marketable as fresh fruit. The elimination of a greater proportion of culls raises the standard of the marketable fruit, which then commands higher prices.

The proportion of the total crop not salable as fresh fruit and treated as culls for conversion into by-products varies widely, depending upon the skill of the grower, the season, and the market conditions. In Italy, the proportion of culls is commonly as high as 30% of the total crop. In California, where cultivation methods are superior to those in general use, the proportion of culls is normally much lower, probably only 10%. Culls in Palestine are estimated at 15%.

Culled citrus fruits are delivered to citrus-products plants either in lorries or in railway cars.

D. CITRUS FRUIT DISEASES

Citrus diseases and their control are fully described by Fawcett (1936). However, mention of some of the major citrus fruit diseases may be useful in connection with the selection of culls suitable for the manufacture of citrus products.

Citrus fruit diseases may be classified into three main groups: (*1*) fruit rots caused by microorganisms (green and blue molds, Aspergillus rot, Alternaria rot, brown rot, Diplodia rot, etc.); (2) internal

derangements caused mainly by abnormal metabolism of the plant (frost injury, for example); (*3*) external abnormalities which may or may not be caused by a definite disease (spots of various kinds, hail and frost marks, scabs, hypertrophies or eruptions, insect scales, and fumigation or spray injuries).

Common green mold is caused by the fungus *Penicillium digitatum* Sacc. and begins almost invariably through some injury to the peel. The powdery spore masses of olive-green color then appear on the surface, encircled by a band of white mycelium. The area attacked becomes very soft, and the entire fruit decomposes into a green mass of spores. Although the mold first attacks the skin, the juice acquires a bitter and disagreeable flavor before the organism penetrates into the endocarp.

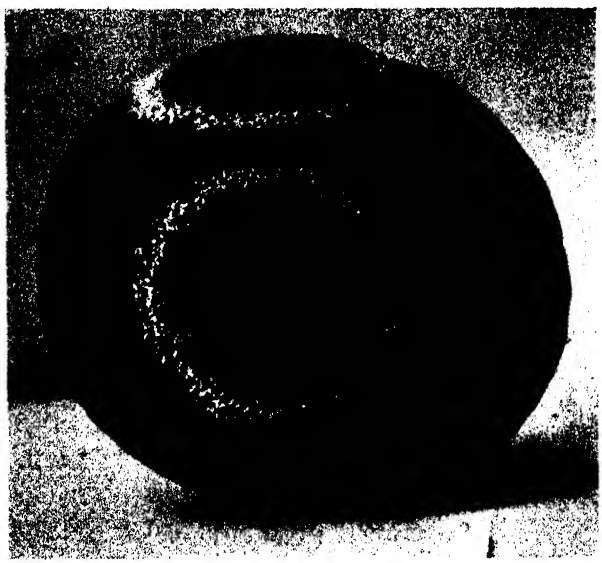


Fig. 12. Orange affected by *Penicillium digitatum*.

Blue contact mold is caused by *Penicillium italicum* Wehmer, in a manner similar to common green mold and tends strongly to spread from fruit to fruit merely by contact. The color of the spore masses is blue. Very often, after the fruit has been attacked by this fungus, green mold also sets in; the result is a mixture of the green and blue colors. However, the green spores soon predominate. Both green and blue molds attack matured fruit more readily than unripened fruit. Careful handling of the fruit during picking and packing operations, avoiding even the slightest injury to the skin, is the most effective preventive measure against attack.

Brown rot is a very widely distributed citrus fruit disease caused by any of the six species of fungi, known as *Phytophthora citrophthora*. The first symptom of brown rot is a slight discoloration on the surface of the fruit which soon develops into a brown-colored spot and penetrates into the fruit. A ready means of identification of this rot is a characteristic and penetrating odor of

rancidity. Because these fungi propagate best in moist soils, citrus fruits are attacked by brown rot mainly during periods of heavy and prolonged rains, and the infection is more likely to occur in fruits on the lower branches of the tree.

Sclerotinia or cottony rot, a rapidly progressing contact decay caused by the fungus *Sclerotinia sclerotiorum,* produces a white fluffy growth of mycelium covering the affected fruits. While the earliest symptom is an almost imperceptible discolored area on the fruit surface, the subsequent stages of this fruit disease are very rapid, spreading, especially in moist air, from fruit to fruit by mere contact. This rot attacks mainly lemons.

Alternaria rot, common also among noncitrus fruits such as dates, is caused by the fungus *Alternaria citri,* mainly in lemons and navel oranges. *Alternaria,* which is normally present in the buttons on lemons in the grove, attacks the weak fruits

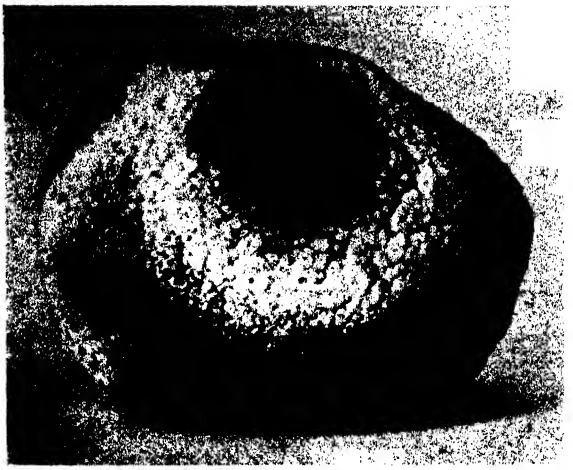


Fig. 13. Orange affected by *Penicillium glaucum.*

suffering from internal decline, frost, or other abnormal conditions of the fruit tissues. The fungus enters the fruit through the stem end, producing a soft breakdown of the central axis or core, while the exterior may appear sound. In navel oranges, this fungus causes a dry, firm, black rot in the navel end extending into the endocarp.

Diplodia rot, a very common disease caused by the fungus *Diplodia natalensis,* usually begins, as in the *Phomopsis stem-end rot* (from which it is distinguished with difficulty in its earlier stages), at the stem end of the fruit. Diplodia rot starts as an almost imperceptible discoloration around the button, develops into a dark brown spot, and spreads rapidly over the entire surface, which becomes leathery and pliable, accompanied by a strong odor of decay.

Water spots. After a prolonged period of wet weather in California, a serious breakdown in the rind of citrus fruits often occurs, especially in navel oranges. This consists in spots resulting from the imbibition of water through the navel or through wounds in the cuticle of the rind, and into adjacent tissues. The attacked areas may later be invaded by various molds, such as green or blue *Penicillia* or by *Alternaria* species.

Internal decline of lemons (endoxerosis) is an abnormal physiological condition characterized by a breakdown or drying of the internal tissue at the stylar end.

Internal decline is accompanied by a pinkish to brownish discoloration and involves not only the rind but also a considerable portion of the inner pulp and the endocarp.

Frost injury. When citrus fruits are affected by frost while on the tree, they show certain external and internal effects. The first symptom is the appearance, on the rind surface, of spots (similar to those produced by liberated oil) which later turn brown. Internally such fruits are full of tiny spots composed of crystals of hesperidin deposited on the membrane and the pulp. Fruits affected by frost have a lower specific gravity, contain less juice, and usually have a thicker skin. The juice is sometimes slightly bitter.

External stains, etc. Adverse weather conditions as well as spraying and dusting may produce various conditions of staining, spotting, pitting, or other markings on citrus fruits. However, these rarely affect quality from the nutritional point of view; and although fruits thus affected are usually culled out as being unsuitable as such for the market, they are well adapted for the manufacture of citrus products.

PART I

CHEMICAL COMPOSITION

CHAPTER II

THE EPICARP OR FLAVEDO

A. PHOTOSYNTHESIS AND PIGMENTS

1. Anatomy of the Epicarp

All citrus fruits have a continuous layer of epidermal cells with a thick cuticle containing stomata. Under this epidermis lies the epicarp or flavedo—a parenchymatous layer rich in chloroplasts and

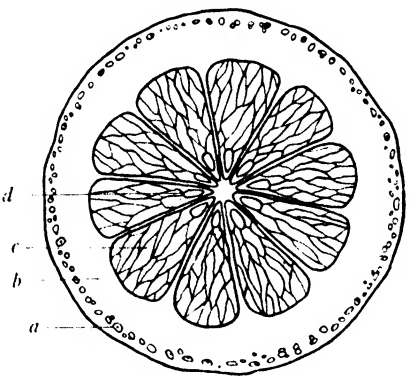


Fig. 14. Schematic presentation of a cross section of a citrus fruit: *a*, oil sacs in the flavedo; *b*, the albedo; *c*, the carpels containing the juice cells; *d*, seeds.

containing numerous oil sacs (Fig. 14). Examination under the microscope shows that the pigment is not equally distributed throughout the cells, but is concentrated in minute structures, plastids, which are green (*chloroplasts*) in unripe fruit and gradually become yellow or orange (*chromoplasts*—plastids colored other than green) as ripening progresses. The chloroplasts vary in size but most frequently are about 5 μ in diameter; they do not consist of solid coloring matter but appear to be spongelike structures in which the chlorophyll and the other pigments are enmeshed, principally at the surface, thus permitting the plastids to absorb as much light as possible.

2. Chlorophyll

In unripe fruits the coloring matter of the chloroplasts consists mainly of chlorophyll, the green pigment of the leaves which, by taking the active part in the photosynthesis, creates with the aid of light various organic compounds from carbon dioxide of the air and water.

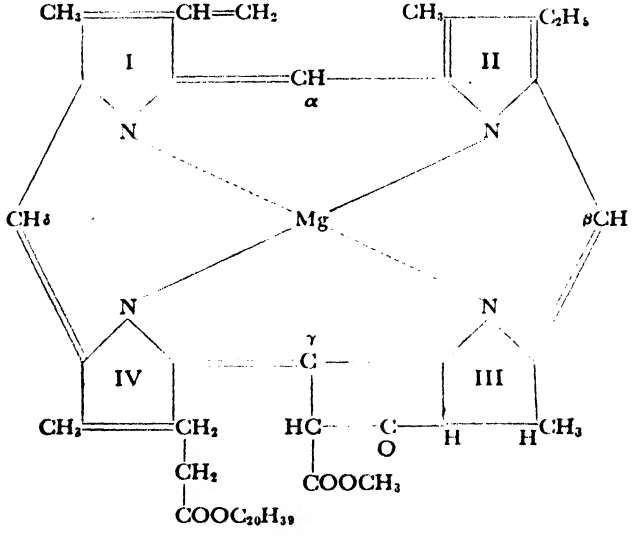
$C_{32}H_{30}ON_4Mg\begin{matrix}COOCH_3\\COOC_{20}H_{39}\end{matrix}$

Chlorophyll *a*

$C_{32}H_{28}O_2N_4Mg\begin{matrix}COOCH_3\\COOC_{20}H_{39}\end{matrix}$

Chlorophyll *b*

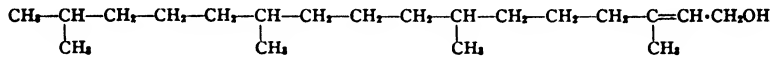
The green pigment consists of two components, chlorophyll *a* and chlorophyll *b*, the ratio of *a* to *b* being approximately constant at 1:2.9. They are invariably accompanied by two yellow pigments, carotene ($C_{40}H_{56}$) and xanthophyll ($C_{40}H_{56}O_2$) both of which have been found to exist in several isomeric forms widely distributed among plants.



Graphic structure of chlorophyll *a*

Chlorophyll *a* is an ester of methyl (CH_3OH) and phytyl ($C_{20}H_{39}OH$) alcohols. The four pyrrole rings are each linked with a so-called methine bridge conventionally labeled with Greek letters α, β, γ, and δ. The molecule of chlorophyll contains magnesium

in conjugated linkage and is similar to hemin, the coloring pigment of blood, except that the latter contains iron in its molecule instead of magnesium. According to the new terminology in the chemistry of chlorophylls, the *a* component is 1,3,5,8-tetramethyl-2-vinyl-4-ethyl-7-propionyl (phytyl) ester 9-oxo-10-carbomethoxy-phorbin. Chlorophyll *b* differs from chlorophyll *a* only by having an aldehyde group in place of the methyl group in pyrrole ring II. The constitution of the phytyl alcohol forming the ester in the chlorophyll molecule is:



This is reminiscent of the isoprene structure (compare with the structure of the carotene molecule on page 30). Phytol, being a colorless oil and insoluble in water, is responsible for the water insolubility of chlorophyll.

While the problem of structure of the chlorophyll molecule can be considered solved, the mode of its creation inside the green parts of the plant is still a matter of conjecture. Botanists have for a long time believed that the plants form in their plastids some simpler substances from which the green pigment is later synthesized. In fact, the buds of most of the etiolated plants show no traces of chlorophyll, which does, however, appear immediately after the buds are exposed to light for a few seconds. It is, therefore, suggested that a precursor of the chlorophyll in some leuco form (now designated as protochlorophyll) is formed in the plastids of the plant. It is also assumed that, while within the living tissue, chorophyll is combined with a protein, however, the existence of a chlorophyll-protein complex of uniform composition is not yet proved by experiments.

The colorless phase is common to all chlorophyll containing higher plants with the exception of a few species of cryptogams and conifers, as well as several varieties of *Citrus* (oranges) in which the cotyledons of the seeds are green inside the fruit. Apparently a certain amount of iron is necessary to transform the colorless phase into chlorophyll.*

Chlorophyllase, an enzyme which sometimes accompanies the green pigment, has the property of detaching the phytol group from the main chlorophyll molecule. Chlorophyllase from some plant

* A well-known citrus disease, chlorosis, which causes the green leaves to turn yellow, is usually overcome by treating the affected leaves with a solution of iron salts, the leaves regaining their green color in a few hours.

sources and at certain seasons of the year is quite active; from other sources it is inactive. It does not occur at all in some vegetables, such as stringbeans, peas, and asparagus.

During the ripening of the fruit the green pigment turns colorless, permitting the color of the yellow material to show. So far, no readily identifiable fragments of the molecule associated with the phase of complete disappearance have been found. The chlorophyll molecule apparently breaks up rather completely.

Chlorophyll also dissolves readily in the essential oils of citrus. Hence, during extraction, when the oil emulsion comes in contact with the ruptured peel—as in the cold-press machine method—the oils are strongly colored. The color may be green from chlorophyll if the extraction is performed early in the season, or dark orange, or even red, if the season is well advanced. The strong green color of early-season oils changes, however, to yellow, dark yellow, or red after a short storage period when the chlorophyll disappears, due most probably to the action of enzymes.

3. Mechanism of Photosynthesis

The universal importance of chlorophyll is its biochemical action in connection with the life process generally known as assimilation of carbon dioxide by green plants, or photosynthesis. For nearly 150 years it has been known that under the influence of light the green leaves of plants, containing chlorophyll, synthesize carbohydrates from carbon dioxide in the air and from water. Oxygen is released during this process, the over-all reaction of photosynthesis being:

$$6\ CO_2 + 6\ H_2O = (CH_2O)_6 + 6\ O_2$$

Photosynthesis and the part played by chlorophyll in the assimilation process are mechanisms known so far only in their general aspects. Extensive studies during the last century were designed to discover the primary product created during photosynthesis. However, the identity of this product is yet an open question. Formaldehyde was suggested as a primary product by Baeyer in 1870—a thesis later supported by Willstätter's school. Since then, this role has also been claimed for various other substances. The second question raised concerned the nature of the first carbohydrate formed during the photosynthesis. On the basis of numerous experiments on the absolute and relative concentrations of the carbohydrates in plants at different times of the day and seasons of the year, glucose, starch, sucrose, and inositol have each been suggested in turn as the first

carbohydrate formed during photosynthesis. Recent investigations suggest that perhaps a hexose monophosphate is the primary precursor of sucrose and starch and that free monosaccharides found in the cell sap are secondary products formed by the hydrolysis of disaccharides.

Much study has also been devoted to the problem of the amount of light required for this process. Although the average conversion yield in direct sunlight is only about 3% of the absorbed light, the photosynthetic mechanism is capable of converting light into chemical energy with an efficiency of up to about 30% if it occurs in weak light and in the presence of an ample supply of CO_2.

Willstätter and Stoll (1913) were the first to suggest that photosynthesis is a surface reaction which takes place on the surface of the plastids and that both chlorophyll *a* and *b* participate. Photosynthesis usually is considered as photochemical reduction of carbon dioxide, with oxygen liberation as a secondary process. According to some recent views, however, photosynthesis is primarily decomposition of water with secondary reactions between one of the products and carbon dioxide. Fundamentally, photosynthesis is an oxidation-reduction reaction between water and carbon dioxide; from the physical point of view, photosynthesis should be regarded as a sensitized photochemical reaction, induced by a light-absorbing substance, the chlorophyll, acting as a photocatalyst (since no decrease in the concentration of chlorophyll in leaves has been observed after intense photosynthesis) and probably even taking an active part in the reaction, in the manner of other oxido-reductases. Whether the primary photochemical process is more closely associated with the hydrogenation of carbon dioxide, or with the dehydrogenation of water, or with both, is as yet undecided.

B. THE CAROTENOIDS

1. Carotene and Xanthophyll

When the citrus fruit ripens, the green chlorophyll disappears or becomes colorless and its permanent yellow companions, carotene ($C_{40}H_{56}$) and xanthophyll ($C_{40}H_{56}O_2$), manifest themselves. These pigments, the so-called carotenoids (usually classed among the polyenes), are largely, if not entirely, responsible for the yellow and orange to red colors of all citrus fruits as well as of many other fruits.

The carotenoids are lipochrome pigments which are insoluble in water but are soluble in fats and oils, thus accounting for the straw-

yellow to dark red color of the essential oils of citrus, depending upon the technique by which they are extracted.

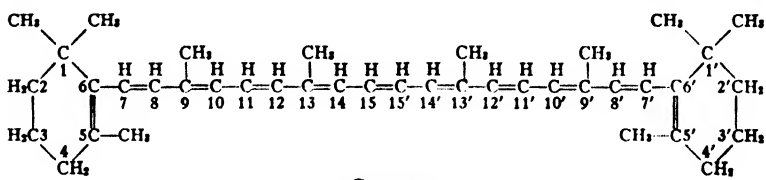
The so-called true carotenoids are hydrocarbons containing 40 carbon atoms, such as carotene (named after carrots) and its isomer lycopene (the red pigment of tomatoes), and their alcohol, ketone, and ester derivatives. All other carotenoids are probably their decomposition products with fewer than 40 carbon atoms.

Carotene was the first carotenoid pigment isolated by Wackenroder in 1831; its hydrocarbon nature was recognized by Arnaud; its empirical formula, $C_{40}H_{56}$, was determined by Willstätter and its structural formula by P. Karrer.

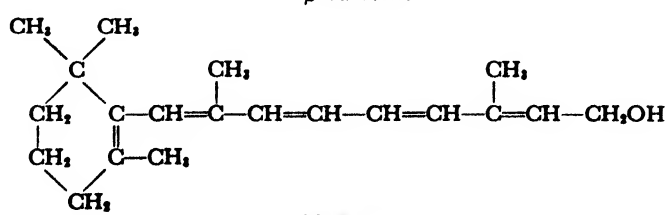
Karrer found that carotene occurs in three isomers, called α, β, and γ, differing in the position of their eleven double bonds. No individual carotenoid has been found to exist alone. β-Carotene is the major component of all the plant-carotene mixtures examined. Due to the conjugation of these numerous carbon double bonds, carotene is readily autoxidizable by atmospheric oxygen, a characteristic of most of the carotenoids; this oxidation is, however, not reversible.

All polyene pigments owe their color to such long conjugated double bonds, the saturation of which is accompanied by loss in color. It is noteworthy that when citrus essential oils, which naturally contain a considerable amount of these pigments, are exposed to the air, they quickly oxidize, losing their color. The slightest fading of their original color is a very good indication of undesirable flavor changes in the oil and of subsequent spoilage.

A most interesting fact about carotene was discovered by H. von Euler and P. Karrer, who found that the pigment is hydrolyzed into the fat-soluble vitamin A, an essential factor in the growth of ani-



β-Carotene

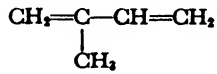


Vitamin A

mals and man. This breakdown of carotene into vitamin A is performed by human and some animal bodies.

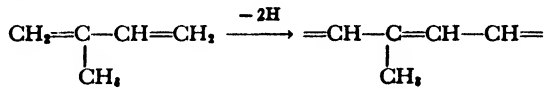
The structural formula of β-carotene, containing exactly two groups of vitamin A, is shown on page 30. Carotene is therefore called the precursor of vitamin A.

It is again noteworthy that all carotenoid chains can be regarded as built of isoprene (C_5H_8), one of the most important structural units of organic plant material:



As previously mentioned, the structure of phytol is similar; also, many essential oils, the terpenes, as well as natural rubber, are built of this important molecule. The similarity of the molecular structure of all these plant substances suggests that they are genetically related.

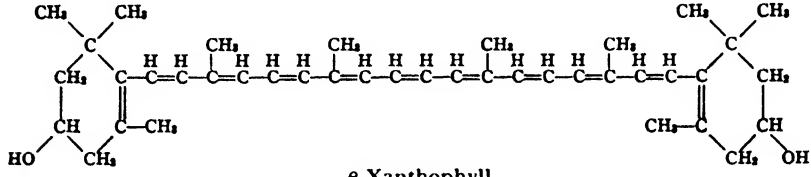
These derivatives of isoprene apparently can form in three different ways: by direct addition of the C_5H_8-groups to form terpenes (as will be shown under "essential oils," pages 42–43); by addition and simultaneous hydrogenation, as shown by Willstätter in the case of phytol; and, by addition and simultaneous dehydrogenation, as in the case of carotenoids. In the third case the isoprene, upon losing two atoms of hydrogen, rearranges itself to form a grouping typical of carotenoids:



Accordingly, the phytol of the chlorophyll molecule may be directly dehydrogenized into carotenoids—a possibility which has been assumed by many scientific workers but has not been proved. On the other hand, recent researches show that the amount of yellow pigments of the fruits is not augmented after the chlorophyll has become colorless or when the phytol has been detached. Quite the contrary was shown recently by Miller *et al.*[1] who found that, as the orange fruit progressively matures, the chlorophyll content decreases and the carotenoid content, even after all chlorophyll has disappeared, increases. However, in other citrus fruits (limes, lemons, and grapefruit) part of the carotene disappears simultaneously with the chlorophyll.

[1] Miller, E. V., J. R. Winston, and H. A. Schomer, "Physiological Studies of Plastid Pigments in Rinds of Maturing Oranges," *J. Agric. Res.*, **60**, 259 (1940).

Xanthophyll ($C_{40}H_{56}O_2$), the second yellow companion of chlorophyll, is a dihydroxy derivative of the optically active α-carotene, the two hydroxyl groups being connected with the carbon rings. Xanthophyll occurs in two isomeric forms, α and β. Its structural formula is:



β-Xanthophyll

The carotenoids are usually accompanied in the cell by lipides, and there is much evidence that xanythophyll, an alcohol, and other similar carotenoids are linked with the fatty acids in the form of esters. Many yellow plant materials contain such waxlike pigments.

Citrus essential oils (see page 58) contain an appreciable amount of stearoptenes which have been very little studied, and there is reason to believe that they might also contain waxlike pigments consisting of esters of carotenoids and lipides.

One more point of interest, in the author's opinion, is the possibility that an important source of carotene, and therefore of vitamin A, may be obtained in the process of producing orange oil by the cold machine process (see page 184). During this process the orange pigment comes in close contact with the essential oil, which becomes highly colored. For successful marketing, such oils are treated with decolorizing activated carbon of very high quality. The residue left after filtration consists of carbon sludge containing all the yellow and orange pigments, particularly carotene in very high concentrations. It is suggested that this may become a valuable source for an additional by-product.

Zechmeister and Tuzson (1931)[1a] and Vermast (1931)[1b] found in *Citrus aurantium* a xanthophyll which gave a blue color with hydrochloric acid, a reaction specific to violaxanthin ($C_{40}H_{56}O_4$). In a later study (1936), Zechmeister and Tuzson showed that the pigments of orange peel are present in the form of esters. After saponification the fractional solution of these pigments in petroleum ether and methanol of 90% yielded a carotene fraction of 28.1 mg and a

[1a] Zechmeister, L., and P. Tuzson, "Über das Pigment der Orangenschale," *Naturwissenschaften*, 19, 307 (1931).

[1b] Vermast, P. G. F., The Carotinoids of *Citrus aurantium*, *Naturwissenschaften*, 19, 442 (1931).

xanthophyll fraction of 49.8 mg per kg peel. Both fractions were examined chromatographically. The bulk (95%) of the carotene fraction was identified as crystalline cryptoxanthin ($C_{40}H_{56}O$); its presence was ascertained both spectographically and analytically. This fraction contained also β-carotene with traces of α-carotene. In the xanthophyll fraction, xanthophyll was identified, isolated, and found to constitute about a third of the whole fraction. Some other pigments were present but were not identified.

Finally, there are indications that the rind of the citrus fruits may contain other yellow-orange pigments besides the carotenoids. Schunck (1903) and Tschirch (1904) obtained proof of the presence of water-soluble noncarotenoid pigments, probably, including anthocyanin, in the peel of several varieties of oranges.

As an exception to the predominance of carotenoids in the peel of citrus fruit, we may mention the characteristic color of the peel of the mature lime which has been found by Hardy and Warneford[2] to be phlobatannin, a pigment resident in the cell sap rather than in the chromoplasts. From the peel of a tangerine Nelson[3] isolated a pigment, pentamethyl flavonol, to which he assigned the name tangeretin.

2. Determination of Plant Pigments

A number of methods exist for the determination of plant pigments, all of them being complicated and outside the scope of this book. However, it may be appropriate to describe here briefly one method[4] for the determination of chlorophyll and carotene in plant tissues, which utilizes recent developments in standard photoelectric colorimeters. This method is based on the fact that the maximum absorption band of a solution of chlorophyll *a* and *b* in the red region of the visual spectrum is independent of the presence of other pigments in the absorbing medium.

A small quantity of the tissue (1 to 2 g), after being moistened with pure acetone, is macerated well with quartz sand and a small amount of Na_2CO_3. The tissue is then repeatedly extracted with small portions of acetone and washed in a suction funnel to assure complete extraction of the pigment and finally made up to 100 cc.

Chlorophyll is determined directly in the extract of the combined

[2] Hardy, F., and F. H. S. Warneford, "The Coloring Matter of Lime Juice," *Ind. Eng. Chem.*, **17**, 48 (1925).

[3] Nelson, E. K., "The Occurrence of a Pentamethyl Flavonol in Tangerine Peel," *J. Am. Chem. Soc.*, **56**, 1392 (1934).

[4] Petering, H. G., W. Wolman, and R. P. Hibbard, "Determination of Chlorophyll and Carotene in Plant Tissues," *Ind. Eng. Chem., Anal. Ed.*, **12**, 148 (1940).

pigments without separating it from the yellow pigments. It is placed in the absorption cell of a photoelectric colorimeter and the transmission of the solution is compared with the transmission of the pure solvent. The reading is converted to concentration by using a standard calibration curve obtained with pure chlorophyll.

For the determination of carotene, the chlorophylls must be separated and the xanthophylls and flavones removed. In a 50 cc portion of the original acetone extract, the chlorophyll is removed by finely divided $Ba(OH)_2$, the mixture is refluxed in an Erlenmeyer flask for 30 min on a water bath, then cooled and filtered free from the green sludge. The carotene is washed away quantitatively with pure acetone, and 50 cc of petroleum ether are added to the filtrate in a separatory funnel. The carotene in the petroleum ether layer is washed free of xanthophylls and solvents, dried over Na_2SO_4, and made up to 100 cc; the concentration is again measured photometrically.

Flavones and their derivatives, quercetinlike substances, have been recently demonstrated and quantitatively determined in the peels of citrus fruit by a special colorimetric method.[5] Lemon peel has shown the highest quercetin equivalent (1.66 mg per g fresh weight); lower values are found in orange and grapefruit peels. It should be mentioned here that these flavone and flavonol derivatives are the fundamental structural units of a group of important substances found in citrus fruit, namely, glucosides (see pages 96–101).

A more exact study of the pigments of the citrus epicarp would seem desirable, using chromatographic and solubility methods as well as the improved microchemical technique. (For carotene determinations in orange juice, see pages 103–104.)

C. ARTIFICIAL COLORING OF CITRUS FRUIT

1. Influence of Color on Marketability of the Fruit

The consuming public usually judges the maturity of citrus fruits by their color. However, it is a well-established fact that color alone is by no means a reliable indication of maturity. When some varieties of citrus fruit mature, the fully developed yellow and orange colors may be completely or partially masked by the green pigments.

In Florida, oranges rarely lose their greenish tint regardless of the state of maturity. In Palestine, Jaffa oranges, although sufficiently

[5] Weatherby, L. S., and Amber L. S. Cheng, "The Determination of Flavones or Quercetinlike Substances in Certain Naturally Occurring Products," *J. Biol. Chem.*, **148**, 707 (1943).

mature (acid to sugar ratio nearly 1:8) and palatable, still preserve their green color during the early shipments. In California, Valencia oranges, for instance, lose their green color in winter while still unpalatable, turning green again when, in the spring, growth starts and hot weather returns.

Such fruit is often undervalued, and the consumer who pays more attention to appearance than to the more desirable qualities pays "for the color." Consequently, methods of artificial coloring of citrus fruit have been extensively used in the United States for many years. These methods actually add no color to that which has been attained naturally by the fruit; they merely cause the green chlorophyll to disappear and permit the previously masked yellow and orange pigments to become fully evident. If, on the other hand, the treated fruit is unripe, the developed color gives the fruit a pale and rather "unhealthy" appearance. (A process of coloring the pale yellow citrus fruits by adding a dye is sometimes used in the United States.)

It is, therefore, an indispensable condition for the coloring of citrus fruit, especially oranges, that the fruit be sufficiently mature. When this process is employed for ripening other fruits (such as bananas) or vegetables (tomatoes, celery), some physiological changes occur, improving not only the color but also the degree of ripeness, but in oranges it influences only the color of the peel, the acidity and the total solids in the juice being practically unchanged. Should the orange be unripe before treatment, the artificial color would be deceiving and, hence, illegal.

2. Old Californian Coloring Method

It has been customary in California to hasten the coloration of green citrus fruit by means of blue-flame kerosene stoves placed directly in the room where the oranges were stored or in the basement beneath it. These are the so-called "sweat rooms."

For some time it was thought that the change in color was brought about by heat, but Sievers and True (1912)[5a] found later that the products of combustion were responsible for the hastening of coloration. The atmosphere in such rooms is extremely disagreeable, requiring ventilation before workers can remove the fruit. The kerosene-stove method of coloring required more than two weeks and had several disadvantages. First, the fire risk during the operations was great; the stoves had to be kept lighted night and day, and tempera-

[5a] Sievers, A. F., and R. H. True, "A Preliminary Study of the Forced Curing of Lemons as Practiced in California," *U. S. Dept. Agr., Bur. Plant Ind. Bull.*, 232 (1912).

ture control was practically impossible. Secondly, the smoky kerosene flame imparted a slight odor to the fruit. These disadvantages of the method could be overcome only if the gases responsible for the change in color could be identified. With ordinary gas-analysis methods, however, the effective gaseous constituents could not be detected.

3. New Coloring Method

Experiments undertaken in 1924 by the Laboratory of Fruit and Vegetable Chemistry in Los Angeles, in cooperation with the Lemon Men's Club of Southern California, disclosed that the gases responsible for the coloration of the fruit were unsaturated hydrocarbons whose specific gravity was approximately equal to that of air. These facts suggested ethylene (C_2H_4), and later experiments proved that this gas, or a mixture of other unsaturated hydrocarbons, could effectively bring about the desired change in color in citrus and other fruits. The mechanism of this reaction is not yet known. The gases merely activate the upper peel of the citrus and stimulate the fruit to renewed life activity. During this period, lasting five to ten days, the fruit continues to respire by inhaling oxygen and expelling carbon dioxide, and, as a result, the fruit itself brings about the discoloration of the green pigment.

The ethylene gas has only a stimulating effect; the quantity used is very minute. Even one part by volume of ethylene in five million parts of air can produce the effect. The proper concentration of the gas, however, depends on the time required by the coloration, the location where the process is conducted, and the relative quantity of air present in space not occupied by the fruit (to provide sufficient oxygen for the respiration of the fruit).

The ethylene is usually introduced from a gas cylinder in charges or "shots" at intervals of six to eight hours and at a gas:air ratio of about 1:4000 or 1:5000. Some packers have reported favorable results with concentrations of 1:20,000. Lower concentrations require longer periods; higher concentrations also retard the process.

4. Atmospheric Conditions

As a physiological process, artificial coloration of fruit requires careful attention to atmospheric conditions during the transformation. Temperature must be carefully controlled and kept uniform. The high temperatures that were formerly thought necessary for the process are now known to be not only unnecessary but positively

injurious. The temperature prescribed by the California packers is 60° to 80° F. At temperatures below 45° F, no hastening of the coloring was obtained, and the rate was also reduced if the temperature was raised above 93° F. It has also been observed that the temperature should vary according to the color of the fruit.

The question of humidity is also very important. During the process the fruit loses some water; the atmosphere of the coloring room must therefore be provided with sufficient humidity to prevent shrinkage of the fruit. Experiments made by the writer showed that even if every precaution was taken, oranges lost on coloration an average of about 0.4% of their weight.

As noted above, both stove gas and ethylene increase the rate of respiration of the citrus fruit. It has been shown that Valencia oranges, for instance, give off in an hour 23 mg of carbon dioxide for every kg of fruit at 29.3° C, but only 2.0 mg for 1 kg at 1.7° C.

Generally it has been found that the CO_2 output under the conditions of coloring is increased about 150 to 250%. Obviously a sufficient supply of oxygen is necessary for the process. Absence of oxygen prevents successful coloration. It is recommended, therefore, that the coloring rooms be thoroughly ventilated at least once daily for a period of 1 to 2 hours.

In this connection it is noteworthy that oranges and lemons, if subjected to restricted ventilation have been found to give off (as shown by Biale and Shepherd[6]) from 3 to 5 mg of acetaldehyde per 100 kg per hour. The same authors found that the vapors from moldy lemons inoculated with green mold (*Penicillium digitatum* Sacc.) cause a very marked increase in the rate of carbon dioxide evolution and accelerate color development of sound green lemons. Emanations of a single moldy lemon can produce these effects in as many as 500 fruit.[6a] Recently Miller, Winston, and Fisher[7] identified ethylene as one of the volatile substances given off by citrus fruits during respiration.

Packing sheds or huts built of metal sheets or wooden boards are unsuitable as coloring rooms: there is almost no possibility of closing such huts hermetically; the walls could not absorb the excessive moisture exuded by the fruit during the "sweating" process, moisture

[6] Biale, J. B., and A. D. Shepherd, "Identification of Acetaldehyde among the Volatile Products of Citrus Fruits," *Amer. Soc. Hort. Sci. Proc.*, **37**, 543 (1939).

[6a] Biale, J. B., and A. D. Shepherd, "Respiration of Citrus Fruits in Relation to Metabolism of Fungi; I, Effects of Emanations of *Penicillium digitatum* Sacc. on Lemons," *Amer. Jour. Botany*, **28**, 263 (1941).

[7] Miller, E. V., J. R. Winston, and D. F. Fisher, *Journ. Agr. Res.*, **60**, 269 (1940).

condensed upon the ceiling and the walls being detrimental to the fruit. Considerable changes in temperature during day and night caused by rapid convection through the metal walls make it impossible to maintain the uniform temperature required. This applies also to coloring under canvas tents, a procedure adopted by some packers.

The atmospheric conditions necessary for the coloring process, as described above, create at the same time conditions optimum for the development of various diseases in fruits which bear the spores of the organisms responsible, mainly the two kinds of *Penicillium* (blue

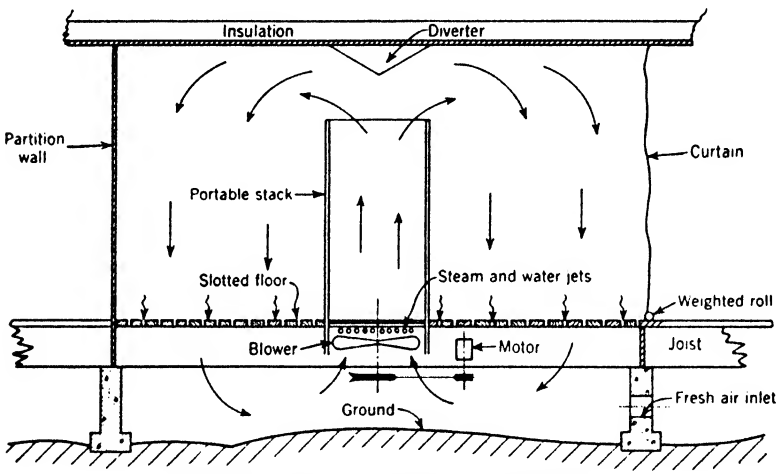


Fig. 15. Plan of a modern curing room.

and green molds) and *Diplodia* (stem-end rot). In other words, since the fruit continues its life activities in the coloring room at a quicker pace than on the tree, the spores will behave similarly if they are present on the fruit. Hence, proper and careful selection of the colored fruit before packing is imperative.

5. Latest Improved Methods

The latest improved methods of coloring as developed in the United States require rooms equipped with air-conditioning apparatus to maintain the requisite constant atmospheric conditions. Figure 15 is self-explanatory. The portable stack is placed over a hole cut in the floor through which fresh air from the blower, preheated if necessary, is introduced; the air proceeds toward the ceiling where it hits a diverter which spreads the current in all directions.

The ethylene gas is not introduced by "shots" but is continuously added to the circulating air (the so-called "trickle system") at a rate of 1 part to 30,000 or 50,000 parts of air. Special devices are obtainable for accurately measuring the amount of ethylene gas released into the entering air (Fig. 16). It is claimed that the time of coloring by this system is reduced to only 24 to 48 hours. The constantly changing air prevents the accumulation of CO_2 and excessive humid-

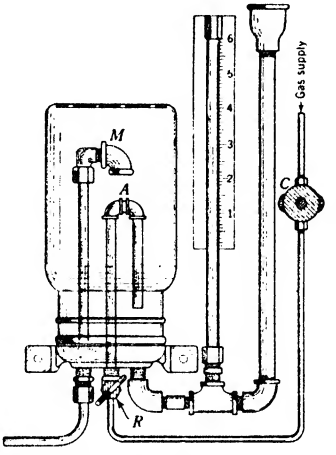


Fig. 16. Metering trickle feeder for ethylene gas; *C*, pressure-reducing valve; *R*, needle valve regulating the flow of gas. The flow of gas passing through *M* is measured on the graduated scale (courtesy Food Machinery Corporation, Dunedin, Florida).

ity and thereby lessens the danger of decay. Probably the chief advantage of this latest system lies in the shorter time required, which correspondingly reduces the exposure of the fruit to the diseases which find the same conditions favorable for their development.

6. Recapitulation

In summary, coloration of citrus fruit is hastened by the application of very simple methods, but too often the air in which the coloring takes place is so polluted with CO_2 and probably other factors harmful to the fruit cells that a physiological breakdown is inevitable. This breakdown is usually accompanied by accelerated growth of molds and bacteria. Too much ethylene, heavy concentrations of CO_2, high temperatures, and humidity overstimulate the respiration and merely serve to hasten breakdown and decay. The best method

for avoiding poor results and accomplishing the coloring satisfactorily is the introduction of air conditioning into the coloring rooms.

Because the warm, humid atmosphere of the coloring rooms naturally causes the skin of the fruit to become tender, fruit should be allowed to cure in fresh air for at least four hours after removal from the coloring or "curing" rooms and prior to any subsequent handling.

As mentioned above, the mechanism of the effect of ethylene, C_2H_4, upon chlorophyll is not yet known, nor does the literature indicate that research on the subject is under way. Systematic study in this field may throw very interesting light on the state of chlorophyll and its behavior during the process of ripening. A few remarks in this connection may be opportune:

1. It is of interest that green fruit in the completely unripe state shows no change in color when treated with ethylene. Assuming that the yellow pigments, carotene and xanthophyll, always accompany the chlorophyll in the same proportion, why does not the ethylene affect the chlorophyll at the early stage?

2. While ripe oranges and lemons processed by the ethylene method first pass a state of pale yellow, then turn orange, tangerines behave differently. Treated mandarins and tangerines turn orange in color without the yellow transition.

3. It is well known that when an unripe citrus fruit is pricked early in the season by the Mediteranean fruit fly, it turns orange and often drops off the tree, although the fruit has not attained maturity. Tests in such cases have shown no increase in sugar content. Evidently the change in color is no indication of maturity.

D. ESSENTIAL OILS

1. Location of Essential Oils in the Epicarp

Adjacent to the chromoplasts, numerous oil sacs or glands are located irregularly at different depths in the epicarp (flavedo) of all citrus fruits. These glands or canal-like intercellular receptacles have no walls of the usual type, but are bounded by the debris of degraded tissues. Being ductless, they have no communication with the surroundings.

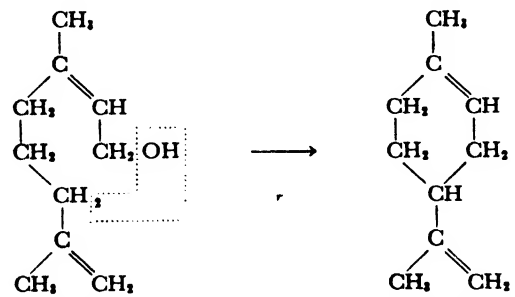
The cells surrounding the oil glands contain an aqueous solution of sugars, salts, and colloids and exert some pressure on the glands; thus the oil is held by the gland under a pronounced turgor pressure. It is well known that when the peel of a citrus fruit is bent

between the fingers the oil is ejected with some force for a considerable distance. If, on the contrary, the peel is not macerated or pressed, the oil will not easily lend itself to extraction even by such drastic means as distillation: the oil cells are thus not easily broken. Before the oil can be obtained by distillation with steam or under reduced pressure, the oil glands must be broken by thorough mincing or trituration of the oil-containing tissues. This turgor force under which the oil is secreted plays an important role in all the existing methods of extraction of the citrus essential oils (see page 183). The oil sacs are not all situated at the same depth below the cuticle, nor are they of the same size: they usually average 0.4 to 0.6 mm in diameter.

Whether the essential oils are formed in the epidermic cells, in some resinogenous membrane not connected with the plasma, as suggested by Tschirch,[8] or whether the plasma of the living cell is responsible for their creation, as thought by Lehmann,[9] is still an open question. Charabot (1918) and his co-workers,[9a] who made a special study of many citrus oils, believe that, in general, essential oils wander from one place to another in the plant during the growth process.

2. Theories on Formation of Essential Oils

Little is known regarding the formation of the essential oils in plants. It is possible that the formation of the terpenes takes place by dehydration of alcohols of the general formula $C_{10}H_{18}O$ to which they are genetically related, since this can be performed in vitro. During the dehydration, the ring formation takes place:



Geraniol *d*-Limonene

Some, however, are of the opinion that the terpenes originate from

[8] Tschirch, A., "Chemie u. Biologie der pflanzlichen Selkrete: Ein Vortrag," Akad. Verlagsgesellschaft m. b. H., Leipzig (1908).

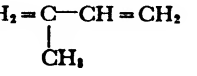
[9] Lehmann, C., *Planta Archiv f. wiss. Botanik*, I, 343 (1925).

[9a] Charabot, E., and M. Gatin, *Le parfum chez la plante*, Paris, 1918.

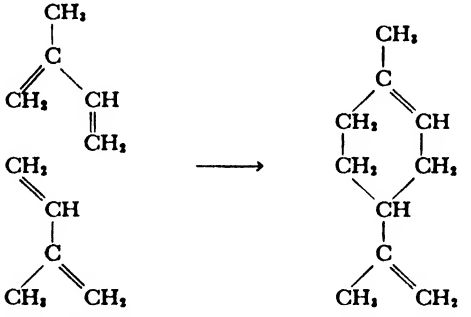
proteins or carbohydrates. According to Ehrlich,[10] Tschirch,[11] and Kodama,[12] isoamyl alcohol, an important structural unit of the essential oils, can be obtained from amino acids containing six carbon atoms (leucine). Buchner[13] states that the same alcohol can be synthesized from hexoses.

Two schools of thought are thus divergent on this point: the first maintains that the essential oils are created in the epidermis, that is, as a consequence of photosynthesis; the second school regards the oils as products of plant metabolism.

Ruzicka presented a hypothesis according to which most of the terpenes may be regarded as made of an isoprene skeleton, the unsaturated aliphatic hydrocarbon C_5H_8, having the following structure:



In fact dipentene, the racemic form of limonene, is obtained synthetically by polymerization of isoprene after heating it to about 300° C:



Two molecules of isoprene — Dipentene

Similarly, isoprene is formed when limonene or dipentene vapor is passed over red-hot platinum wire. The yield is particularly good when dipentene vapor is diluted with nitrogen or is passed over the catalyst at low pressure.

Many sesquiterpenes and polyterpenes are also made up of isoprene molecules linked to each other in various ways, as has been demonstrated for most members of this group of which the structure

[10] Ehrlich, *Ber. Schimmel & Co. Akt.-Ges.*, 156 (1935).
[11] Tschirch, A., *ibid.*, 153 (1925).
[12] Kodama, S., "Studies on Amino Acids; I, On Formation of Some Volatile Oils from Leucine," *J. Biochem.*, 1, 213 (1922).
[13] Buchner, L. A., *Ber. Schimmel & Co. Akt.-Ges.*, 156 (1925).

is known. Most terpenes are multiples of isoprene and have the general formula $C_{10}H_{16}$ or $(C_5H_8)_2$; the sesquiterpenes, $C_{15}H_{24}$ or $(C_5H_8)_3$; and the polyterpenes—diterpenes, $(C_5H_8)_4$, triterpenes, $(C_5H_8)_6$, tetraterpenes, $(C_5H_8)_8$.

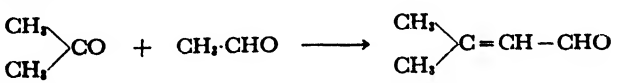
This isoprene hypothesis is the more striking when we consider that phytol (the alcohol fraction of chlorophyll), carotenoids, and sterols as well as rubber (caoutchouc)—all may be regarded as derivatives of isoprene which can thus be looked upon as "Baustein" or structural unit. One may probably accept this hypothesis in support of the opinion that the essential oils are formed during photosynthesis in the epidermal cells of fruits and leaves.

On the other hand, Charabot concludes that the primogenial components of the essential oils are alcohols from which terpenes are later created through ring formation (see page 41). However, Charabot also believes that the alcohols originate in plant tissues rich in chlorophyll during the assimilation process.

A controversy exists also regarding the question of the quantity of terpenes in plants during growth. According to Gildemeister, the less developed the plant is at the time of oil distillation, the greater is the terpene content of the oil. On the other hand, Charabot, who made extensive investigations on bergamot fruit, is of the opinion that the amount of terpenes increases steadily as growth proceeds.

In general, the formation of the more easily soluble components, especially alcohols, accompanies the early growth of the plant. In this more easily soluble and mobile state, they are usually transported through the plant as glucosides. According to Charabot, the mandarin and sour-orange trees contain the greatest amount of organic acids in the leaves, the main organs of photosynthesis. He also states that the amount of esters increases during ripening of the fruit.

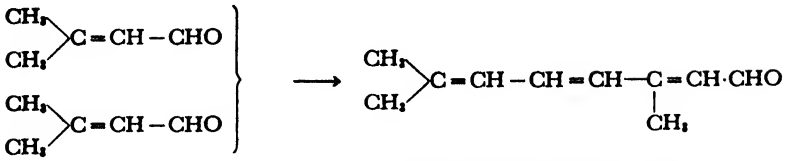
The most attractive theory regarding the formation of the essential oils in the plant and the actual precursor of the terpenes has been proposed by Read,[14] who considers as the key substance the acyclic alcohol, geraniol $(C_{10}H_{18}O)$, or its stereoisomer, nerol; both compounds occur widely distributed in the plant kingdom, either in the free state or as esters of acetic or other acids, and may be formed by condensation of acetone and acetaldehyde, citral being in this case the intermediary product:



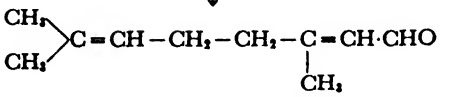
Acetone Acetaldehyde β-Methyl crotonaldehyde

[14] Read, F. M., *J. Soc. Chem. Ind.*, **48**, 786 (1929).

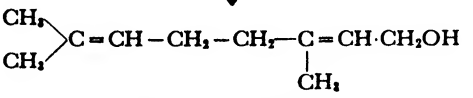
two molecules of which give upon condensation:



3,7-Dimethylocta-2,4,6-triene-1-al



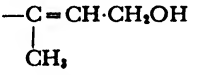
Citral ($C_{10}H_{16}O$)



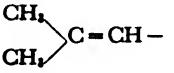
Geraniol ($C_{10}H_{18}O$)

Geraniol can similarly be converted into terpenes (limonene) by dehydration, as shown above.

To account for the adoption of geraniol as the key substance, Read points out that it possesses an unusual molecular structure, since it contains a primary alcohol group activated by an α,β-ethylenic linkage:



situated in close proximity to a second active group:



also containing an ethylenic linkage.

Owing to the great mobility of this grouping, geraniol can be regarded as the parent substance of a great variety of cyclic terpenes and related bodies, the joint occurrence of which has been observed in plant products.

In contradiction to Read's hypothesis, however, may be cited the composition of orange oil, which probably contains no aldehydes other than *n*-decylaldehyde, $CH_3(CH_2)_8.CHO$, and no such active ethylenic linkages as shown in geraniol or citral; hence it is doubtful whether geraniol is always the key substance. Furthermore, according to Read's theory, one should find instances during the growth of the fruit when the alcohols or aldehydes are present in a proportionally much greater quantity than the terpenes, which is never the case.

3. Function of Essential Oils

The physiological function of the essential oils in plant economy is rather obscure. While the odoriferous principle in the leaves or flowers can be assumed to be useful in attracting insects to the pollen, no such property can be ascribed to the oil present in other parts of the plant. The oil may possibly act as a protection against insect attack, but it would appear more satisfactory, in the absence of any definite proof, to regard essential oils, like alkaloids and tannins, as waste products of plant metabolism.

4. Components of Essential Oils

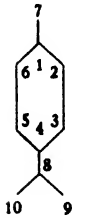
The citrus oils are typical volatile or essential oils made up of mixtures of terpenes, sesquiterpenes, higher alcohols, aldehydes, ketones, acids, esters, and camphors or waxes. Before examining the composition of the citrus oils, it may be worth while to discuss each group separately. However, only representatives occurring in the citrus oils will be mentioned.

(a) Hydrocarbons

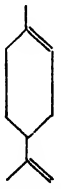
(1) TERPENES

Citrus essential oils are made up mainly of hydrocarbons designated as terpenes, of the general formula $C_{10}H_{16}$, and of a smaller amount of sesquiterpenes ($C_{15}H_{24}$); these two components serve as carriers for the more important class of oxygenated compounds (alcohols, aldehydes, ketones, acids, and esters) which are usually the bearers of the characteristic odor of the oil in which they are contained.

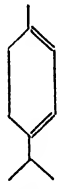
In constitution, most of the terpenes have a striking resemblance, as shown by the accompanying comparison of their structural formulas. They are all unsaturated hydrocarbons with two double bonds and may be grouped under the name of menthadienes:



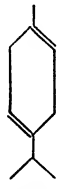
Limonene Δ1,8(9)



α-Terpinene Δ1,3

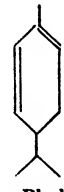


γ-Terpinene Δ1,4



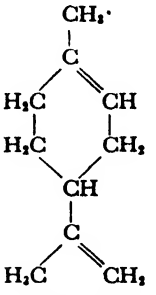
β-Terpinene Δ1(7),3

α-Phellandrene Δ1,5



β-Phellandrene Δ1(7),2

***d*-Limonene.** The principal hydrocarbon occurring in citrus oils is an alicyclic terpene, *d*-limonene ($C_{10}H_{16}$), whose structural formula is:



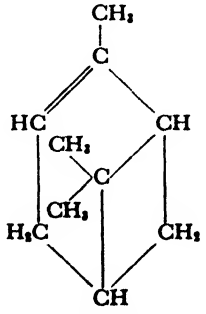
d-Limonene

d-Limonene represents 90% of the oil of orange, from which it can be obtained in the purest form; it is contained, as well, in all other citrus oils. When carefully purified, this hydrocarbon has an agreeable lemonlike odor.

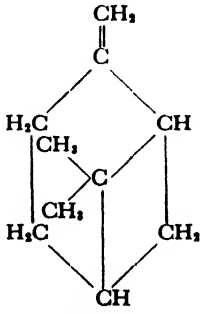
PHYSICAL CONSTANTS. B.p. = 175–176° C; $d_{15°} = 0.850$; $n_D^{20°} = 1.475$; $[\alpha]_D = +123°40'$ (see Table III).

The levorotatory modification of limonene is not found in citrus oils. It occurs in Finland turpentine oil, American peppermint oil, and other oils. Chemically, both limonenes are alike, and, when mixed, dipentene, the racemic modification, results. This racemic form, which is optically inactive, is obtained also when one of the active limonenes is heated to a high temperature or is acted upon by acids.

Besides *d*-limonene, some hydrocarbons are found only in lemon oil and others in neroli oil. They are all alicyclic hydrocarbons, also represented by the general formula $C_{10}H_{16}$. Their boiling points range from 170 to 180° C.



α-Pinene

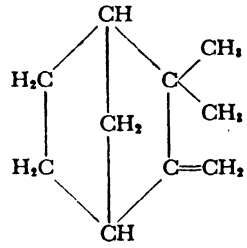


β-Pinene

Pinene is a terpene very widely distributed in nature and occurs in lemon oil in two isomeric forms—see page 46.

Neroli oil contains only α-pinene, a colorless, mobile liquid. Like most terpenes, it undergoes autoxidation upon standing, by taking oxygen from the air, and partly resinifies. It is readily converted at higher temperatures into dipentene. α-Pinene exists in two optically active forms as well as in the racemic state.

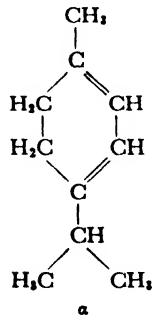
Camphene. Of all hydrocarbons of the general formula $C_{10}H_{16}$, camphene is the only one which occurs in nature as a solid. It has two optically active modifications, as shown by its structural formula:



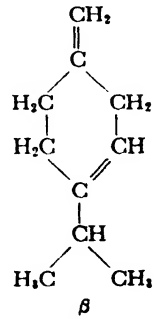
Camphene

d-Camphene is found in the oils of orange (petitgrain and sweet-orange blossoms). Both *d*-camphene and *l*-camphene occur in lemon oil.

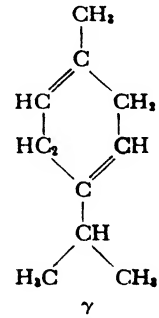
Terpinene is another hydrocarbon, $C_{10}H_{16}$, which occurs in three isomeric forms differing only in the position of its two double bonds:



α



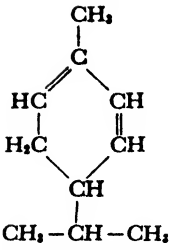
β



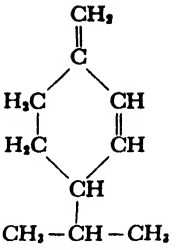
γ

Only γ-terpinene is found in lemon oil. Terpinene greatly resembles dipentene.

Phellandrene is a widely distributed hydrocarbon although it is one of the least stable terpenes. It also contains two double bonds and occurs as two isomers:

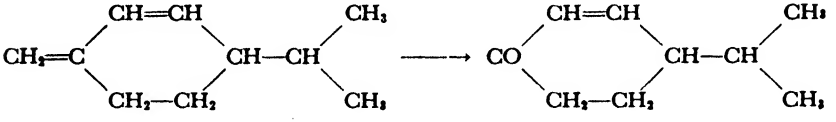


α-Phellandrene



β-Phellandrene

α-Phellandrene is optically active. Only β-phellandrene is found in lemon oil. By the action of atmospheric oxygen, β-phellandrene is converted into a ketone:



(2) SESQUITERPENES

The higher-boiling fractions of many essential oils, those boiling between 250° and 280° C, contain hydrocarbons whose general formula is $C_{15}H_{24}$, the so-called sesquiterpenes. They are very widely distributed but have been investigated only to a limited extent. The sesquiterpenes have a density of approximately 0.90, they are usually slightly colored, their viscosity is higher than that of terpenes, and, like the terpenes, they have but a faint odor. Because it is rather difficult to obtain sesquiterpenes in a very pure state, little is definitely known of their constitution and the conditions of their isomerism.

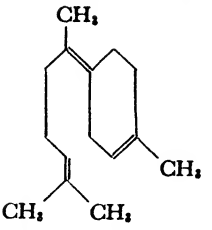
Among the cyclic sesquiterpenes found in citrus oils, mention should be made of bisabolene and cadinene. Their constitution has been elucidated by Ruzicka.[15]

Bisabolene ($C_{15}H_{24}$), identical with limene, is found in lemon oil. It is the most important member of the subgroup of monocyclic sesquiterpenes.

PHYSICAL CONSTANTS. B.p. = 110–112° C (at 4 mm); d_{15}° = 0.8813; $[\alpha]_D$ = − 41°31′; $n_D^{20°}$ = 1.49015.

According to Ruzicka, the constitution of bisabolene is:

[15] Ruzicka, L., "Ueber Konstitution und Zusammenhänge in der Sesquiterpenreihe," Borntraeger, Berlin (1928).



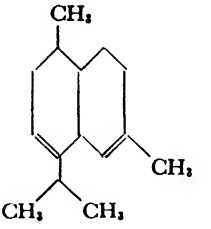
Bisabolene

This sesquiterpene has been synthetically obtained by moderate action of concentrated acids on nerolidol, an aliphatic sesquiterpene alcohol and an important constituent of neroli oil.

Cadinene ($C_{15}H_{24}$), the most important representative of the second subgroup—namely, the dicyclic sesquiterpenes—is widely distributed and occurs in two optically active modifications. Cadinene is found also in lemon oil.

PHYSICAL CONSTANTS. B.p. = 271–273° C; d_{15}° = 0.9215; n_D = 1.50647; $[\alpha]_D$ = − 105°30′.

The structural formula of cadinene has been found to be:



Cadinene

Note that cadinene is the cyclic isomer of bisabolene.

(b) Oxygenated Constituents

The principal bearers of the specific odor of the essential oils are the oxygenated compounds, namely, alcohols, aldehydes, ketones. and acids and their esters.

(1) ALCOHOLS

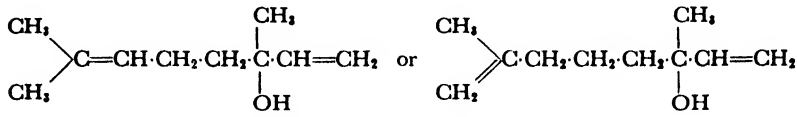
Alcohols in their free state supposedly seldom occur in citrus oils; usually, the alcohols are combined with acids in the form of esters. The fact that, on analysis, free alcohols and free acids are found may be attributed largely to the saponification of the esters during the process of distillation.

***n*-Nonyl Alcohol.** $[CH_3(CH_2)_7 \cdot CH_2OH]$. Of all the saturated aliphatic alcohols, only *n*-nonyl alcohol has been found in the oil of unripe sweet oranges as an ester of caprylic acid.

PHYSICAL CONSTANTS. B.p. = 213° C (at atmospheric pressure), 98–101° C (in vacuo at 12 mm pressure); $d_{15}^{\circ} = 0.842$; $[\alpha]_D = \pm 0°$; $n_D^{15°} = 1.43582$.

Of much greater interest, due to their fragrance, are the unsaturated aliphatic alcohols of the terpene type $C_{10}H_{18}O$, namely, linalool, geraniol, and nerol, as well as citronellol, $C_{10}H_{20}O$.

Linalool ($C_{10}H_{18}O$) has the following structural formula:



3,7-Dimethylocta-1,7-diene-3-ol

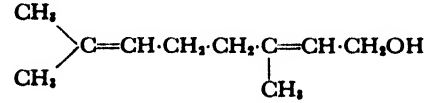
The position of the double bond at carbon atom 7 in linalool and in other alicyclic terpene derivatives is a matter of much controversy that is still undecided. One school of thought is of the opinion that both forms are present in a mixture. Others think that certain terpene derivatives are homogeneous and consist only of one or the other of the above forms. Recently reported results[16] collected over a period of years and with various alicyclic terpene derivatives strengthen the case against the existence of mixtures and support the contention of many workers, mainly those engaged in the industry, that the compounds of this type are essentially homogeneous. It is further believed that most, if not all, of these compounds occur in nature in the first form shown above.

Having one asymmetric carbon atom linalool appears in two optically active modifications. Its dextrorotatory modification, *d*-linalool, is found in the oil of sweet oranges. As an ester of acetic acid, linalool acetate occurs in the oil of bergamot, petitgrain, lemons, neroli, and Italian limette.

PHYSICAL CONSTANTS (of *d*-linalool). B.p. = 197–199° C or 69–71° (at 4 mm); $d_{15}^{\circ} = 0.869$–0.873; $n_D^{20°} = 1.462$–1.464; $[\alpha]_D = +19°18'$.

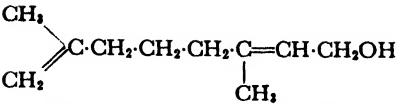
Geraniol ($C_{10}H_{18}O$) is a primary alcohol isomeric with linalool but optically inactive, as shown by its structural formula:

3,7-Dimethylocta-2,6-diene-1-ol



[16] Carroll, M. F., "Structure of Certain Alicyclic Isolates," *Perfumery Essent. Oil Record*, **38**, 226 (1947).

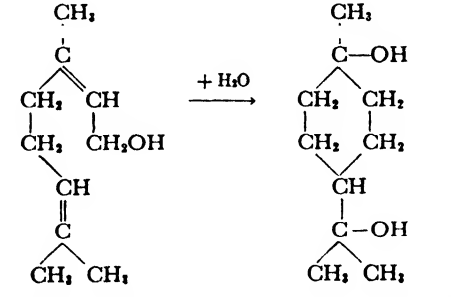
or



3,7-Dimethylocta-2,7-diene-1-ol

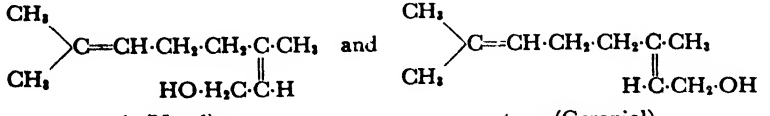
It is a widely distributed component of essential oils. As an alcohol, geraniol occurs in neroli and petitgrain. As an acetate, it is found in lemon and petitgrain oils. As a primary alcohol, geraniol yields upon oxidation the aldehyde citral (see page 54) and can be obtained from citral by reduction. The action of acids upon geraniol causes ring formation (see page 41); thus, formic acid produces α-terpineol, dipentene, and terpinene.

Nerol ($C_{10}H_{18}O$) is a geometrical stereoisomer of geraniol and is found in neroli oil and the oils of petitgrain and bergamot. It possesses an agreeable, roselike odor, and its chemical behavior closely resembles geraniol. Both geraniol and nerol undergo a ring closure on treatment with H_2SO_4 and are converted into 1,8 terpine hydrate. In nerol this ring formation proceeds more rapidly than in its stereoisomer geraniol, which indicates that nerol has the *cis*-configuration:



Geraniol or nerol 1,8 Terpine hydrate

Thus, the following formulas should be assigned to nerol and geraniol:

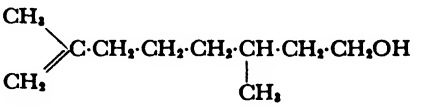


cis-(Nerol) *trans*-(Geraniol)

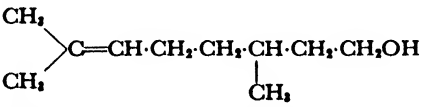
Citronellol ($C_{10}H_{20}O$), a very widely distributed alcohol, has the odor of roses, and is almost always accompanied by geraniol. It

exists in two optically active modifications. While *d*-citronellol occurs in lemon oil, its levorotatory form is found in rose oil and both enantiomorphic forms in geranium oil.

Harries and Himmelmann[17] are of the opinion that citronellol is a mixture of two isomers (cf. page 50 under Linalool) whose structural formulas are:



3,7-Dimethyloct-7-ene-1-ol for citronellol

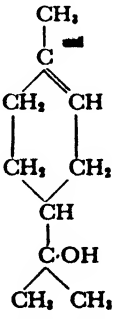


3,7-Dimethyloct-6-ene-1-ol for rhodinol

Citronellol, being a primary alcohol, yields upon oxidation the aldehyde citronellal ($C_{10}H_{18}O$). Upon reduction with sodium amalgam, citronellal can be reconverted into citronellol.

PHYSICAL CONSTANTS (of *d* - citronellol). B.p. = 117–118° C (at 17 mm pressure); $d = 0.8565$; $[\alpha]_D^{17^\circ} = +4^\circ$; $n_D = 1.45659$.

Terpineol. An alicyclic alcohol of special interest to citrus chemistry is α-terpineol ($C_{10}H_{18}O$), of the same empirical formula as the alcohols mentioned above except that it has a terpene ring and is a tertiary unsaturated alcohol, as shown by its structural formula:

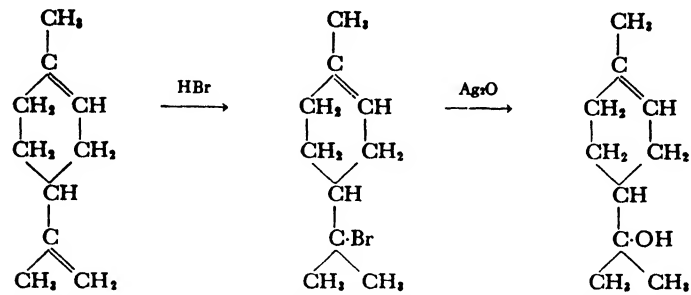


α-Terpineol

[17] Harries and Himmelman, *Ber.*, 41, 2187 (1908).

α-Terpineol is a solid substance and is optically active. Solid *d*-α-terpineol occurs in the oils of sweet oranges, petitgrain, and neroli. Solid *l*-α-terpineol is found in limette oil. β- and γ-terpineols, which differ from the foregoing by the position of the double bond, have not been found in nature.

α-Terpineol can be prepared from limonene by adding a molecule of hydrogen bromide and then replacing Br by hydroxyl:

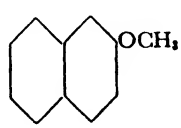


Limonene — Limonene hydrobromide — α-Terpineol

Nerolidol ($C_{15}H_{26}O$), an aliphatic sesquiterpene alcohol, is found in the high-boiling fraction of orange-blossom oil. It possesses only a faint odor but is capable of fixing other perfumes.

PHYSICAL CONSTANTS. B.p. = 276–277° C and 128–129° C (at 6 mm pressure); $d_{15}° = 0.880$; $[\alpha]_D = +13°32'$.

In this connection it is perhaps of interest that an odor similar to orange blossom is now prepared artificially from β-naphthol. The odor of β-naphthol methyl ether, Yara-Yara (m.p. = 72°, b.p. = 274° C), is similar to orange blossoms. It is prepared by methylation of β-naphthol with dimethyl sulfate. Nerolin, the ethyl modification of the same compound, has a similar odor.



Yara-Yara

(2) ALDEHYDES

As components of essential oils, most aldehydes occur only in very small quantities. They are, nevertheless, of great importance because they possess a characteristic odor and flavor. Of the aliphatic aldehydes found in citrus oils, the following may be mentioned:

***n*-Octyl Aldehyde** ($C_7H_{15} \cdot CHO$) occurs in lemon oil.

PHYSICAL CONSTANTS. B.p. = 60–61° C (9 mm); $d_{15}° = 0.8211$; $n_D = 1.41955$.

***n*-Nonyl Aldehyde** ($C_8H_{17} \cdot CHO$) is also found in lemon oil.

PHYSICAL CONSTANTS. B.p. = 80–82° C (at 13 mm); $d_{15}° = 0.8277$; $n_D^{15°} = 1.42452$.

***n*-Decyl Aldehyde** ($C_9H_{19} \cdot CHO$) is the chief aldehyde found in the oil of orange, in which it may comprise 1.3–2.7% of the oil. It is the principal oxygenated constitutent of orange oil, and it occurs also in mandarin and neroli oils.

PHYSICAL CONSTANTS. B.p. = 207–209° C (at 755 mm), 93–94° C (at 12 mm); $d_{15}° = 0.828$; $n_D^{15°} = 1.42977$.

This aldehyde has recently been prepared synthetically in the U.S.S.R.[18] by passing a mixture of capric and formic acids through manganese dioxide at 350–375° C. The reaction takes place as follows:

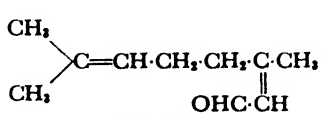
$$CH_3 \cdot (CH_2)_8 \cdot COOH + HCOOH \rightarrow CH_3 \cdot (CH_2)_8 \cdot CHO + H_2O + CO_2$$

The necessary capric acid is obtained from the residues (about 3%) of fusel oil. These residues contain 13% of fatty acids, two-thirds of which is capric. The yield of *n*-decyl aldehyde is 70–75%.

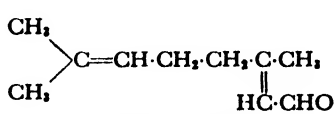
More recently the aldehydes have been prepared by dehydrogenation of the corresponding alcohols with a copper catalyst.

Citral ($C_{10}H_{16}O$) is commonly distributed in nature; it is an important constituent of many essential oils, particularly lemon, petitgrain, West Indian limette, and mandarin oils, and probably also sweet-orange oil. It was first identified by J. Bertram in 1888.

In all these oils citral appears as a mixture of two stereoisomers, β-citral and α-citral (*cis*- and *trans*-configurations), as follows:



β-Citral (*cis*)



α-Citral (*trans*)

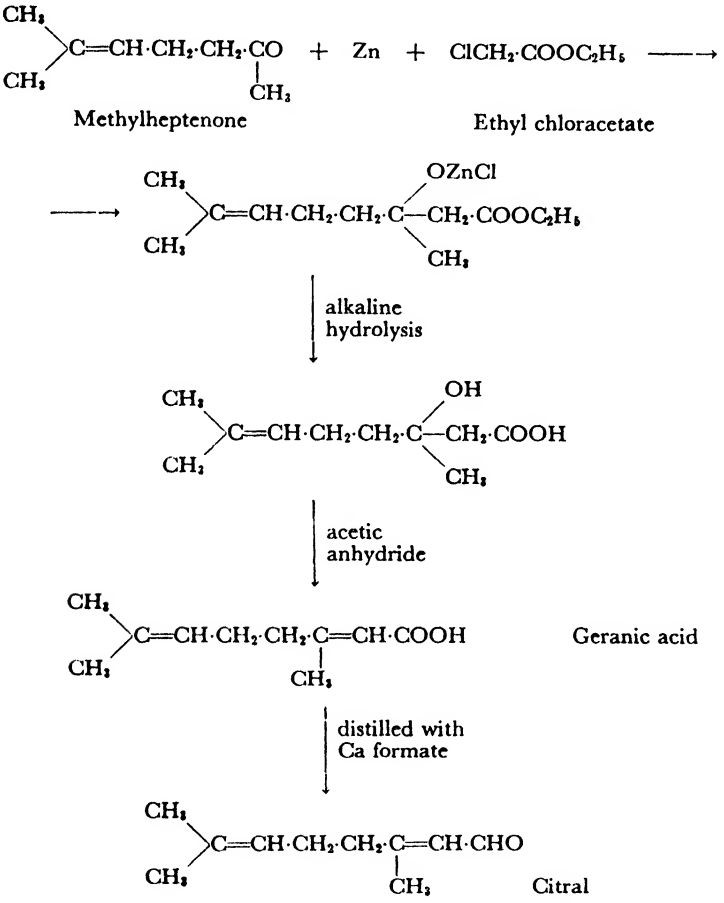
The two stereoisomeric alcohols, geraniol and nerol, on careful oxidation give mixtures of the two citrals. α-Citral predominates in the mixture if the starting material is geraniol; with nerol, β-citral is chiefly obtained.

[18] Ossipowa, W., *Masloboino-Zhirovoe Delo* (Oil and Fat Industry, **11**, 378 (1935).

Citral is a thin yellow oil which smells very strongly of lemons.

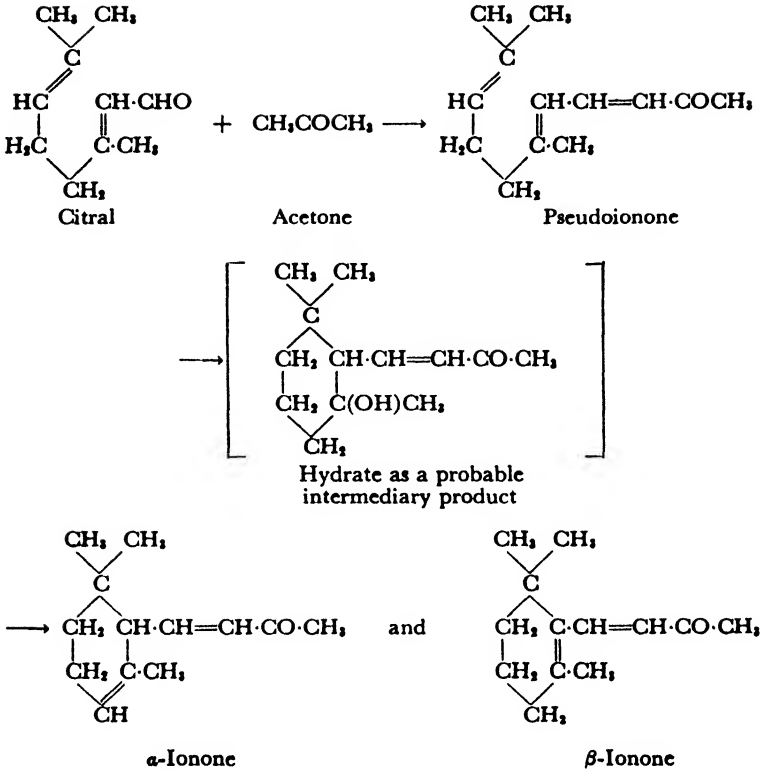
PHYSICAL CONSTANTS. B.p. = 228–229° C (at atm. pressure) and 110–112° C (in vacuo at 12 mm); $d_{15}^{\circ} = 0.8972$; $n_D = 1.4931$.

Citral can be prepared synthetically from methylheptenone:

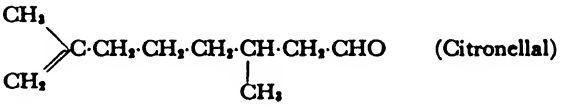


Citral is an aldehyde with two double bonds; it reacts with the usual reagents for aldehydes and, as already mentioned, can be reduced with sodium amalgam (in acetic acid solution) to geraniol.

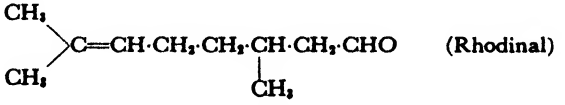
Citral is also a starting point for the very important compound ionone, the synthetic perfume with the odor of violets. If citral is condensed with acetone in the presence of weak alkalis, pseudoionone is formed, which, when heated with dilute H_2SO_4, gives a mixture of α- and β-ionone:



Citronellal ($C_{10}H_{18}O$), another aliphatic unsaturated aldehyde, has one double bond and occurs occasionally with citral. Unlike citral, citronellal is optically active, having one asymmetric carbon atom in both its stereoisomeric forms:



or

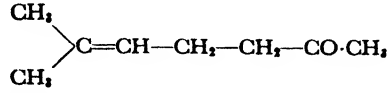


With one exception, only the dextrorotatory modification (*d*-citronellal) is found in nature. *l*-Citronellal is found in "Java lemon olie."

PHYSICAL CONSTANTS (of *l*-citronellal). B.p. = 205–208° C; $d_{18°} = 0.8567$; $[\alpha]_D = -3°$; $n_D^{20°} = 1.44791$.

(3) KETONES

Methylheptenone ($C_8H_{14}O$), the only ketone of interest in citrus chemistry, has been found in lemon oil. It is a colorless, mobile liquid with a penetrating odor reminiscent of amyl acetate. Methylheptenone is not optically active, its structural formula being:



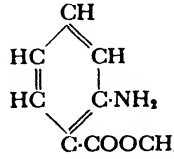
As mentioned above, methylheptenone is the starting material in the synthesis of citral.

(4) ORGANIC ACIDS AND ESTERS

Organic acids are probably present in the essential oils only in the form of esters. However, when an oil is distilled during isolation of its components, the esters probably decompose into their respective acids and alcohols. Thus, for instance, orange oil contains either *n*-caprylic acid ($C_7H_{15} \cdot COOH$) or its esters. Esters are, in fact, very important constituents of the essential oils, for, although they occur in very minute quantities, their odor is distinctive, giving a specific fragrance to the oils concerned.

Citrus oils particularly rich in esters are bergamot oil and, to some extent, lemon and orange oils. In addition to the esters mentioned above, such as linalyl acetate and geranyl acetate ($CH_3COOC_{10}H_{17}$), of much importance is the methyl ester of anthranilic acid, discovered by Walbaum (1895)[19] in neroli oil and found since then in practically all citrus oils.

The Methyl Ester of Anthranilic Acid is of extreme importance; it is an orthoamido-derivative of benzoic acid with the following structure:

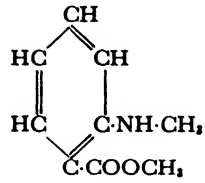


It forms large crystals with numerous faces and reveals a beautiful blue-violet fluorescence. It can be prepared synthetically. Anthranilic acid itself can be prepared by reduction of orthonitrobenzoic acid with Sn and HCl; it is in practice prepared from phthalimide by treatment with Cl or Br and caustic soda.

PHYSICAL CONSTANTS. M.p. = 24° C; b.p. = 132° C (at 14 mm); $d_{15}^{\circ} = 1.168$.

[19] Walbaum, *J. prakt. Chem.*, 59, 352 (1899).

Methyl Anthranilic Acid Methyl Ester occurs in mandarin oil, its structural formula being:



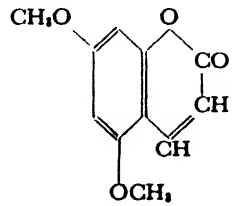
It is noteworthy that when oranges packed at the end of the season are kept for a long time, they spread a pronounced scent of methyl anthranilate, possibly the result of a considerable increase of this ester in the orange peel. It would be of interest to pursue this matter by further research.

PHYSICAL CONSTANTS. M.p. = 18.5–19.5° C; b.p. = 130–131° C (at 13 mm); $d_{18}° = 1.120$.

(5) STEAROPTENES

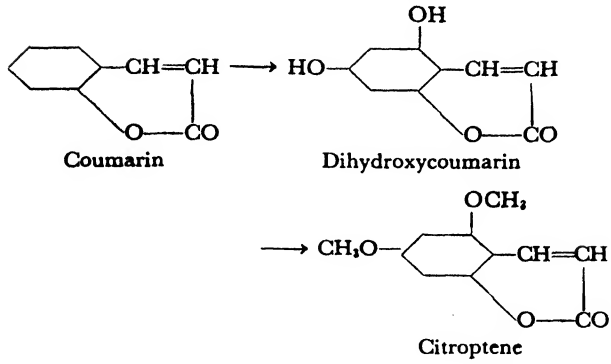
All the essential oils of the genus *Citrus* leave, after distillation, a considerable quantity of a nonvolatile residue. Moreover, similar waxlike substances separate from each oil on prolonged standing. These residues are called stearoptenes. They are soft, waxlike, and more or less slimy masses. Much work has been done on their composition, and in some instances pure substances have been isolated.

Citroptene ($C_{11}H_{10}O_4$), identical with limettin, has been isolated from lemon oil. Citroptene is obtained as a granular, crystalline mass when the residue, left after distilling lemon oil, is treated with ether. Recrystallized from acetone and methyl alcohol, it gives colorless needles melting at 146° to 147° C. Its constitution has been shown by Schmidt[20] to be:

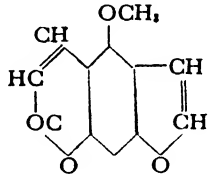


It is a derivative of coumarin (benzo-α-pyrone); methylated dihydroxycoumarin and its solutions possess a handsome violet-blue fluorescence.

[20] Schmidt, E., "Ueber das Citropten," *Apoth. Ztg.*, 16, 619 (1901).



Bergaptene ($C_{12}H_8O_4$) is a stearoptene found in bergamot oil to the extent of 5%. It crystallizes in fine white needles, m.p. = 188° C. Thoms and Baetcke[21] assign the following structural formula to bergaptene:



Thus, it is the monomethyl ether of dihydroxycoumarin and, like citroptene, can be derived from phloroglucinol.

Aurade or neroli-camphor is another stearoptene found in orange blossom oil or neroli oil. A paraffin, it occurs in almost all flower oils. It melts at 55° C.

Other Stearoptenes. The composition of other stearoptenes has not been sufficiently studied. From orange oil, for instance, a compound can be separated after saponification which has an empirical formula approximately corresponding to cerotic acid ($C_{26}H_{52}O_2$). From the same saponification liquid a substance (m.p. = 138° C) has been extracted with ether, showing, on elementary analysis, the constitution $C_{28}H_{48}O_2$, and in chloroform the Liebermann cholesterol reaction.

(c) Recapitulation

The most odoriferous substances are the oxygenated, usually open-chain compounds, as contrasted with the terpene hydrocarbons whose

[21] Thoms, H., and E. Baetcke, *Ber.*, 45, 3705 (1912).

TABLE III. Table of Known Components

Chemical group	Components	Chemical formula	Physical		
			M.p. °C	B.p. °C	B.p. in vacuo °C
Terpenes	(1) *d*-Limonene	$C_{10}H_{16}$		175°	
	(2) Dipentene	$C_{10}H_{16}$		175°	
	(3) α-Pinene	$C_{10}H_{16}$		155°	
	(4) β-Pinene	$C_{10}H_{16}$		164°	
	(5) Camphene	$C_{10}H_{16}$	48°	160°	56° at 15 mm
	(6) β-Phellandrene	$C_{10}H_{16}$			57° at 15 mm
	(7) γ-Terpinene	$C_{10}H_{16}$		177°	
	(8) Octylene	C_8H_{16}	123°		
	(9) Paraffin	C_{27}—			
Sesquiterpenes	(10) Bisabolene	$C_{15}H_{24}$			110° at 4 mm
	(11) Limene	Identical with bisabolene			
	(12) Cadinene	$C_{15}H_{24}$		271°	
Alcohols	(13) Geraniol	$C_{10}H_{18}O$		230°	110° at 10 mm
	(14) Nerol	$C_{10}H_{18}O$		226°	125° at 25 mm
	(15) *d*-Linalool	$C_{10}H_{18}O$		197°	69° at 4 mm
	(16) *l*-Linalool	$C_{10}H_{18}O$			
	(17) Nerolidol	$C_{15}H_{26}O$		276°	128° at 6 mm
	(18) Phenylethanol	$C_6H_5 \cdot C_2H_4 \cdot OH$		220°	109° at 12 mm
	(19) *d*-Terpineol	$C_{10}H_{18}O$		218°	104° at 10 mm
	(20) Dihydrocuminic	$C_{10}H_{16}O$		226°	92° at 5 mm
	(21) *n*-Nonyl	$CH_3(CH_2)_7CH_2OH$		213°	98° at 12 mm
	(22) *d*-Citronellol	$C_{10}H_{20}O$			117° at 17 mm
	(23) Octyl	$CH_3(CH_2)_6 \cdot CH_2OH$		196°	
	(24) Phenols	$C_6H_5 \cdot OH$			
Aldehydes	(25) Citral	$C_{10}H_{16}O$		228°	110° at 12 mm
	(26) *l*-Citronellal	$C_{10}H_{18}O$		205°	
	(27) *n*-Octyl ald.	$C_7H_{15} \cdot CHO$			60° at 9 mm
	(28) *n*-Nonyl ald.	$C_8H_{17} \cdot CHO$			80° at 13 mm
	(29) *n*-Decyl ald.	$CH_3(CH_2)_8 \cdot CHO$		207°	93° at 12 mm
Ketones	(30) Methylheptenone	$C_8H_{14}O$		173°	
Acids and Esters	(31) Linalyl acetate	$CH_3 \cdot COO \cdot C_{10}H_{17}$		220°	96.5° at 10 mm
	(32) Geranyl acetate	$CH_3 \cdot COO \cdot C_{10}H_{17}$		242°	127° at 16 mm
	(33) Ester of *n*-caprylic acid				
	(34) Methyl anthranilate	$C_6H_4(NH_2) \cdot COO \cdot CH_3$	24°		132° at 14 mm
	(35) Methyl anthranilic methyl ester	$C_6H_4(NH \cdot CH_3) \cdot COO \cdot CH_3$	19°		130° at 13 mm
	(36) Octyl acetate	$CH_3(CH_2)_6 \cdot COOCH_3$			
	(37) Neryl acetate				
Stearoptenes	(38) Citroptene	$C_{11}H_{16}O_4$	146°		
	(39) Limettin	Identical with citroptene			
	(40) Bergaptene	$C_{12}H_8O_4$	188°		
	(41) Aurade		55°		
	(42) ?	$C_{26}H_{46}O_2$ (?)			
	(43) ?	$C_{22}H_{42}O_2$ (?)			
	(44) Pyrrole derivatives				
	(45) Furfurol	$C_4H_3O \cdot CHO$		160.5°	

main importance lies in their capacity to fix the odor. The oxygenated compounds occur in very small quantities compared with the hydrocarbons. Furthermore, while it is relatively easy to convert the oxygenated compounds into terpenes by closing the ring, the reverse is practically impossible. On this practical question of converting the

Occurring in Various Citrus Oils

constants			Individual citrus oils											
Index of refraction n_D	Specific optical rotation $[\alpha]_D$	Sp. gr. $\frac{}{d}$	Lemon oil	Orange oil (sweet and bitter)	Bergamot oil	Mandarin oil	Grapefruit oil	Citron oil	Lime oil	Neroli Oil	Petitgrain oil	Petitgrain Portugal	Lemon petitgrain
(1) 1.475	+123°40′	0.850	×	×	××	×	×	××	×		××	×	×
(2) 1.47194		0.844						×		××	××		
(3) 1.46553	±0°	0.858	××		×××					××			
(4) 1.47548	−19°29′	0.865	××								××		
(5) 1.4555		0.850	×××		×					×	××	×	?
(6) 1.4788	+14°45′	0.8520	×××										
(7) 1.47650	+0°32′	0.8485	×××										
(8) 1.4066		0.7275	~										
(9)										×			
(10) 1.49015	−41°31′	0.8815	×		×								
(11)													
(12) 1.50647	−105°30′	0.9215	×				×		×				
(13) 1.4766		0.8894					×			××	××	×	×
(14)	+0°	0.8813			×					××	××		
(15) 1.462	+19°18′	0.869	?	×								?	?
(16)	−20°7′				×					××	×		
(17)	+13°32′	0.880								××			
(18) 1.53212		1.0242											
(19) 1.48084	+95°9′	0.935	×		××					×	×		
(20) 1.49629	−13°18′	0.9510			××								
(21) 1.43582	±0°	0.842		×			?						
(22) 1.45659	+4°	0.8565	×										
(23)		0.8278		×			×						
(24)										?			
(25) 1.4931		0.8972	×				×	×	×			×	×
(26) 1.44791	−3°	0.8567	××										
(27) 1.41955		0.8211	××				×						
(28) 1.42452		0.8277	×										
(29) 1.42977		0.828		×			×			×			
(30) 1.44003		0.8530	×										
(31)	−6°35′	0.913	××	×	×					××	××		
(32) 1.4628	±0°	0.9174	××				×			××	××		
(33)				×									
(34)		1.168					×		×	×	×		
(35) 1.57963	±0°	1.120	×			×							
		(36)		×			×						
		(37)								×			
		(38)	×					?					
		(39)							×				
		(40)			××								
		(41)											
		(42)											
		(43)											
		(44)									×		
		1.1594 (45)									×		

less important terpenes into more essential odoriferous substances, much work has been done although with rather limited success.

To some extent it is possible to convert terpenes into alcohols or esters. Limonene, for instance, can be converted into terpineol, as shown on page 53. Terpinyl acetate ($CH_3COOC_{10}H_{17}$) can be

obtained artificially by heating a mixture of pinene and acetic acid for 64 hours. Bertram in a series of patents (German Patent Nos. 67,255 and 80,711) suggested a procedure for converting useless terpenes into esters by hydration and subsequent esterification.

5. Tabulation of Constituents

Citrus plants, like practically all other plants, contain their essential oils not only in the peels of the fruit, but also in the blossoms, leaves, and young twigs and even in the juice of the fruit. The accessory organs of most other plants usually do not contain materials of any commercial value, but with citrus plants the practice is to extract, besides peel oils, the flower oil (such as *neroli,* the oil of the blossoms of bigaradier, the sour orange) and *petitgrain,* the oil distilled from young leaves and twigs.

Each of these oils has a different composition and thus has a specific fragrance. Table III enumerates all the known components of the various citrus oils. The components are classified according to their chemical group. An $\times$ denotes the presence of the component in the particular oil.

The aroma of citrus juices, which is quite different from that of the oils, will be discussed later.

6. Historical Note

Although the essential oils must have been known since the introduction of citrus culture, they apparently found no early application. The first statements concerning lemon and orange oils were made by Conrad Gesner[22] as early as 1555. Later, Portae[23] described the preparation of citrus oils by distillation of the freshly grated rinds. In 1721 C. J. Geoffrey[24] described a mechanical method of preparation by rupturing the oil cells of the fruit peel by means of a grate—a method which in all probability had been practiced even before the publication of Geoffrey's memoir.

In the municipal price ordinances concerning apothecary wares and spices, oils of lemon and orange were enumerated for the first time among the distilled oils in the Frankfurt am Main statutes of 1582. Both oils were listed in the *Dispensatorium Noricum* of 1589 and the *Pharmacopoeia Augustana* of 1613.

[22] Gesner, C., *Enonymi Philiatri,* "Ein Köstlicher, Teurer Schatz," Zurich (1555).
[23] Portae, J. B., "Magiae Naturalis Libri Viginti," Romae (1563), edit. Napoli (1589).
[24] Geoffrey, C. J., *Mém. Acad. Sci. Paris,* p. 95 (1726).

7. Description of Individual Oils

(a) Oils Derived from Peel of Fruits

(1) OIL OF LEMON

Lemon oil is probably the most important and most extensively consumed of all citrus oils. It is extracted from the peel of the lemon, *Citrus medica* Linnaeus, subspecies *limonum* (Risso), Hook f. It is produced mainly in Italy and other Mediterranean countries (Palestine, Spain, Portugal, and southern France), also in the United States (California, Florida, and Texas).

Lemon oil is still produced in Italy by the primitive sponge method; however, most of the producers are converting to the less expensive cold-machine process. Only in the United States is some of the oil produced distilled.

The yield varies enormously according to the variety, to seasonal changes and geographical conditions, and to the methods of extraction. The hand-pressing method ("processo alla spugna") in Sicily yields some 320 to 640 g per 1000 lemons—in other words, 0.15 to 0.3% of the weight of the fruit. Machine pressing yields, on the average, 0.2%. Distillation in vacuo gives higher yields.

Similarly, the physical and chemical properties of lemon oils vary considerably according to the variety, the season, and the method of extraction. Table IV gives minimum and maximum figures for lemon oils produced in various countries, the method of extraction being indicated in each case.

Composition. Quantitatively the principal constituent of lemon oil, as of most citrus oils, is *d*-limonene, which comprises about 90% or more of the oil. However, unlike many other citrus oils, lemon oil contains several other hydrocarbons, among them β-phellandrene, γ-terpinene, camphene, and probably also octylene.

Although minimum amounts of α- and β-pinene also are frequently found in natural unadulterated lemon oil, occasionally they are entirely absent. The conditions under which the lemon tree does or does not produce pinene in the oil have yet to be investigated.

Of the sesquiterpenes, both bisabolene and cadinene are present.

The principal oxygenated constituent is the aldehyde *citral,* which is the most important from the standpoint of aroma. Citral amounts to 3.5 to 5% of the oil. Lemon oil also contains *n*-octylic and *n*-nonylic aldehydes, *l*-citronellal, and the ketone methylheptenone.

Further, linalyl and geranyl acetates and methylanthranilate have

TABLE IV. Analyses of Lemon Oils from Various Sources

Source	Process of manufacture	Sp. grav.	Non-volatile residue %	Refractive index		Optical rotation (plus values)	Citral		Esters %	Author and year
				Original	10% distillate		%	Method		
Italy	hand pressed	0.856 –0.861	2.1 –4	1.474 –1.476		54 to 67°	3.5 –4	Kleber		Gildemeister, 1909
"	" "	0.8578–0.8589	2.23–3.15			58.87 to 60.60°	4.51–5.78		3.09–3.49	LaFace, 1930
"	machine pressed	0.8590–0.8600	3.05–4.96			57.80 to 59.61°	4.07–4.51		2.76–3.39	
"	" "	0.8576–0.8604	3.20–7.1			55 to 62.8°	1.9 –3.2			Donovan, 1931
"	hand pressed	0.8508–0.8539		1.474 –1.4753	1.472 –1.4732	54 to 63.6°	3.4–5.2	Kleber		Chace, 1910
California	machine pressed	0.8475–0.8525	2.01–4.52	1.4738–1.4749	1.4726–1.4737	52.7 to 70.38°	2.0 –3.7		1.76–3.12	Poore, 1932
Palestine	" "	0.8500–0.8596	3.09–3.68	1.4730–1.4744		65.3 to 66.7°	3.03–3.54	Walter-Bennett		Stern, 1944

TABLE V. Analyses of Orange Oils from Various Sources

Source	Process of manufacture	Sp. grav.	Non-volatile residue %	Refractive index	Optical rotation	Aldehydes as *n*-decylic %	Esters %	Author and year
Italy	hand pressed	0.848 –0.853	1.5 –4.2	1.473 –1.475	+95°31′ to 98°	1.3 –2.7		Gildemeister, 1909
Jamaica	" "	0.8481–0.8491	1.4 –2.0	1.46984	+97°43′ to 98°2′	2.3 –3.8		" "
Dominica	" "	0.8486	1.6		+98°21′			" "
California	machine pressed	0.8413–0.8482	2.07–5.65	1.4730–1.4742	+96° to 99°	1.0 –1.8		Poore, 1932
Italy	hand pressed	0.8435–0.8476		1.4723–1.4737	+92° to 98°	1.47–2.89		Chace, 1910
Palestine	machine pressed	0.840 –0.850	2.5 –3.6	1.4737–1.4747	+94°50′ to 96°50′	1.10–1.52		Stern, 1944
Florida	" "	0.8425	2.8	1.4734	+95°50′	6.28 as capraldehyde	0.65–0.74	Foote & Gelpi, 1943
Terpenes from orange oil	after distillation	0.847 –0.854		1.473	+95°50′ to 100°18′	—	—	Gildemeister, 1909

been identified. Poore (1932) found in California lemon oil also acetic, capric, and caprylic acids. The nonvolatile residue of lemon oil consists mainly of the stearoptene *citroptene*, or lemon camphor, which has previously been described.

Adulteration. Some fifty years ago, when the components of pure lemon oil were not yet known, the oil of lemon was freely adulterated by turpentine oils. With the introduction of physical and optical methods of examination—such as the use of the refractometer and polarimeter and the examination of the first 10% of the distillate—turpentine oil was replaced as an adulterant by an admixture of terpenes (*d*-limonene), left after distillation when preparing terpeneless oils, and by cheap citral, made of lemon-grass oil. Such adulterants are most dangerous, for their detection is exceedingly difficult.

(2) OIL OF ORANGE

Orange oil is derived from the peel of *Citrus aurantium* Linnaeus (*C. vulgaris* Risso), subspecies *sinensis* (Gallesio). The oil is produced by both cold expression (hand or machine) and distillation in vacuo.

For a long time the principal source of production was Italy (Sicily and Calabria); later, production started also in the West Indies and the United States (California). Several years before World War II new sources of supply were opened, namely, Palestine, Spain, Brazil, Rhodesia, South Africa, and French Guinea (French West Africa). The production in the last-named country has increased to enormous proportions never before attained even by Italian producers; the oranges in French Guinea grow wild and the oil is hand pressed by convicts. The orange oil from this source, however, is of inferior quality.

Sweet orange oil is a yellow to red-brown liquid with a distinct aroma of oranges. Its physical constants differ according to origin, season, and method of extraction, as shown in Table V.

The main constituent of orange oil is *d*-limonene (present to the extent of at least 90%), which is the only hydrocarbon present. Orange oil is, therefore, the best source for preparing pure *d*-limonene. The principal odoriferous constituent is *n*-decyclic aldehyde, which formerly was believed to be the only aldehyde present. Recently, however, capraldehyde and citral are claimed to have been identified.[21] The alcohols *d*-linalool and *n*-nonylic are found in

[21] Foote, P. A., and R. Z. Gelpi, "Florida Volatile Oils; IV, Sweet Orange," *J. Am. Pharm. Assoc.*, 32, 145 (1943).

orange oil in the free state and most probably also in the form of esters of formic, acetic, capric, and caprylic acids. Poore found in California orange oil also octyl alcohol and an unidentified white amorphous compound melting at 62–63° C. Parry established the presence of anthranilic acid methyl ester.

The residue left after evaporation has not been sufficiently investigated. It is only very slightly soluble in alcohol, the insoluble part having a melting point of 67–68° C and a saponification value of 65.

The adulteration of orange oil is rather difficult and is easily detected because the physical constants of this oil lie within narrow limits. With its low specific gravity and its exceedingly high angle of rotation, any admixture of foreign substances may easily be exposed. Also, the absence of pinene is an important factor in determining adulteration with turpentine oil.

The bitter variety of orange oil is made from *Citrus bigaradia* Risso variety, and differs but slightly from sweet orange oil, mainly in its bitter taste and slightly lower angle of rotation.

PHYSICAL CONSTANTS. $d_{15}° = 0.852$ to 0.857; $[\alpha]_D^{20°} = +89°$ to $+94°$; $n_D^{20°} = 1.473$–1.475. Aldehyde content (as decylic aldehyde) about 1%; the residue after evaporation varies between 3% and 5%.

The composition of bitter orange oil has not been elucidated but is presumed to be similar to that of sweet orange oil. Its bitter taste is assumed to be due to a glucoside contained in the nonvolatile portion of the oil.

Recently, bitter orange-peel oil of French origin has been investigated and the following composition reported:[22]

Phenols (hesperitin?)	0.09%
Free acids (formic, acetic, pelargonic, cinnamic)	0.05
Terpenes (*d*-limonene)	92.00
Sesquiterpenes ($d_{15}° = 0.921$; $n_D^{20°} = 1.4980$)	0.03
Aldehydes (nonanal, decanal, dodecanal)	0.78
Free alcohols (linalool, terpineol)	0.37
Esters (linalyl acetate, decyl pelargonate, and neryl, geranyl, and citronellyl acetate) as lynalyl acetate	2.10

(3) BERGAMOT OIL

This important oil is produced from the bergamot tree, *Citrus aurantium* Linnaeus, subspecies *bergamia* (Risso et Poiteau), which grows almost exclusively in Calabria (Italy), its fruit being used for oil production only. The oil is expressed by a special, primitive machine designed in Calabria (see page 185).

Bergamot oil is a brownish-yellow liquid, bitter in taste, and hav-

[22] Igolen, G., and D. Sontag, "French Bitter Orange-Peel Oil," *Chimie et Industrie*, 45, suppl. to No. 3, 157 (1941).

ing a very pleasant odor. While other peel oils are used both in perfumery and as flavorings in confectionery and the mineral-water industry, bergamot oil is used only in perfumery.

PHYSICAL AND CHEMICAL CONSTANTS. d_{15}° = 0.881–0.886; α_D = +8 to +22°; $n_D^{20^\circ}$ = 1.464–1.468; acid value = 1–3.5. The linalyl acetate content which actually determines the value of bergamot oil, is within the limits of 34 to 40%. Soluble in 1 volume of 90% alcohol. The residue after evaporation is 4.5–6%, having an acid value of 19–30.

Bergamot oil contains, of course, *d*-limonene but it has been suggested that also dipentene, the racemic form of limonene, is one of its constituents. The principal bearer of the odor of bergamot oil is, however, the acetic ester of *l*-linalool. *l*-Linalool is most probably present to some extent also in its free state. Three other alcohols are contained in bergamot oil—namely, dihydrocuminic alcohol, nerol, and terpineol. Besides these odoriferous substances bergamot oil contains some 5% of totally odorless bergaptene, already described. The following have also been identified in bergamot oil: *l*-α-pinene (α_D = — 8°3′), *l*-camphene (α_D = — 22°8′), and the sesquiterpene bisabolene ($C_{15}H_{24}$).

Bergamot oil is often adulterated by turpentine oil mixed with orange and lemon oils, as well as by the addition of various esters, such as terpinyl acetate, glycerin acetate, citric acid esters, and the like.

(4) OIL OF LIMES (LIMETTE)

Oil of limes is produced in the West Indies either by the cold-expressing method ("écuelle à piquer") or by distillation. Accordingly, the two oils differ substantially in composition. Oil of limes is obtained from the rind of the fruit of *Citrus medica* Linnaeus, var. *acida* Brandis.

PHYSICAL CONSTANTS (of the expressed oil). Golden-yellow in color: d_{15}° = 0.878–0.901; $[\alpha]_D$ = +32° to +38°; $n_D^{20^\circ}$ = 1.482–1.486; evaporation residue 10 to 18%; which has a saponification value of 160 to 181; soluble in 4 to 10 volumes of 90% alcohol; citral value 6.5 to 9% if determined by the phenylhydrazine method, 2.2 to 6.6% according to the method of Burgess.

In addition to citral, methylanthranilate and limene (identical with bisabolene) have also been found in the oil of limes. The stearoptenes contain limettin identical with citroptene), $C_{11}H_{10}O_4$, m.p. = 147.5°. The terpenes of lime oil have not yet been sufficiently investigated.

PHYSICAL CONSTANTS (of the distilled oil). Colorless; unpleasant in odor: d_{15}° = 0.860–0.870; α_D = +33° to +47°; ° = 1.4702–1.4707.

According to Burgess and Page,[23] the distilled oil contains *l*-α-terpineol and another unidentified alcohol, also limene (identical with bisabolene).

An oil similar in composition to bergamot oil is also produced in south Italy from the sweet variety of *Citrus limetta;* it contains limonene and linalyl acetate.

PHYSICAL CONSTANTS (of the sweet variety). $d_{15}° = 0.872$; $[\alpha]_D = +58°19'$; saponification value 75.

Mexican lime oil distilled from the juice of the green crushed fruit has recently been studied.[24] The following compounds were identified: α- and β-pinene, furfural, octyl aldehyde, *d*-limonene and dipentene, nonyl aldehyde, borneol, decyl aldehyde, geraniol, citral, linalool, α-terpineol, lauric aldehyde, lauryl alcohol, bisabolene, and an azulenic compound. High-boiling paraffin hydrocarbons could not be detected.

PHYSICAL CONSTANTS (of the Mexican oil). $d_{15}° = 0.866$; $n_D^{20°} = 1.4761$; $[\alpha]_D^{20°} = +41.6°$; citral value 1.15%.

Increasing quantities of lime oil from a variety called the Persian lime *(Citrus aurantifolia)* which is about twice the size of the West Indian variety, are now produced in southern Florida. An analysis of a sample of cold-pressed Persian lime oil showed[25] the following physical constants: $d_{20°} = 0.844$; $a_D^{20°} = +40° 34'$; $n_D^{20°} = 1.4865$. Aldehydes (calculated as citral) = 8%; nonvolatile residue = 11.6%. Solubility—clearly soluble in 95% alcohol but not clearly soluble in 10 volumes of 90% alcohol. The first 10% distillate has: $a_D^{20°} = 50° 54'$; $n_D^{20°} = 1.6834$.

(5) GRAPEFRUIT OIL

Grapefruit oil is expressed or distilled from the peel of grapefruit or shaddock, *Citrus decumana* Linnaeus. It is of only recent development and is produced to a limited extent in the United States, Palestine, Union of South Africa, and Spain.

The machine-pressed oil is amber colored; its odor is not specific to grapefruit but rather is reminiscent of orange and lemon oils. In

[23] Burgess, H. E., and T. H. Page, "Note on the Composition of Distilled Oil of Limes and a New Sesquiterpene," *Proc. Chem. Soc.*, 20, 181 (1904); *J. Chem. Soc.*, 85, 414 (1904).

[24] Guenther, E. S., and E. E. Langenau, "Investigation of the Chemical Constituents of Distilled Lime Oil," *J. Am. Chem. Soc.*, 65, 959 (1943).

[25] Atkins, C. D., E. Wiederhold, and J. L. Heid, "The Recovery of Flavoring Oil from Persian Limes," *Fruit Products J.*, 23, 306 (1944).

comparison with other citrus-peel oils the yield of grapefruit oil is very poor. The cold-pressing method yields only 0.05% of oil per weight of fruit or about one lb. per ton.

PHYSICAL CONSTANTS. $d_{20}° = 0.8563$; $[\alpha]_D^{20}° = +93°28'$; $n_D^{20}° = 1.4758$; aldehyde content 1.67% (calculated as octyl and decyl aldehydes).

According to Nelson and Mottern (1934),[26] grapefruit oil contains about 90% of *d*-limonene, 2–3% of oxygenated compounds and sesquiterpenes, and 7–8% of stearoptenes. Among the oxygenated constituents the following have been identified: octyl and decyl aldehydes, geraniol and octyl alcohol (both free and as acetates), cadinene, citral, methylanthranilate, and probably also nonyl alcohol. The stearoptenes have not been investigated.

Terpeneless oil of grapefruit has been prepared by the same authors (2.7% of the total oil).

PHYSICAL CONSTANTS (of the terpeneless oil). $\alpha_D = +24°$; $n_D^{20}° = 1.4615$; soluble in 6 parts of 80% alcohol.

(6) OIL OF MANDARINS

Oil of mandarins, a most agreeable product, is prepared from the peel of the well-known mandarin or tangerine, *Citrus madurensis* Loureiro [*C. nobilis* Andrews (non Loureiro)], the yield being about 0.2% of the weight of fruit.

Mandarin oil is produced mainly in Italy by hand pressing. To a limited extent it has been prepared by distillation in Brazil.

PHYSICAL CONSTANTS (of hand-pressed Italian mandarin oil). $d_{15}° = 0.854$–0.859; $\alpha_D = +65$ to $+75°$; $n_D^{20}° = 1.475$–1.478; acid value up to 1.7; ester value 5 to 11; ester value after acetylation 12.5; evaporation residue 2.4–3.5%; soluoble in 7–10 parts of 90% alcohol.

The main component of mandarin oil is *d*-limonene, but its principal odoriferous constituent is methyl anthranilic acid methyl ester (1% of the oil).

According to Nelson and Mottern (1934),[26] who investigated the oil of Florida mandarins (Dancy tangerines), no trace of methylanthranilate was found. Nelson and Mottern found some 95.75% of limonene and also octyl and decyl aldehydes, linalool, and a small amount of citral. This tangerine oil probably also contains citronellol and sesquiterpene cadinene. The alcohols are present partly as esters, although the ester content of the oil is low.

[26] Nelson, E. K., and H. H. Mottern, "Florida Grapefruit Oil," *Ind. Eng. Chem.*, **26**, 634 (1934); "Florida Tangerine Oil," *Am. Perfumer Essent. Oil Rev.* (Sept., 1934); "Tangeritine," *J. Am. Chem. Soc.*, **56**, 1932 (1934).

(7) OIL OF CITRONS

Oil of citron is expressed from the rind of the citron fruit, *Citrus medica* var. *vulgaris* Risso (so-called *cedro*) and from var. *gibocarpa* Risso (so-called *cedrino*).

PHYSICAL CONSTANTS (of the first). $d_{15}^{\circ} = 0.8706$; $\alpha_D = +67°$; (of the second) $d_{15}^{\circ} = 0.850\text{–}0.854$; $\alpha_D = +77$ to $81°$; $n_D^{20\circ} = 1.47519$.

This oil seems to consist mainly of *d*-limonene with some dipentene and about 6% of citral. It deposits a waxy constituent, $C_{18}H_{18}O_6$, probably identical with citroptene.

(b) Oils Derived from Flowers, Leaves, and Twigs

So far the discussion of individual oils has been concerned only with those of the peel of the fruits. In the following sections the various oils derived from the flowers, leaves, and twigs will be briefly described.

(1) OIL OF NEROLI

The true oil of neroli is obtained by steam distillation of the blossoms of the bitter or Seville orange, *Citrus bigaradia Risso* (*C. aurantium* Linnaeus subspecies *amara* Linnaeus). However, more or less successful attempts have been made to obtain a similar oil from the flowers of other varieties of *Citrus*.

The production of neroli oil is principally confined to southern France, where the blossoms are carefully picked by hand. Upon distillation some 0.09 to 0.15% can be obtained, about a third of the oil remaining in the water from which the oil is separated in the receiver. This orange-flower water (*Aqua Florum Aurantii, Aqua Naphae*) is sold as such in perfumery.

Distillation of neroli oil has been practiced to some extent also in Spain, Italy, Venezuela, Paraquay, Algeria, and Syria. Besides distillation other methods, such as maceration and extraction with volatile solvents, have been tried. Extraction has yielded some 0.23 to 0.18% of the weight of flowers.

Neroli is a yellowish oil which becomes brownish-red upon exposure to light; it is slightly fluorescent. It has an intense, very pleasant odor of orange blossom and a somewhat bitter, aromatic taste.

Table VI gives the physical properties of neroli oil from various sources and prepared by different methods.

TABLE VI

Physical Indices of Neroli Oil from Different Sources

Source	Method of preparation	Sp. grav.	$[\alpha]_D$	$n_D^{20°}$	Sapon. value	Methyl-anthranilate %
France	Distillation	0.870 –0.881	+1°30′ to +9°8′	1.468 –1.474	19–69	0.45–1.1
"	From orange-flower water	0.945 –0.968	+1°47′ to +2°30′		49–100	11.6–16
"	Extraction with petroleum ether	0.889 –0.929	−0°48′ to −4°6′		55–118	2.7–15
"	Enfleurage	0.909	+8°34′		58.2	5.2
Spain	Distillation	0.870 –0.885	+9°30′ to 29°	1.4705–1.472	18–47	0.45–0.5
Italy	"	0.860 –0.924	+2°54′ to 56°30′	1.468 –1.474	6–127	0.2
Venezuela	"	0.884 –0.887	−0°55′ to −1°54′	1.463 –1.465	96–102	
Paraguay	"	0.9076	+0°25′		72.5	
Algeria	"	0.8723–0.877	+5°42′ to 6°6′		72–91	
Syria	"	0.8758	+1°6′		51.5	

Neroli oil dissolves in 1 to 2 volumes of 80% alcohol; more solvent causes turbidity. The alcoholic solution is characterized by a handsome violet-blue fluorescence.

In view of the importance of neroli oil to the perfumery trade, a more comprehensive study of its composition has been made. Neroli oil proper, produced by steam distillation, has the composition shown in Table VII, prepared by Hesse and Zeitschel.[27]

TABLE VII

Composition of Neroli Oil

Class	Constituents	Approx. %
Hydrocarbons (35%)	1. Pinene, 2. Camphene, 3. Dipentene, 4. Paraffin C_{27}	35
Terpene alcohols and their acetates (47%)	5. *l*-Linalool	30
	6. *l*-Linalyl acetate	7
	7. *d*-Terpineol	2
	8. Geraniol, 9. Nerol	4
	10. Geranyl acetate, 11. Neryl acetate	4
Sesquiterpene, 6% alcohol	12. *d*-Nerolidol	6
N-compounds (0.7%)	13. Anthranilic acid methyl ester	0.6
	14. Indol	0.1
Acids (0.1%)	15. Acetic acid	—
	16. Palmitic acid	—
Other minor constituents and resinous products	17. Decylic aldehyde(?), esters of phenyl acetic acid, benzoic acid, jasmone, and farnesol	11.2

[27] Hesse, A., and O. Zeitschel, *J. prakt. Chem.* [2], **66**, 481 (1902).

The most common, as well as the most dangerous, adulterants of neroli oil are bergamot oil and oil of petitgrain.

The oil of sweet-orange flowers or neroli Portugal is not an article of commerce, but it has been prepared in South Africa by de Villiers (1932),[28] from whom the following indices have been derived.

PHYSICAL CONSTANTS. $d_{15}° = 0.8789$; $n_D^{20°} = 1.4770$; $\alpha_D = +16°45'$; ester value = 27.1; dissolves in less than 1 volume of 80% alcohol.

The yield obtained from the blossoms of navel oranges was about 0.1 to 0.15%.

(2) OIL OF PETITGRAIN

The name petitgrain proper is given to the oil obtained by steam distillation from the twigs and leaves of the bitter orange, *Citrus bigaradia* Risso. However, similar oils are occasionally distilled from the leaves or twigs (or both) of other citrus varieties.

Originally, petitgrain oil was produced principally in the south of France by distilling the residues left after pruning the trees; later, it was introduced into Paraguay, where the bitter orange grows wild. The Paraguayan plantations were so much abused, however, that the vast orange forests have practically been annihilated.

The oil is usually distilled in very primitive installations. The process lasts 36 hours, the yield being 0.33 to 0.4%.

Petitgrain oil resembles neroli oil in odor, but is much less fragrant; it has an aromatic, bitter taste and a yellowish color. It is largely used in perfumery. Its physical constants deviate considerably, according to differences in the raw material from which it has been distilled or to seasonal climatic variations.

PHYSICAL CONSTANTS. $d_{15}° = 0.886$–0.900; $\alpha_D = +5$ to $-2°45'$; $n_D^{20°} = 1.459$–1.464; acid value up to 2; ester value 106–163, which corresponds to 37–57% of linalyl acetate; soluble in 2–4 volumes of 70% alcohol.

Petitgrain oil contains, on examination, the following components: pyrrole derivatives (most probably due to the decomposition of chlorophyll during distillation), furfural, camphene, β-pinene, dipentene and *d*-limonene, *l*-linalool (both as an alcohol and as its ester), *d*-α-terpineol, nerol (about 2%), geraniol and geranyl acetate, some sesquiterpenes which have not yet been identified, and probably also methylanthranilate. (Charabot and Pillet claim that oil of petitgrain normally contains no limonene, but that if it is occasion-

[28] de Villiers, F., "Oil from Orange Blossoms," *Citrus Growers, S. Africa*, 13 (Feb., 1932).

ally found, it is due to the small fruits adhering to the twigs and finding their way to the distilling column.)

Petitgrain oil is frequently adulterated with orange and lemon oils and with turpentine.

Petitgrain Portugal is the oil distilled in France and Algeria from the leaves of sweet orange (*C. aurantium* Risso). According to Litterer, it contains *d*-camphene, *d*-limonene, citral (about 4%) geraniol (about 12%), and probably linalool.

PHYSICAL CONSTANTS. $d_{15}^{\circ} = 0.86012$–0.8584; $\alpha_D = +56°46'$ to $+53°52'$; ester value 21.6% (as linalyl acetate).

Mandarin Petitgrain Oil is distilled in Spain from the leaves of the tangerine tree, yielding 0.2 to 0.35%. It usually separates into two layers, one lighter, the other heavier. Its main constituent is methyl anthranilic acid methyl ester, which may amount to as much as 65% of the oil.

PHYSICAL CONSTANTS. $d_{15}^{\circ} = 1.0142$; $\alpha_D = +7°46'$; ester value 216.

Lemon Petitgrain or Petitgrain Citronnier resembles true petitgrain in odor but has a distinct lemonlike aroma. Its main components are limonene, citral, geraniol, and probably camphene and linalool.

PHYSICAL CONSTANTS. $d_{15}^{\circ} = 0.868$–0.894; $\alpha_D = +14°$ to $+35°$; saponification value 14–46; aldehyde content 20–30%.

Oil of Limette Leaves (*C. medica* Linnaeus, var. *acida* Brandis) resembles lime oil in odor. The oil consists of probably 43% of citral, and according to Watts, also contains dipentene and methyl nonyl ketone.

PHYSICAL CONSTANTS. $d_{15}^{\circ} = 0.8783$; $\alpha_D = +37°30'$; acid value 3.6%; ester value 23.0.

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CHAPTER III

THE MESOCARP OR ALBEDO

A. CARBOHYDRATES

1. Composition of the Albedo

Penetrating further into the peel of citrus fruit, one comes to the white, spongy, parenchymatous layer (mesocarp), generally known as the albedo. This soft pith is composed of cells of very irregular shape and size, with large intercellular spaces filled with air.

The albedo portion of the fruit represents some 20 to 60% of the whole fruit, the thickness of the peel varying according to the different fruits. The thickness of the peel of California lemons, for instance, ranges from 2 to 8 mm and that of Palestine oranges (Jaffa variety) from 4.5 to 12 mm. Grapefruits have a still thicker albedo, while some varieties of shaddocks consist practically wholly of mesocarp, the endocarp being negligible in comparison with the huge size of the fruit.

Fresh albedo contains some 75 to 80% water, while its main components calculated on the dry basis consist, according to Rosenfeld,[1] roughly of 44% sugars (in a ripe fruit), 33% of cellulose (including lignin and pentosans), and 20% pectinous substances.

Haas and Klotz[2] have shown that the sugar content of the peel at the stem end is higher than that at the stylar end. However, the direction of the sugar gradient in the juice of citrus fruit is the reverse of that in the peel.

A complete analysis of grapefruit peel has been given by H. D. Poore[3] of the United States Department of Agriculture (Table VIII).

In factories where citrus fruit is fully utilized, all of these sub-

[1] Private communication from Dr. Bruno Rosenfeld of the Daniel Sieff Research Institute, Rehovoth, Israel.

[2] Haas, A. R. C., and L. J. Klotz, "Physiological Gradients in Citrus Fruits," *Hilgardia*, 9, 181 (1935).

[3] Poore, H. D., "Recovery of Naringin and Pectin from Grapefruit Residue," *Ind. Eng. Chem.*, 26, 637 (1934).

TABLE VIII

Composition of Grapefruit Peel

Analysis	California, Peel and rag combined %	Florida, Peel %	Florida, Rag %
Total solids vacuum-dried at 70° C.	22.02	16.71	15.61
Ash	0.70	0.74	0.75
Citric acid	0.43	0.74	0.63
Volatile oil (by steam distillation)	0.56	0.43	....
Ether extract	0.23	0.28	0.16
Crude fiber	2.00	1.71	1.44
Protein ($N \times 6.25$)	1.63	1.13	1.06
Total sugars as invert	8.68	6.35	6.30
Pentosans	1.31	0.83	0.44
Calcium pectate	3.93	3.19	3.56
Naringin (glucoside)	0.63	0.40	0.10

stances find their application: sugars are fermented into alcohol or subjected to other fermentations, pectin is extracted to be used as a jellifying agent in the manufacture of jams, marmalades, etc., and the residues, containing mainly cellulose, pentosans, proteins, and remaining sugars, are dried and converted into a cattle fodder.

The albedo of citrus fruits also contains a rather large proportion of ascorbic acid (vitamin C) which will be discussed later in the book. The peel of sweet orange has been found to contain phytosterolin accompanied by two phytosterols. These have been identified by Matlack and Kremers,[4] who also isolated palmitic, steric, oleic, linolic, and linolenic acids (evidently from the seeds, which are known to contain a semidrying oil).

Since most of the constituents of both peel and juice are of a carbohydrate nature, it will be appropriate to dwell on this class of natural substances somewhat more in detail. The discussion, however, will be limited to those carbohydrates which are present in citrus plants.

2. Description of the Carbohydrates of Citrus Fruits

Carbohydrates, as discussed elsewhere, are photosynthesized by the green plant from carbon dioxide of the air with the aid of chlorophyll.

They are usually classified into three groups: *monosaccharides* or

[4] Matlack, M. B., and E. Kremers, "The Chemistry of Agrumens with Special Reference to That of Sweet Orange," *Amer. J. Pharm.*, **100**, 599 (1928).

simple sugars, such as glucose, fructose, mannose, etc.; *disaccharides* or sugarlike polysaccharides, made up of monosaccharides and resembling them closely, such as sucrose, maltose, and lactose; *polysaccharides,* which are condensation products of simple sugars without the properties of sugar, including pentosans, starches, glycogen, cellulose, and pectins.

Although sugars form the major part of the citrus peel, their nature has scarcely been investigated. Since 1895 the peel has been known to contain fructose. Flateau and Labbé (1898) believed that citrus peel contains mannose, since neutral water extract gave a precipitate with lead acetate; no further data have been found on this subject. Only recently has a thorough study of sugars contained in the peel been made by Rosenfeld, who showed that their identification is possible only after a complete isolation in very pure state; otherwise the pectic substances and glucosides which adhere tenaciously to the sugars cause erroneous interpretations.

Rosenfeld claims the absence of mannose, and the presence of glucose, fructose, and sucrose in the following proportions: glucose, 63.5 %, fructose, 20.5%, and sucrose, 16.0%.

(a) Monosaccharides

Glucose and fructose are very widely distributed in nature, especially in fruits. Glucose exists abundantly in nature also in the form of glucosides, which are combinations of glucose with various other substances of different structure, such as alcohols or phenols. Some of the glucosides will be discussed later.

The monosaccharides are neutral, crystallizable, and diffusible substances, soluble readily in water and difficultly in alcohol, and insoluble in ether. Not all of these sugars are sweet; they cover a wide range of sweetness, and some are even bitter.

Monosaccharides may be divided chemically into polyhydroxyaldehydes (aldoses) and polyhydroxyketones (ketoses). Depending on the number of constituent formaldehyde units (CH_2O) sugars may be classified as bioses, $(CH_2O)_2$; trioses, $(CH_2O)_3$; tetroses, $(CH_2O)_4$; pentoses, $(CH_2O)_5$; and hexoses, $(CH_2O)_6$.

(1) HEXOSES

Glucose is especially abundant in grapes (20% of total weight), in the juice of other fruits, and in some vegetables, such as onions, unripe potatoes, etc., as well as in honey. Like other monosaccharides,

glucose is utilized in the production of glycogen in the animal body and to maintain the normal glucose content of the blood.

For the last fifty years glucose has been regarded as a straight-chain aldehyde with an OH-group attached to each of five carbon atoms, the terminal carbon being attached to the aldehyde group, thus:

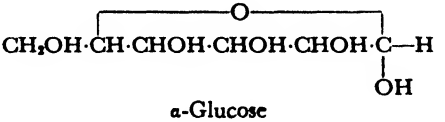
$$\overset{6}{CH_2OH}\cdot\overset{5}{C}HOH\cdot\overset{4}{C}HOH\cdot\overset{3}{C}HOH\cdot\overset{2}{C}HOH\cdot\overset{1}{C}HO$$

(asterisks mark carbons 5, 4, 3, 2)

It is evident from this formula that glucose contains four asymmetric carbon atoms (indicated by asterisks); consequently, a possibility of $2^4 = 16$ stereoisomers exists. Glucose is only one of these 16 isomers, all of which are actually known, some occurring in nature, others having been prepared synthetically. Ordinary glucose is designated as *d*-glucose, its enantiomorph as *l*-glucose.

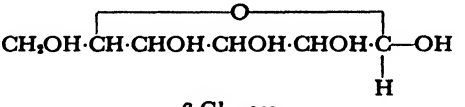
Configurational formulas, although still commonly used for convenience, do not suffice to explain the existence of two further isomers of D-glucose differing in melting point, solubility, and particularly in specific rotation. Thus, for one of them (α-glucose), the specific rotation $[\alpha]_D = +109.6°$, while for β-glucose $[\alpha]_D = +20.5°$. Each of these isomers, if left in solution for several hours, will finally attain a specific rotation $[\alpha]_D = +52.3°$. (In the stereochemistry of the carbohydrates, the prefixes D- and L- refer to the configuration of the compounds, as contrasted to *d*- and *l*-, which indicate the sign of their optical activity.)

It is now generally accepted that glucose exists in a cyclic hemiacetal form with a lactone ring:



α-Glucose

this form shows the terminal carbon atom to be also asymmetric and therefore explains the existence of a second isomer (not an antipode):



β-Glucose

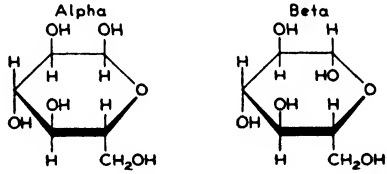
These two isomers in solution attain in time an equilibrium (mutarotation) having a specific optical rotation of +52.3°. Some evidence indicates that the two isomers, α- and β-glucose, exist side by

side with a small amount of the open-chain aldehyde form; there is probably a tautomeric equilibrium between the carbonyl and the cyclic hemiacetal forms:

$$\alpha\text{-glucose (cyclic)} \rightleftharpoons \text{open-chain aldehyde} \rightleftharpoons \beta\text{-glucose (cyclic)}$$

The cyclic form of the aldoses shows, as stated above, five asymmetric carbon atoms and, therefore, $2^5 = 32$ isomers are predicted, in lieu of $2^4 = 16$ isomers on the basis of the open-chain formula. In fact, the number of isomeric aldohexoses now known is greater than 16, and it may be expected that all of the 32 isomers will be isolated in the future.

The pyranose form of glucose (1–5 lactone) is represented stereochemically as follows:

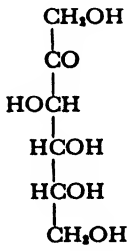


Fructose (fruit sugar or levulose) occurs in plant juices and fruits and especially abundantly in honey. Fructose and glucose are derived in equal quantity when cane sugar (sucrose) is hydrolyzed.

Fructose is a ketohexose represented by the general formula:

$$\overset{6}{\mathrm{CH_2OH}}\cdot\overset{5}{\mathrm{CHOH}}\cdot\overset{4}{\mathrm{CHOH}}\cdot\overset{3}{\mathrm{CHOH}}\cdot\overset{2}{\mathrm{CO}}\cdot\overset{1}{\mathrm{CH_2OH}}$$

having only three asymmetric carbon atoms, as indicated by asterisks. It is obvious, therefore, that $2^3 = 8$ isomeric ketohexoses can exist. Of these, D-fructose is of general interest, having the following configuration:



D-Fructose (levulose)

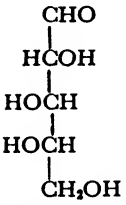
As already mentioned, the prefix D- does not refer here to the sign of rotation; in fact, D-fructose is levorotatory, $[\alpha]_D = -93°$.

(2) PENTOSES AND PENTOSANS

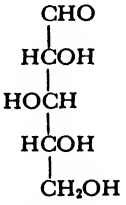
The general formula of pentoses is, as already mentioned, $C_5H_{10}O_5$, i.e., they are sugars containing five carbon atoms. They are not very common in the free state but are very widely distributed in the vegetable kingdom as polymerized anhydrides, called pentosans. They form the cell-wall constituents and enter into the composition of various gums, mucilages, and pectins.

There is no evidence that the pentosans occur as a direct product of photosynthesis. Spoehr[5] has shown that their formation is dependent upon certain conditions, especially water content and temperature: low water content coupled with a high temperature results in a decrease in the amount of hexoses and an increase of pentosans, and vice versa.

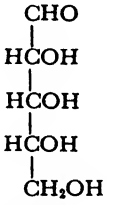
The following are the structural formulas of the four pentoses found in plant material which have so far been investigated:



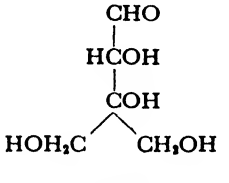
Arabinose



Xylose

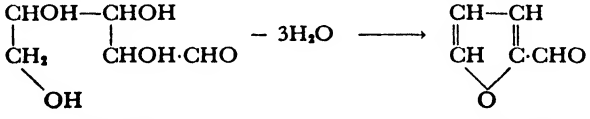


Ribose



Apiose

Pentoses when treated with HCl or H_2SO_4 give rise to furfural:



Pentose — Furfural

This reaction of the pentoses or pentosans with hydrochloric acid, by which furfural is produced, finds considerable application in food analysis. By carefully regulating the conditions, the yield of furfural may be so nearly constant as to be utilized for the quantitative determination of pentosans. Pentoses reduce Fehling's solution and yield osazones but are not fermentable by yeast.

Citrus albedo contains a surprisingly high amount of pentoses and pentosans. Bartholomew[6] and his co-workers found that about 60%

[5] Spoehr, H. A., "Photosynthesis and Metabolism," *Carnegie Inst. Washington Publ. No. 287* (1919).

[6] Bartholomew, E. T., and W. J. Robbins, "Internal Decline (Endoxerosis) of Lemons; IV, The Carbohydrates in the Peel of Healthy and Endoxerotic Fruits," *Amer. J. Bot.*, **13**, 342 (1926); "Methods for Determining Pentoses as Furfural in

of the dry albedo consists of carbohydrate material soluble in 95% alcohol or hydrolyzable in 1% HCl; of this 60%, at least 25% are pentoses and pentosans. Valencia orange peel has been found to contain 15.86% and lemon peel 17.45% of pentoses and pentosans expressed in terms of furfural.

(b) Disaccharides

Of the three most important disaccharides, sucrose (cane sugar), lactose (milk sugar), and maltose (malt sugar)—all having the general formula $C_{12}H_{22}O_{11}$—only sucrose will be discussed here.

SUCROSE (OR SACCHAROSE)

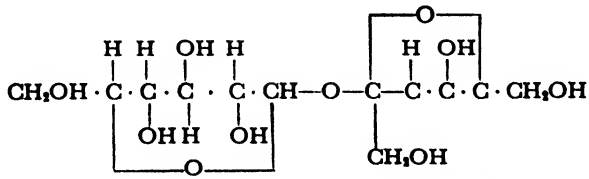
Sucrose is the well-known cane sugar or beet sugar used extensively in our daily diet. It is very widely distributed in the plant kingdom and occurs in considerable quantities, often together with glucose and fructose, in the fruit juices of many plants; its occurrence in the juice and peel of citrus fruits has been noted (pages 77–79).

By the action of acids or of an enzyme invertase, sucrose is readily hydrolyzed into one molecule of glucose (dextrose) and one of fructose (levulose):

$$C_{12}H_{22}O_{11} + H_2O \rightarrow \underset{\text{D-Fructose}}{C_6H_{12}O_6} + \underset{\text{D-Glucose}}{C_6H_{12}O_6}$$

Although fructose is levorotatory and glucose dextrorotatory, an equimolecular mixture after hydrolysis is not inactive. On the contrary, the mixture of the two sugars rotates the plane of polarized light to the left because the specific rotation of fructose is higher than that of glucose. This hydrolysis is called, therefore, *inversion* and the mixture of fructose and glucose, *invert sugar.*

The inversion of sucrose establishes also the fact that a molecule of sucrose is built up of one of glucose joined with one of fructose through the loss of water. According to Haworth, the constitutional formula of sucrose is:



Citrus Fruits," *ibid.*, 22, 829 (1935). Bartholomew, E. T., W. B. Sinclair, and E. C. Raby, "Granulation (Crystallization) of Valencia Oranges," *Calif. Citograph.*, 19, 88 (1934).

Sucrose itself is dextrorotatory, $[\alpha]_D^{20°} = +66.5°$. It does not reduce Fehling's solution or react with phenylhydrazine.

(c) Polysaccharides

The polysaccharides do not resemble the sugars: they are mostly insoluble in the usual solvents and, when soluble to any degree, form colloidal suspensions. Of interest in this group are starch, cellulose, and pectins. (The commercially important pectins are discussed in a separate section.)

On complete hydrolysis with acids many of the polysaccharides yield D-glucose as the final product.

The accepted view on the constitution of all these complex substances, which have an unusually high molecular weight, is that they consist of very many hexose molecules (or their derivatives) linked together into chains of varying lengths. Their general empirical formula may be represented as $(C_6H_{10}O_5)_x$.

Modern x-ray spectroscopy has confirmed that the majority of the polysaccharides (with the exception of glycogen) are of crystalline nature.

(1) STARCH

To a greater or lesser extent starch is found in all the tissues of citrus fruits during the process of their development, but it is particularly abundant in the albedo, especially in the inner portion adjacent to the juice segments. However, as maturing proceeds, the amount of starch diminishes, and by the time the fruit is fully ripe only traces of starch, if any, are found. The disappearing starch is no doubt transformed into other substances. However, in some plants, as, for instance, in potatoes and rice, starch is the main carbohydrate reserve.

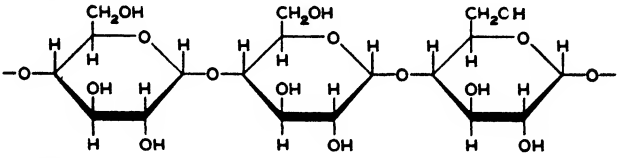
Starch dissolves in hot water, forming a colloidal jelly, but is practically insoluble in cold water. It gives a blue coloration with iodine (dissolved in KI)—probably an adsorption phenomenon, the reaction being very sensitive. The blue color disappears when the solution is heated and reappears when cooled.

Through the action of the enzymes and of even very dilute acids, starch is readily and quantitatively hydrolyzed into glucose:

$$(C_6H_{10}O_5)_x + xH_2O \rightarrow xC_6H_{12}O_6$$

which shows that the starch molecule is built up entirely of D-glucose residues. On the basis of these and other facts, Haworth ascribed to starch the following structural formula, in which about 13 to 15

glucose residues are combined, chainlike, by glucoside linkages similar to maltose:

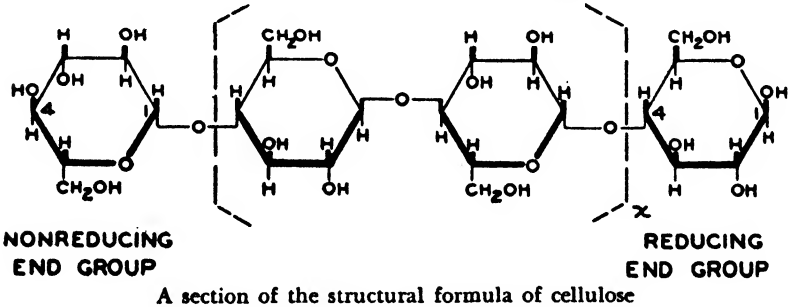


(2) CELLULOSE

The fabrics or the structural material of citrus albedo, in common with those of the entire plant world, is built largely of cellulose. The fibers of cellulose vary according to source, those of the citrus albedo being very short. From the standpoint of quantity, cellulose occupies the first place in nature among all organic substances: it is estimated that half of the carbon dioxide of the atmosphere is fixed by the cellulose of the vegetable kingdom.

Cellulose is insoluble except in Schweitzer's reagent (an ammoniacal solution of copper hydroxide) and is relatively resistant to hydrolysis by dilute acids. On careful hydrolysis, however, cellulose yields a disaccharide, cellobiose, which on further hydrolysis yields two molecules of glucose.

According to Haworth and others, the cellulose molecule is composed of a long chain of about 50 to 100 cellobiose units. About fifty such chains are arranged in a bundle, called a micelle. A cellulose micelle is about 50A thick and 500 to 1000A long.



A section of the structural formula of cellulose

The difference between the starch molecule and that of cellulose is first in its length, as determined by the x-ray diagram, and secondly in the stereochemical nature of the glycosidic link: starch is related to α-glucoside and cellulose to β-glucoside.

Successful experiments on a semicommercial scale have been performed in Palestine during the recent war in order to create woodlike boards by treating the citrus peel with sodium hydroxide and

then matting the cellulose fibers together. A new plastic material, named "Weberite," has recently been developed in the United States from waste citrus peel and pulp.[7]

3. Alcoholic Fermentation

As the sugars of citrus peel are important in the process of alcoholic fermentation, attention will now be devoted to this vital biological process.

It has been very well known for centuries that weak sugar solutions allowed to stand for some time change into alcohol through the so-called fermentation process. The reaction, shown by Pasteur to be caused by yeasts, is generally represented by the equation:

$$C_6H_{12}O_6 = 2\,C_2H_5OH + 2\,CO_2$$

This apparently simple reaction proved, however, to be a most complicated mechanism. A great impetus was given to comprehensive study of this highly important biochemical reaction when Buchner, in 1897, discovered that sugar solutions can be fermented into ethyl alcohol without the living yeasts by using the cell-free and even the sterilized extract (juice) from yeasts. Buchner called this "unorganized ferment," or enzyme, *zymase*. Since then it has been shown that zymase is not a single ferment but a series of enzymes.

In the course of further observations it was noticed that ethyl alcohol and carbon dioxide are not the only end products of the fermentation and that, side by side with the above, small quantities of other substances are also derived from sugar. It was also shown that phosphoric acid and its esters play most important roles in this reaction and that a number of intermediate products are created during fermentation. To clarify the mechanism of alcohol fermentation, the numerous intermediate stages have been studied by many investigators, notably Harden and Young, Robison, Neuberg, Embden, Meyerhof, and others. Since the various schemes for the mechanism of ethyl alcohol fermentation can be found in many textbooks, no attempt is made here to describe them.

Yeasts, as well as yeast juice, contain a great number of enzymes, most of which take an active part in the process of fermentation.

It is outside the scope of this work to treat in detail the activities and the constitution of the enzymes involved in the fermentation. Enzymes in general, and the selection of the proper variety of yeasts,

[7] Weber, G. L., "Plastics from Citrus Waste," *Pacific Plastics Mag.*, I (No. 4–5), 8 (1943).

as well as the most favorable conditions under which alcoholic fermentation can proceed smoothly, are discussed elsewhere in this book.

Besides ethanol, which is the main end product of the fermentation process, some other by-products are obtained, such as "fusel oil," forming 0.1 to 0.7% of the crude distilled spirits. Fusel oil is a mixture of amyl and isoamyl alcohols, with smaller quantities of isobutyl and normal propyl alcohols and traces of acids, esters, and aldehydes. Fusel oil derived after fermentation of citrus peel or peel-juice contains large quantities of *d*-limonene naturally originating from the essential oils of the peel, even when these have been previously largely separated.

When citrus peels are used as raw material for ethanol distillation, some traces (0.04–0.08% of the mash) of methyl alcohol are also present in the raw spirits. This is due to the presence of pectinous compounds in every kind of citrus peel. Methanol, a natural constituent of pectin, occurs as the methyl ester of polygalacturonic acid (see pages 90–96). This methyl ester is readily split into pectic acid and methanol either during the treatment of the citrus peel with milk of lime to facilitate the separation of the sugar-containing peel juice from the pulp (as will be shown later), or during the actual fermentation by enzyme action.

4. Other Fermentations

Citrus peel as a source of sugars can, of course, be subjected to other fermentation processes such as, for instance, the acetic acid or the butanol-acetone fermentation.

Acetic acid fermentation is discussed under the manufacture of vinegar from orange juice (see pages 314–318).

The other fermentation processes are not commercially used in the industry, though in at least one case known to the writer (Palestine during World War II) citrus peels were subjected to butanol-acetone fermentation according to Weizmann's process, in which *Clostridium acetobutylicum* (Weizmann) is used.[8] The fermentation proceeds as readily using disintegrated citrus peels from which the essential oil has been extracted as it does using maize mash. During the butanol-acetone fermentation the pectin of the albedo is hydrolyzed and partially demethylated; more than 500 cu m of hydrogen gas (45%) and carbon dioxide (55%) are evolved during

[8] Rosenfeld, Bruno, "Fermentation of Waste Products from the Apple and Citrus Fruit Juice Manufacturing Industries." U. S. Patent No. 2,276,240 (Mar. 17, 1942).

the fermentation of 1 ton of sugar contained in the peel. According to Rosenfeld, 500 parts of peel gave 12 parts of butanol, 5 of acetone, and 1 of ethanol. The mixture contained some methanol from the decomposition of the pectin.

B. PECTIC SUBSTANCES

1. Definition. Commercial and Physiological Significance of the Pectins

The term "pectic substances" designates complex colloidal carbohydrates commonly occurring in all plant tissues and especially in fruits. They are composed mostly of polygalacturonic acids of differ-

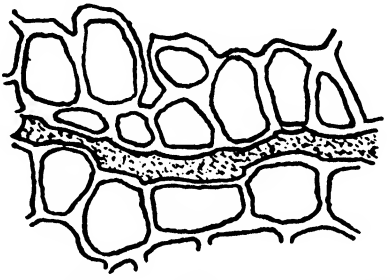


Fig. 17. Pectin channels under microscope.

ent degrees of esterification and neutralization and show wide variations in their solubility in water. The forming of jellies with sugar and acid is the characteristic property of pectins.

From the commercial point of view pectin is the most important component of albedo, for commercial citrus pectin is now being extensively produced in powder form and is used as an important ingredient in the manufacture of jams and many other products (see later). Citrus fruits are second only to apples as the main source for this industry.

Pectin is also physiologically very important. The middle lamellae of the albedo—that is, the layers between each of the adjacent cellulose walls of the cells—are almost wholly composed of pectic substances. In young citrus peel, pectins are often formed in such great abundance that they create wide channels by pushing apart the cells (Fig. 17).

As a colloid *par excellence,* pectin has the property of imbibing large quantities of water. Owing to this capacity pectin plays an important role in the early stages of development of the fruit when the

cells still lie far apart and at a comparatively great distance from the water-conducting vessels. The pectic substances then quickly absorb water and transfer it among the cells more easily than could be effected by the osmotic property of the cells themselves.

The discovery of pectin as the "gelatinous principle of fruits" was made by Braconnot in 1825. Since then, a great deal of research has been carried out by numerous investigators on the technical as well as on the scientific (chemical and biological) aspects of the problems involved. A critical and historical survey of the field may be found in several of the works cited in the accompanying bibliography. We shall endeavor here only to present concisely the most important facts and the latest theories on the subject. Technical considerations are discussed with the manufacture of citrus pectin (see pp. 361–9); details on the jelly-forming capacity of the pectins, especially on the pectin-sugar-acid gel, are treated in the chapter on jams and marmalades (see page 322).

2. Transformation of Protopectin into Pectin

While the pectic substances are in the plant they are believed to be intimately associated with cellulose in the outer cell walls or in the region of middle lamellae. In this form, as a precursor of pectin proper, the substance is named *protopectin* (or pectose).

Protopectin is insoluble in water and, according to Carré and Horne,[9] can be observed microscopically using ruthenium red as a stain. Sucharipa (Ripa) [10] claims to have isolated pure protopectin from lemon albedo by treating its insoluble copper compound with acetic acid. This, however, has not been confirmed by several subsequent investigators. It is suggested that protopectin is either an anhydride of pectin or that it is formed by the union of pectin and cellulose by elimination of molecules of water.

When the citrus albedo is heated with acid or acidfied water, the protopectin is loosened from its connection with cellulose and is hydrolyzed into pectin, which is readily soluble in water and, under proper conditions, gives with sugar and acids a colloidal gel.

The same transformation or hydrolysis of the protopectin can take place also by the action of an enzyme called *protopectinase,* which is assumed to be formed in the plant tissues during ripening. When the citrus fruit is still unripe the major part of the pectic substances are

[9] Carré, M. H., and A. S. Horne, "An Investigation on the Behavior of Pectic Materials in Apples and Other Plant Tissues," *Ann. Bot.,* **41,** 1 (1927).

[10] Sucharipa, R., "Protopectin and Some Other Constituents of Lemon Peel," *J. Amer. Chem. Soc.,* **46,** 145 (1924).

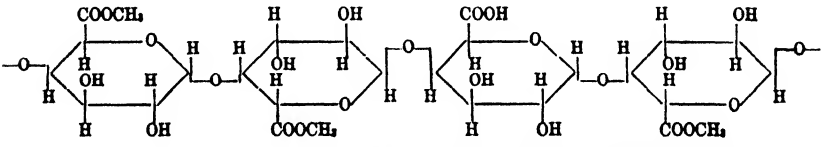
present in the albedo as insoluble protopectin and only a very small amount of soluble pectin can be leached out from it by cold water. As the ripening of the fruit proceeds, more and more protopectin is converted into soluble pectin, so that in normally ripened citrus fruits about two-thirds of the pectic substances present are in soluble form.

3. Chemical Properties of Pectin

From its water solutions pectin is readily precipitated by alcohol or acetone as a suspended jelly, which in turn is again soluble in water. This coagulation can take place also at certain acidities by the action of various salts such as magnesium sulfate, ammonium sulfate, basic lead acetate, or aluminum sulfate, whose particles carry an electric charge with a sign opposite to that of pectin (which is a positively charged colloid).

Pectin is, therefore, a reversible colloid of the lyophilic type. Pectin solutions rotate polarized light to the right. Crude pectin contains various hemicelluloses, pentosans, arabans, galactosans, and other similar impurities and can be purified by repeated precipitations and redissolutions. Purified pectins have been shown to be composed almost exclusively of acid methyl esters of pectic acids which upon hydrolysis yield D-galacturonic acid and methyl alcohol.

The molecular weight of pectin is not easily determinable; it is in all probability very high. The molecular weight of pectins from oranges is reported to be definitely higher (40,000–50,000) than that of other fruits. Furthermore, the pectin substances in oranges[10a] were found to be less methoxylated than those of lemons.[10b] Its chemical configuration has long been a subject of controversy. According to the researches of Mark and Link, which have been confirmed by Schneider and his co-workers,[11] pectin is now regarded as a long chain of polygalacturonic acids with carboxyl groups partially esterified with methyl alcohol:



[10a] Norris, F. W., "Pectic Substances of Plants. IV, The Pectic Substances in the Juice of Oranges," *Biochem. J.*, 20, 993 (1926).

[10b] Norman, A. G., "Studies in Pectin. III, The Degree of Esterification of Pectin in the Juice of the Lemon," *Biochem. J.*, 22, 749 (1928).

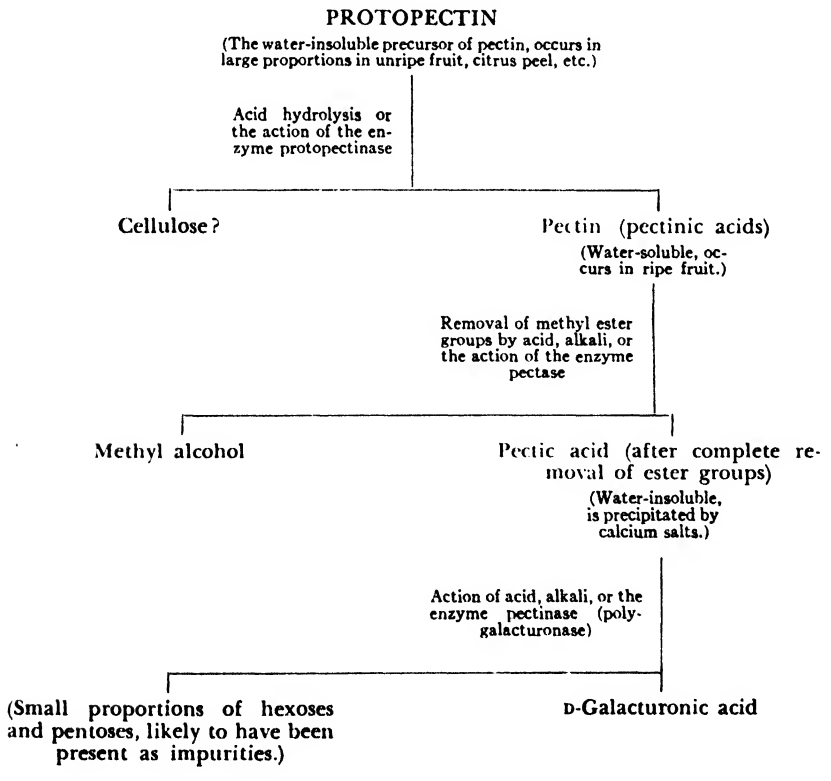
[11] Schneider, G. G., and H. Bock, "Ueber die Konstitution der Pektinstoffe," *Ber. dtsch. chem. Ges.*, 70, B. 1617 (1937).

The configuration of the basic structure of pectic substances shows similarity to the structure of cellulose. The number of galacturonic acid residues which make up the polygalacturonic acid structure is still a matter of debate. One group of investigators (Schneider, Henglein, Bock, etc.) believes that there are about 80 units in the structure. Others think that a larger or smaller number of units compose the basic structure and that the association of smaller polygalacturonic acid chains in a manner different from the 1,4-glycosidic linkages is responsible for the colloidal properties.

Pectins derived from various sources differ on account of the different proportions of free acid groups, methyl ester groups, and acid groups which have been neutralized to form salts. A further cause of variation is the size and the distribution pattern of the polygalacturonic acid chains. During preparation a fractionation typical of the

TABLE IX

The Changes of Peptic Substances in Fruit



method used occurs; the chemical and colloidal properties of pectins will, therefore, vary greatly with different methods of preparation.

Pectins may undergo hydrolysis by acid or alkali or by the action of enzymes. Heat alone may also cause the degradation of pectic substances. The interrelation of various pectic substances is shown in Table IX.

In view of the variations found in the properties of pectin specimens, pectin should be regarded as a generic term covering a wide range of minor variations of molecular composition.

Raw citrus peel may contain from 1.5 to 3%, dried rinds from 9 to 18% of pectin. Wilson found in lemon albedo 2.5 to 5.5% of pectin or 30 to 40% calculated on a dry basis. The largest quantity of pectin was found in the Hung lemon, a citrus variety of the Philippine Islands.

In a series of tests performed on Valencia and Navel oranges and lemons, Heid[12] found that pectin recoverable from ethylene-treated fruit has a lower jelly-unit yield than that from untreated fruit or from fruit stored for the same length of time without ethylene treatment. It seems probable that ethylene, by accelerating respiration (see page 37), also speeds up the pectic hydrolysis in the albedo.

4. Pectic Enzymes

NOMENCLATURE

The various enzymes partaking in pectic hydrolysis have been extensively investigated. To reduce the widespread confusion which has existed in their terminology, the Committee on Nomenclature of Pectin of the American Chemical Society (Agriculture-Food Division) has established the following terms:[13]

PROTOPECTINASE (synonymous with pectosinase or pectosase) is responsible for the hydrolysis or conversion of protopectin into pectin with the resultant separation of the plant cells from one another.

PECTINASE (synonymous with pectolase and polygalacturonase) hydrolyzes pectins (pectinic acids) or pectic acids into smaller polygalacturonic acids and finally into D-galacturonic acid and galacturonic acid methyl ester. It also usually produces a small proportion of pentoses and hexoses which were likely to be present in the preparation as impurities.

[12] Heid, J. L., "The Effect of Ethylene Treatment upon the Recovery of Citrus Pectin," *Fruit Prod. J.*, **21** (No. 4), 100 (1941).

[13] *J. Amer. Chem. Soc.*, **49**, Proc. 37 (1927). Revised: *Chem. Eng. News*, **22**, 105 (1944).

PECTASE (synonymous with pectin-methylesterase and pectinesterase) hydrolyzes the ester groups, freeing the carboxyl groups and producing methyl alcohol. Of all pectolytic enzymes, pectase has been most widely studied by Kertesz.[13a]

The pectic enzymes in citrus fruits have been recently studied more closely, and pectase and pectolase were isolated separately.[13b] It has been found[13c] that pectase is thermolabile, is stable at *p*H 8.0, but destroyed at an acid *p*H, and that pectolase is stable at *p*H 5.1 but destroyed at an alkaline *p*H.

More recent work[13d] on the elucidation of pectolytic enzymes showed the necessity of only two enzymes to be involved in the hydrolysis of pectin which basically consists of polygalacturonic acid with most of its carboxyl groups esterified by methyl alcohol. These are:

Pectin esterase (PE), which catalyzes the hydrolytic removal of methyl alcohol from the pectin molecule (synonyms: pectase, pectinmethoxylase, pectin methylesterase).

Polygalacturonase (PG), which catalyzes the glycosidic hydrolysis of polygalacturonic acid into monogalacturonic acid (synonyms: polygalacturonidase, pectinase, pectolase, pectin polygalacturonase).

The existence of a specific enzyme "protopectinase" is assumed to be superfluous and it is thought that the natural pectolysis of the intercellular layer in plants is caused entirely by the combined action of PG and PE.

It is of interest to note that enzyme demethylation brought about by the pectase enzyme usually stops at 1.8% methyl ester and will not go below this point even on prolonged contact with the enzyme. Apparently one methyl ester linkage in each eight galacturonic units of the pectin molecule is not attacked by pectase.[13e]

EFFECT OF PECTOLYTIC ENZYMES ON CITRUS VARIETIES

In studying the effect (i.e., the rate of pectic hydrolysis) of pectolytic enzymes on different citrus varieties, Joslyn and Sedky[14] found

[13a] Kertesz, Z. I., Peptic Enzymes, *Ergeb. Enzymforsch*, **5**, 233 (1936).

[13b] McColloch, R. J., and Z. I. Kertesz, *J. Biol. Chem.*, **160**, 149 (1945).

[13c] Rothschild, H., "Properties of Pectin Constituents as Antigens. IV, Isolation of Pectin Enzymes," *Enzymologia*, **5**, 359 (1939).

[13d] Phaff, H. J., and M. A. Joslyn, "The Newer Knowledge of Pectin Enzymes," *Wallerstein Labs. Communs.*, **10**, 133 (1947).

[13e] Hills, C. H., J. W. White, Jr., and G. L. Baker, "Low-Sugar Jellying Pectinates," *Proc. Inst. Food Technol.*, **1942**, p. 47.

[14] Joslyn, M. A., and A. Sedky, "The Relative Rates of Destruction of Pectin in Macerates of Various Citrus Fruits," *Plant Physiol.*, **15**, 675 (1940).

that the decomposition of the pectic substances, as judged by the clarification of citrus juices, is most rapid at *p*H 3.5–4.5.

The presence of pectin esterase in the peel of citrus fruits has made it possible to develop[14a] an efficient method for the manufacture of a series of pectinic acids employing the enzyme *in situ*. By adding alkali to the slurry of peel the reaction of deesterification is allowed to proceed until the *p*H has dropped to 7 and then it is stopped by adding acid to *p*H 3–4. The partially deesterified pectins extracted by this method form very viscous solutions and may find wide usage wherever water-soluble oil-repelling films are desired.

While the formation of a stable gel with ordinary pectin requires the use of approximately 65% sugar and a certain amount of acid, partially deesterified pectin, *i.e.*, pectinic acids, are independent of sugar concentrations or of acid and their jellification is brought about by the addition of calcium or other polyvalent cations. Besides the above treatment with alkali, this partial demethylation of pectin can be made by acids or by the action of the enzyme pectase. Ordinary pectin contains from 9.5 to 11% methoxyl groups (CH_3O), and if it is deesterified to the extent of 3.5 to 6% methyl ester it will give pectinic acids best suitable for preparing gels.

5. Determination of Pectin

Since the composition and the properties of various pectic substances are so different, no ideal method of determination of pectin exists which can be used with sufficient precision. For technical purposes, especially in jam-making, it is important to determine the jelly-forming capacity without differentiating between the chemical composition of the various pectic substances involved; for that purpose a jelly test is used which will be described on page 368. For purposes of general estimation of the pectin-containing material a sufficiently good method is described by Hinton (1939).

(1) ACETONE PRECIPITATION METHOD

To about 100 cc of pectin extract, sufficient acetone is stirred in slowly to make the final concentration of the acetone about 50%. The mixture is allowed to stand for some minutes and is filtered. When the precipitate has been drained fairly free from liquid, it is washed back into the beaker and redissolved in 100 cc of cold water, warmed if necessary, cooled, and reprecipitated with acetone as be-

[14a] Owens, H. S., R. M. McCready, and W. D. Maclay, "Enzymic Preparation and Extraction of Pectinic Acids," *Ind. Eng. Chem.*, **36**, 936 (1944).

fore. The precipitate is filtered off on a tared ashless filter, washed with 60% acetone, and dried to constant weight overnight in an oven at 100° C. For more exact results, the dried filter and precipitate, after being washed, are ashed and weighed again. The difference in weight is the acetone precipitate regarded as pectin.

Results by this method may occasionally be slightly high, owing to the occlusion of nonpectic substances precipitated by acetone.

A similar determination is described by the Association of Official Agricultural Chemists, using 96% alcohol instead of acetone.

(2) ESTIMATION AS CALCIUM PECTATE (METHOD OF CARRÉ AND HAYNES)[15]

The pectin-containing solution is precipitated with four times its volume of alcohol, containing the amount of HCl required to make the resulting mixture 0.1 *N*. After standing overnight, the precipitate is filtered, washed once with acidified alcohol, and dissolved off the filter paper with hot water. It is then saponified with an excess of alkali, acidified with 1.0 *N* acetic acid, and precipitated by the addition with constant stirring of first 0.2 *N*, then 1.0 *N*, calcium chloride solution. The mixture is boiled for 2 minutes, filtered on hard paper, and the precipitate washed with boiling water. The precipitate is then washed back into the beaker with water, boiled again for 2 minutes, and filtered on a fritted-glass crucible. The precipitate is washed with hot water, and finally with alcohol. It is then dried at 100° C and weighed. In pure pectins the yield of calcium pectate is usually about 10% over the weight of the pectin used in the determination.

(3) ESTIMATION OF METHOXYL GROUPS

For the determination of the methoxyl content of pectins the accepted Zeisel method[15a] is used consisting of a volumetric determination of the alkyl iodide formed by the action of hydriodic acid on methoxyl and ethoxyl groups. Recently, however, it has been shown[15b] that the methoxyl content measured by the above method is as much as 20% higher than the true values due to retained alcohol in the pectins. A saponification method has, therefore, been proposed consisting in dissolving 1 gram of pectin (moistened with a little alcohol) in 300 cc of water in a 500-cc Erlenmeyer flask and

[15] Carré, M. H., and D. Haynes, "The Estimation of Pectin as Calcium Pectate and the Application of This Method to the Determination of the Soluble Pectin in Apples," *Biochem. J.*, **16**, 60 (1922).

[15a] Clark, E. P., *J. Assoc. Official Agr. Chem.*, **15**, 13 (1932).

[15b] Jansen, E. F., S. W. Waisbrot, and E. Rietz, "Errors in the Zeisel Methoxyl Values for Pectin Due to Retained Alcohol," *Ind. Eng. Chem.*, **16**, 523 (1944).

titrating the pectin solution with 0.1 *N* sodium hydroxide using the Hinton indicator (a mixture of 3 parts phenol red to 1 part each of bromothymol blue and cresol red). After adding 20 cc of 0.5 *N* sodium hydroxide the flask is stoppered and the reaction mixture allowed to stand for 2 to 3 hours at room temperature, whereupon 20 cc of 0.5 *N* hydrochloric acid is added and the solution back-titrated with 0.1 *N* sodium hydroxide. This last titer corresponds to the saponification value.

C. GLUCOSIDES

1. General Characteristics of the Group

In addition to the components of the citrus albedo enumerated thus far, another class of compounds, occurring in only minor quantities within the mesocarp, have recently attained considerable biological importance, although they have been known since 1771. These are the glucosides—hesperidin, naringin, and a few others—some of which are responsible for the bitter taste in citrus juices.

Although the glucosides are present in the juice and seeds of the endocarp as well as in the albedo, their principal location is in the carpellary membranes, at the boundary between the albedo and the juice segments.

Glucosides, which are generally very widely distributed in nature, are derivatives—chiefly ethers—of glucose, combined with an extraordinary variety of hydroxy compounds such as alcohols, phenols, etc. On boiling with dilute acids, the glucosides are easily hydrolyzed, forming glucose and the corresponding hydroxy compound called *aglucone*.

Hall,[16] who investigated the glucosides of the navel orange, believes that the glucosides create, with the sugars in the plant, a glucose-glucoside complex, which may serve as a medium for translocation of the carbohydrates synthesized in the chlorophyllous tissue. He advances the hypothesis that, in combination with the phenolic glucosides, glucose forms a soluble, easily hydrolyzable compound and is thus temporarily withdrawn from the metabolism until it is brought to that portion of the plant where it is stored or utilized.

Whether the bitter taste in citrus juices on standing is caused by hydrolysis of the glucosides or simply by prolonged diffusion of the glucosides from the bits of pulp and cellular tissue into the juice, is still an open question. There is reason to believe that the increased

[16] Hall, J. A., "Glucosides of the Navel Orange," *J. Amer. Chem. Soc.*, **47**, 1191 (1925).

bitterness is neither caused by oxidation (citrus juices extracted early in the season and preserved with so strong an antioxidant as SO_2 also develop a bitter taste on storage) nor could an enzymatic action be responsible for it (pasteurization which is sufficient to inactivate the enzymes does not prevent the increase of the bitterness). On the other hand one might think that the bitter glucosides, which in themselves are very slightly soluble in water, diffuse slowly from the bits of carpellary membranes into the juice, thus causing the increase of bitterness only some time after the juice has been extracted.

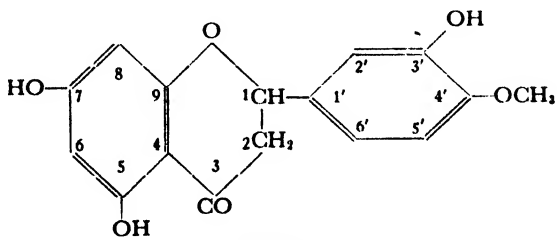
2. Description of the Principal Species of Glucosides

(1) HESPERIDIN

The peel of the unripe orange, as well as of all other citrus fruits with the exception of grapefruit (*Citrus decumana*), is known to contain a glucoside, hesperidin [$C_{28}H_{34}O_{15}$ ($+H_2O$?)], which is a flavanone derivative, 5,7,3′-trioxy-4′-methoxyflavanone-7-rhamnoglucoside; and its sugar portion consists of one molecule of glucose and one of rhamnose. The structural formula of the flavanone alone is given below. The fact that the sugar portion is connected to carbon atom 7 has been demonstrated by King and Robertson.

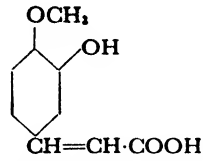
To prepare hesperidin, cover macerated oranges with dilute alcohol, neutralize with KOH, and add some KOH in excess. Filter after two days and precipitate crude hesperidin with HCl. Boil the precipitate with acetic acid for ten minutes; filter after cooling. On standing, the hesperidin gradually crystallizes in white odorless needles (m.p. = 252° C). A yield of about 200 g of hesperidin has been obtained from one ton of fruit.

Hesperidin is soluble in water with difficulty, is more soluble in alcohol, and gives a pronounced yellow coloration when dissolved in alkali. It does not reduce Fehling's solution and is not appreciably fermented by yeast. On heating with dilute H_2SO_4, hesperidin is hydrolyzed into glucose, rhamnose, and the flavanone hesperetin, which crystallizes in plates melting at 227° C:



Hesperetin

Hesperetin on decomposition gives phloroglucinol and hesperitic acid (or isoferulic acid), m.p. = 228° C:



Hesperitic acid

Tanret[17] found in the albedo of the bitter orange (*Citrus aurantium* Linn.) isohesperidin, a crystalline glucoside isomeric with hesperidin, and aurantiamarin, another glucoside to which, in part, the bitterness of the peel is due.

(2) CITRIN (VITAMIN P?)

Citrin is a mixture of the flavanones hesperidin and eriodictin (eriodictyol), which is hesperidin after demethylation: 5,7,3′,4′-tetraoxyflavanone.[18]

Hesperidin and citrin have received considerable attention since 1938 when A. Szent-Györgyi showed that the citrus flavanones have a beneficial effect on the maintenance of normal conditions in the walls of the small blood vessels, the capillaries, controlling permeability. H. v. Euler injected citrus flavanones in a guinea pig suffering from scurvy and obtained a pronounced increase of erythrocytes in the blood; in general, the antiscorbutic results were even better than when vitamin C alone was used. Comparing these effects with those influenced by vitamins, Szent-Györgyi suggested that hesperidin and citrin be given the title of vitamin P (permeability vitamin). Recently, however, the potency of pure citrin has become very much doubted (see pages 152–155).

(3) NARINGIN

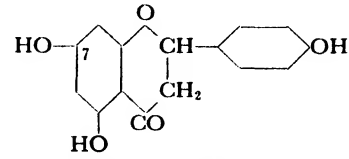
Naringin ($C_{27}H_{32}O_{14} \cdot 8H_2O$) (from the Sanskrit word "naringi" for orange) is a lemon-yellow, crystalline glucoside, the bitter principle characteristic of grapefruit; it has not been found in the flowers or fruit of any other variety of citrus.

This glucoside was discovered by de Vry in 1857 in the flowers of

[17] Tanret, C., "Sur quelques principes immédiats de l'écorce d'orange amère," *Compt. rend.*, **102**, 518 (1886).

[18] Tutin, F., "The Constitution of Eriodictyol, Homoeriodictyol, and of Hesperitin," *J. Chem. Soc.*, **97**, 2054 (1910).

grapefruit trees in Java. When boiled with dilute mineral acids, it hydrolyzes and yields glucose, rhamnose, and naringenin (5,7,4′-trihydroxyflavanone):



Naringenin ($C_{15}H_{12}O_6$)

Naringenin, when boiled with KOH, forms phloroglucin and *p*-coumaric acid. As in the case of hesperidin, naringin is a 7-rhamnoglucoside, i.e., the sugar portion is attached to the seventh carbon atom of the flavanone.

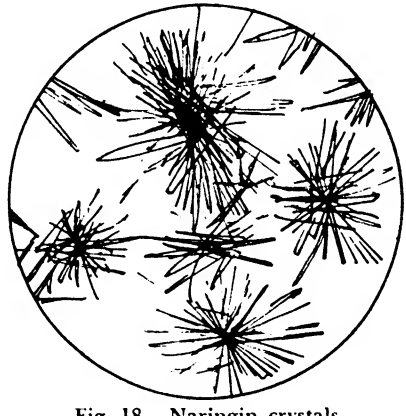


Fig. 18. Naringin crystals.

As the grapefruit ripens, or when it is stored, the naringen content appears to diminish in both the peel and the juice. Naringin is soluble in alcohol, acetone, and hot water, but in the last only to the extent of about 1 part in 2,000 parts of water at 20° C. The bitterness of naringin is very strong and is greater than that of quinine: it can be detected even in a dilution of 1:50,000. When dried at 110° C it melts at 171° C, but when crystallized from water it has six additional molecules of water and then melts at 83° C. Naringin dissolved in ethyl alcohol is levorotatory: $[\alpha]_D^{18°} = -65° 2'$.

Although naringin has not yet been commercially produced to any great extent, its manufacture will be described further in the discussion of citrus pectin (pages 369–370).

One of the effects of freezing citrus fruits in California is the appearance of naringin crystals inside the frozen grapefruit[19] (Fig. 18).

(4) LIMONIN AND ISOLIMONIN

The bitter principles of the Valencia and navel oranges have been isolated and identified by Higby.[20] In naval oranges, he identified isolimonin ($C_{26}H_{30}O_8$)—m.p. = 507.2° F (264° C)—which is optically active and contains one lactone group. From Valencia oranges, he isolated limonin—m.p. = 554° F (290° C)—which contains two lactone groups.

The degree of bitterness in the navel orange decreases with maturity: extraction of the albedo of a completely mature navel orange with acetone gives, according to Higby, an extremely heavy yield of hesperidin but no bitter substance. Hence, disappearance of the bitter substances in the fruit can be determined by acetone extraction.

Both isolimonin and limonin are apparently present in the albedo in a nonbitter, water-soluble state; in this form they are liberated to the juice during extraction. Subsequently, they slowly hydrolyze in the acid juice to form the bitter lactone. A bitter taste can be detected in the juice of immature fruit within a half-hour at room temperature, but this period increases to several hours as the fruit becomes more mature.

Seeds of *Citrus decumana* also contain neolimonin ($C_{26}H_{30}O_8 \cdot 2C_2H_5OH$)—m.p.[21] = 240–242° C—as well as limonin and isolimonin.

(5) CITRONIN

Citronin, a glucoside found by Yamamoto and Oshima[22] in the peel of *Citrus limonum*, yields upon hydrolysis citronetin ($C_{16}H_{14}O_5$), 5,7-dioxy-2′-methoxyflavanone—m.p. = 224° C.

A convenient colorimetric method using alkaline diethylene glycol for the determination of flavanones in citrus fruits has been recently described by Davis.[22a] This method which is applicable to the assay of naringin in the juice and the peel of grapefruit, and of hesperidin in other citrus fruits, has been used to determine the distribution of flavanones in the various tissues of citrus fruit and to follow the

[19] Webber, H. J., "A Study of the Effects of Freezes on Citrus in California," *Calif. Agr. Exp. Sta. Bull. No. 304*, 245 (1919).

[20] Higby, R. H., "The Bitter Constituents of Navel and Valencia Oranges," *J. Amer. Chem. Soc.*, **60**, 3013 (1938).

[21] Mookerjee, Asima, "Bitter Principles of *Citrus decumana*," *J. Indian Chem. Soc.*, **17**, 593 (1940).

[22] Yamamoto, R., and Y. Oshima, *J. Agric. Chem. Soc. Jap.*, **7**, 312 (1931).

[22a] Davis, W. B., "Determination of Flavanones in Citrus Fruits," *Ind. Eng. Chem., Analyt. Ed.*, **19**, 476 (1947).

course of glucoside hydrolysis. Using this method Davis found the following amounts of flavanones in various citrus fruits:

Citrus fruit	In fresh juice (per 100 cc)	In the albedo (per 100 g fresh wt.)
Grapefruit	0.041 mg of naringin	2.100 mg of naringin
Lemon	0.054 " " hesperidin	3.000 " " hesperidin
Orange	0.118 " " "	1.600 " " "
Tangerine	0.065 " " "	1.700 " " "

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CHAPTER IV

THE ENDOCARP

A. ORGANOLEPTIC ASPECTS OF CITRUS JUICES

1. Morphology of the Endocarp

Within the citrus fruit, beneath the enclosing flavedo and albedo, lies the principal edible portion, consisting of segments (carpels, locules) distributed around a soft pithy core which forms the central axis. The axis is of the same composition as the albedo. Each of the segments is enveloped in a thin carpellary membrane (locular wall) —a tissue of epidermal origin. Closely compacted within the segments and attached to the walls with small hairlike papillae lie the club-shaped, extremely thin-walled, multicellular vesicles which contain the juice. They comprise the endocarp of citrus fruits.

2. Color of Citrus Juices

The juices of some citrus fruits, such as lemon, grapefruit, lime, and bergamot, are yellowish green, while others, such as orange and tangerine, are orange to red in color. Each juice cell contains the color-bearing chromoplasts. The colors of citrus juices are in the main produced by the two carotenoids, carotene and xanthophyll, mentioned in the discussion of the flavedo (page 29). The ratio of these two carotenoids determines the color of the juice, which becomes a deeper orange with increased xanthophyll content.

Taylor and Witte[1] tested 164 samples of orange juice and found that the carotene content ranged from 0.34 to 1.65 mg per liter. In these tests the authors evaporated 50 cc of juice in a 300 cc Erlenmeyer flask with circulating air on a steam bath, and the carotene was extracted by the Guilbert[2] method, using 100 cc alcoholic KOH solution for the saponification. The final petroleum ether solutions were immediately compared in a Duboscq colorimeter with a dye standard, using 0.036% solution of potassium dichromate, equivalent

[1] Taylor, A. L., and P. J. Witte, "Carotene in Oranges," *Ind. Eng. Chem., Ind. Ed.*, **30**, 110 (1938).

[2] *Ind. Eng. Chem., Analyt. Ed.*, **6**, 452 (1934).

to 2.66 mg of carotene per liter. The amounts of carotene found in the tested juices were found to be:

	Number of Samples	Average
California Valencias	14	1.65 mg
California Washington Navels	68	1.07 mg
Florida Valencias	34	0.57 mg
Florida pineapple oranges	32	0.34 mg

The pink color of certain varieties of grapefruit and the reddish color of blood oranges are due to pigments which are not in the chromoplasts but are chiefly in solution in the cell sap. The pigment of blood oranges has been shown to be an anthocyanin compound which is probably genetically bound to hesperidin.

According to Matlack,[3] the pink color of the Foster and Marsh varieties of grapefruit is due to lycopene and β-carotene, while the expressed juice of the blood orange contains beautiful red to reddish-brown spherical and needle crystals.

From a study of the coloring matter of lime juice, Hardy and Warneford[4] believe that it contains a polyhydroxyphenolic compound or compounds resembling the tannins or anthoxanthins, for it gives a conspicuous color reaction with ferric chloride and produces color changes on oxidation.

Juices from different varieties of the same species of *Citrus* possess quite different shades of color, which to some extent is also influenced by the country of origin; for instance, the juice of the Valencia orange, grown in California, is a different shade of orange from that of Spain or Palestine. Color of the juice is subject to alteration also as a result of changes (which will be discussed in detail on page 271) during storage, or through the addition of various preservatives. It has been suggested,[5] therefore that the color of concentrated or diluted citrus beverages be stabilized by the addition of approximately 0.01–0.05% of a quinoid compound (such as naphthol-yellow S). It is claimed that both natural and added color then become stable.

3. Flavoring Constituents of the Juices

The nature of the components responsible for the characteristic aroma of freshly expressed citrus juices appears to be due to certain

[3] Matlack, M. B., "Observations on the Red Color of the Blood Oranges," *Plant Physiol.*, **6**, 729 (1931); "Pigments of Pink Grapefruit," *J. Biol. Chem.*, **110**, 249 (1935).

[4] Hardy, F., and F. H. S. Warneford, "The Coloring Matter of Lime Juice," *Ind. Eng. Chem.*, **17**, 48 (1925).

[5] Higby, R. H. (assignor to California Fruit Growers Exchange), "Stabilized Citrus Beverage and Syrup," U. S. Patent No. 2,005,786 (June 25, 1935).

substances within the juice which are distinctly different in composition from the essential oils of the corresponding peel.

Attention has been drawn by Davis[5a] to oil deposits in the juice sacs of most citrus varieties. These deposits in the form of glistening yellowish oil globules can be easily detected macroscopically with a magnification of 10 to 16 diameters. In some varieties of oranges, such as Washington Navel, Valencia, and the King orange, these oil deposits may also be observed even without a lens. In lemons and limes they are relatively less abundant and, being smaller in size, they are likely to be lost sight of. No oil deposits have been found in citrons.

Hall and Wilson[6] made an extensive study of the aroma of Valencia orange juice by distilling a total of approximately 40 tons (!) of juice. They obtained 182 g of oil, which is equivalent to 4.4 parts per million of the flavoring of juice by weight. They found that this flavoring oil contains volatile constituents which are very soluble in water: ethyl alcohol, acetone, acetaldehyde, and formic acid, and constituents which are less soluble in water: an olefin alcohol, $C_{10}H_{18}O$ (representing 90% of the portion insoluble in water), an amyl (probably isoamyl) alcohol, phenylethyl alcohol, and esters of formic, acetic, and caprylic acids. Geraniol and terpineol were indicated but were not positively identified.

Further research is necessary to determine the flavoring constituents of various citrus juices, but it is evident from the foregoing that they are present in only very minute quantities, and this factor, of course, causes great difficulty in their investigation. The problem is further complicated by the rapid change in flavor manifested by most citrus juices after removal from the fruit.

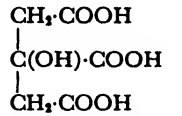
4. Acidity of Citrus Juices

Citrus fruits are regarded as acid fruits, in contrast to numerous nonacid fruits, such as bananas or pears. The acidity of citrus juices is due chiefly to the presence of citric acid, which is one of the most widely distributed plant acids. It was originally discovered in lemon juice, its chief source, by Scheele in 1784; it occurs, however, in many other fruits, such as red currants, cranberries, pineapples, grapes, etc., and is also present in the human and animal organisms.

[5a] Davis, W. B., "Deposits of Oil in the Juice Sacs of Citrus Fruits," *Am. J. Botany*, **19**, 101 (1932).

[6] Hall, J. A., and C. P. Wilson, "The Volatile Constituents of Valencia Orange Juice," *J. Amer. Chem. Soc.*, **47**, 2575 (1925).

Citric acid ($C_6H_8O_7 \cdot H_2O$) has the following structure:



Citric acid

Having no asymmetric carbon atom, citric acid is not optically active. Evidently it is a tribasic acid and is very soluble in water. It is known in the anhydrous form, melting at 153° C, and crystallizing with one molecule of water; the hydrated form melts at 100° C.

Citric acid can be prepared synthetically; however, the existing methods have attained no commercial importance, and citric acid continues to be manufactured in Sicily, California, Hawaii, the West Indies, and Palestine from cull lemons by precipitating it as a calcium salt and then decomposing the citrate of lime with H_2SO_4:

$$Ca_3(C_6H_5O_7)_2 + 3\,H_2SO_4 \longrightarrow 2\,C_6H_8O_7 + 3\,CaSO_4$$

However, during the last twenty years a mycological method has been elaborated, starting with materials containing carbohydrates (glucose, maltose, dextrins) and fermenting them to the extent of 50% or more into citric acid by the action of certain molds (*Citromyces*), such as *Aspergillus niger*. The methods using the molds will be described in detail later.

The presence of other organic acids in citrus juices has been indicated by several investigators. Nelson[7] reported traces of malic acid in orange juice, and Hartman and Hillig[8] found appreciable amounts of it in Florida Valencia oranges. Investigating the organic acids in Palestinian grapefruit, Menchikowsky and Popper[9] found that although the organic acids of the juice consist largely of citric acid (98.72%), they contain also malic (1.00%), oxalic (0.23%), and tartaric (0.05%) acids (percentage of the total amount of acids).

The total acidity of citrus juices is usually expressed as citric acid with one molecule of H_2O (mol.wt. 210). Table X gives the variation of acidity in these fruits.

The total titratable acidity of citrus juices and its relation to the total solids will be discussed in the section on maturity tests (pages

[7] Nelson, E. K., "The Acids of Fruits," *Am. Med. J.* (New Series), **23**, No. 11, 812 (1928).

[8] Hartman, B. G., and F. Hillig, "Acid Constituents of Food Products," *J. Ass. Offic. Agr. Chem.*, **17**, 522 (1934).

[9] Menchikowsky, F., and S. Popper, "Organic Acids in Palestinian Grapefruit," *Hadar*, **5**, 181 (1932).

TABLE X

Range of Acidity in Citrus Fruits

	Minimum %	Maximum %	Average %
Grapefruit			
Florida	0.70	2.43	1.42
California	0.85	2.64	1.77
Arizona	1.24	1.92	1.61
Palestine	1.82	2.44	2.13
Oranges			
U. S. (all varieties)	0.39	1.00	0.68
California	...	...	1.23
Florida	...	...	1.11
Palestine	0.70	1.5	1.2
Lemons			
California	4.20	8.33	5.96
Palestine	5.56	7.25	6.40
Limes	6.10	8.32	7.20

172–175), in conjunction with the selection of fruit for industrial purposes.

As the ripening of the fruit progresses, the percentage of acid in the juice diminishes. However, the fruit continues to grow and its amount of juice increases. One can, therefore, suppose that the quantity of citric acid in the fruit may be constant, and that its percentage in the juice seemingly diminishes because of the increase in the quantity of juice itself. For example, a lemon containing at beginning of the season, 30% juice of 7% acidity—i.e., 2.10 g acid per 100 g fruit—will, at the end of the season, contain approximately 35% juice of only 6% acidity—i.e., 2.1 g acid per 100 g fruit—or the same amount as earlier in the season. This apparent constancy of the absolute amount of acid is, however, very difficult to ascertain, for no means of making all these tests on the identical fruit throughout the season exists, since the specimen is destroyed in testing. This view is supported by Sinclair and Eny[9a] in a recent study on organic acids of grapefruit juice. Their results show that the decrease of the free-acid concentration in grapefruit during development is due chiefly to the increase in size of fruit rather than to a change in the absolute amount of free acid per fruit.

Sinclair and Ramsey[10] tested samples of orange juice from fruit collected in California monthly for seven months, October to May, during ripening and found that the maximum amount of free acid

[9a] Sinclair, W. B., and Eny, D. M., "Organic Acids of Grapefruit Juice," *Plant Physiol.*, **21**, 140 (1946).

[10] Sinclair, W. B., and R. C. Ramsey, "Changes in the Organic Acid of Valencia Oranges During Development," *Botan. Gaz.*, **106**, 140 (1944).

changed little during the entire period, being 1.1 to 1.5 g, as citric acid, per fruit. The concentration of free acids decreased, of course, from 3.99 to 1.72 g citric acid per 100 cc juice, and from 0.17 to 0.14 g malic acid per 100 cc juice.

The total acidity of citrus juices is usually determined by titrating 10 cc of the strained juice with 0.1 *N* NaOH in the presence of phenolphthalein as indicator, using 210/3 = 70 as factor (citric acid being a tribasic acid), the result representing "total acidity expressed as citric acid" with water of crystallization.

The quantitative determination of citric acid in the presence of tartaric, malic, or other organic acids is described in the 1945 edition of the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (Washington, D.C.).

5. The Active Acidity—*p*H

The acidity of citrus juices measured by the titration method or gravimetrically gives no indication as to the active acidity of the juice, which depends largely on the ionic dissociation of the acids present. It is a well-known fact that acids as well as alkalis and various salts when in solution dissociate into their ions. All acids dissociate to a greater or lesser extent to give free hydrogen ions (H^+), and alkalis to give hydroxyl ions (OH^-).

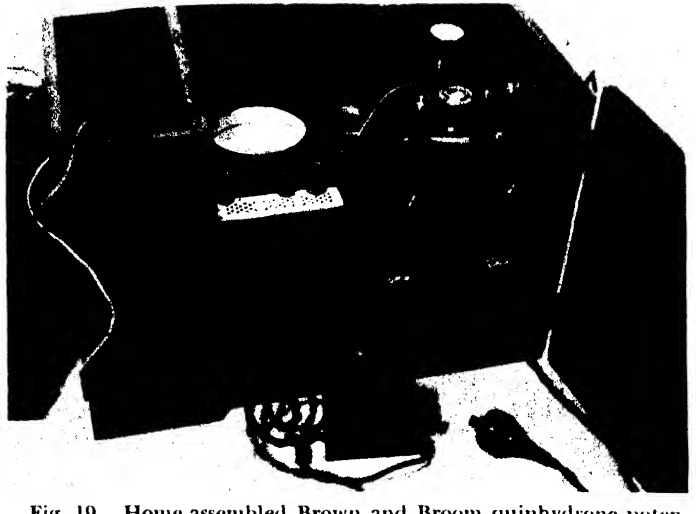


Fig. 19. Home-assembled Brown and Broom quinhydrone potentiometer for *p*H determination. Other more modern *p*H meters (with glass electrodes) can be seen in current laboratory supply catalogues.

The hydrogen-ion concentration is now universally designated by the sign *p*H, which is the logarithm of the reciprocal of the concentration. For the determination of *p*H values in practice, two methods are in general use—namely, the colorimetric and the electrometric methods. The colorimetric method consists of comparing the solution, to which suitable indicators are added, with solutions of standard *p*H, or by using specially prepared paper slips. More exact determinations are made with a potentiometer. (The potentiometer illustrated in Figure 19 makes use of quinhydrone and is suitable for the determination of *p*H in acid solutions such as citrus juices It has been described by H. C. Brown and J. C. Broom.[11])

When the *p*H value is determined electrometrically, the reading is often made in millivolts, one *p*H being equal to 57.7 millivolts at 18° C. (The temperature correction for each degree centigrade is 0.2 millivolts; hence, at 20° a *p*H value of 1 is equal to 58.1 millivolts.) Citrus juices usually possess a yellow to orange color which renders the colorimetric determination of *p*H difficult. Also, since the pulp is not easily filtered, the *p*H is much more conveniently determined using the potentiometer.

Certain solutions possess the power to resist a change in *p*H when a liquid of another *p*H is added to them in small amounts; this power is denoted by the term *buffer action.* Mixtures of acids and their alkaline salts possessing this power are called *buffers.* A large number of substances, which are described as *amphoteric,* and have an important function in biology, are able to dissociate in various ways, yielding, under certain conditions, H^+ ions and, under others, OH^- ions. Proteins, for example, are amphoteric and can dissociate according to either of the general equations:

$$HROH \rightleftharpoons HR + OH^-$$
$$HROH \rightleftharpoons ROH + H^+$$

depending strictly on the reaction of the medium: the acidic dissociation manifests itself more strongly if the medium is relatively poor in H^+, while the basic dissociation predominates if the medium is acid. When such amphoteric substances or "buffers" are present in a solution they resist the acidity by means of their basic dissociation, that is, by liberation of OH^- ions and vice versa.

Fruit juices in general, and citrus juices in particular, are strong buffers. Thus, the *p*H of citrus juice will show but little change if

[11] Brown, H. C., and J. C. Broom, "A Portable Apparatus for Determination of *p*H," *Trans. Roy. Soc. Trop. Med.,* **23,** No. 2 (1929).

diluted with water even to the extent of 50%, notwithstanding that the total titratable acidity of the juice will thereby be reduced by one-half. Fruit juices vary considerably in their buffering effect. Also if various fruit juices are blended, the resultant hydrogen-ion concentration cannot be predicted with any degree of accuracy.[12]

The *p*H of citrus juices changes only very slightly during ripening: Valencia orange juice (U.S.A.), for instance, changes from 2.72 in October to 3.11 in May; Palestine orange juice, from 3.10 to 3.80.

In a recent study Sinclair and Eny[12a] showed that the buffer system of lemon juice is similar to that of citric acid–citrate solutions of equivalent concentrations. Systems in which the ratio of free acid to salt is 1:1 show on dilution the smallest change in *p*H. In lemon juice, however, which contains a higher ratio of free acid to salt, the changes in *p*H on dilution are relatively large. The buffer capacity of lemon juice is not affected by heat or by the addition of sucrose in concentrations as high as 50% by weight.

6. The Sour Taste

While on the subject of acidity it may, perhaps, be appropriate to discuss a question of much importance to the producer of citrus beverages. The problem is very complicated: it should be considered less from the chemical and more closely from the physiological and psychological points of view.

Of the five senses in man's possession, those of taste and smell are probably the least exact; at least, they are not easily measurable. Furthermore, these two senses are so closely allied that it is difficult to ascertain which is affected by a given stimulus.

Of the two, the sense of smell is the more selective and is capable of greater variations. It is generally supposed that we judge our food more by its odor than by its taste, for during swallowing, the passage to the respiratory channel is closed by the epiglottis (little tongue), and immediately after swallowing, a stream of air is expelled, which, as it passes the olfactory nerve, carries the odor and thereby permits us to discern the flavor.

Odor and flavor are, therefore, interconnected and at the same time closely related to the sense of sight. By means of simple ex-

[12] Pederson, C. S., and H. G. Beattie, "Buffering Effect of Fruit Juices," *Food Research*, 8, 405 (1943).

[12a] Sinclair, W. B., and Eny, D. M., "Stability of the Buffer System of Lemon Juice," *Plant Physiol.*, 21, 522 (1946).

periments it is possible to show, for instance, the difficulty of judging food in darkness. A similar factor is involved as one watches a lemon being cut; the mouth of the onlooker tends to water. Much of this lies, however, in the province of psychology, which is scarcely within the scope of this book.

In general, a person is able to discern four distinct categories of taste: sweet, sour, bitter, and salty. Variations of these four depend more on the sense of odor than on that of taste. We can taste a material only when it is in solution; it is, therefore, obvious that without the sense of odor we could scarcely discern such food as fats, proteins, etc. Of the four categories of taste, we shall concern ourselves here with the sour taste only, and even that solely from the chemical point of view.

The compounds which give us the perception of sourness in foods are mainly the organic acids: citric, lactic, tartaric, malic, malonic, oxalic, acetic, etc., as well as phosphoric acid and the acid salts.

There are three main methods for comparing solutions for their acid taste: (*1*) comparing very weak acid solutions so that they barely differ from distilled water (threshold method), (*2*) comparing solutions of equal taste but differing in concentration, and (*3*) comparing solutions of equal concentration in a scale of different tastes.

The main difficulty in all of these methods is the fact that the personal factor must be considered, and tests must consequently be made on a great number of individuals. The old proverb *De gustibus non est disputandum* appears to hold strongly in this case. The difficulty is still greater when we deal in higher concentrations. Whereas in tasting weak acids our selectivity may appear sufficient, it is of no practical use for acids of greater concentrations: we can, for example, easily distinguish between juice of about 1% acidity and of 1.5%, but we can scarcely discern between two solutions of 25% and 30% acidity. It is, therefore, obvious that in studying this effect we must work with weak solutions only.

Most of the physicochemical properties of a substance depend largely on its chemical structure. Just as color depends on constitution, so do particular flavors depend on certain chemical radicals. The groups OH (in carbohydrates) and NH_2 (in saccharin), for instance, together with other complex radicals, are responsible for the sweet taste. Characteristic of the acid taste is the hydrogen ion H^+, when the substance is in a state of electrolytic dissociation.

Until fifty years ago it was thought that the influence of the dis-

sociated hydrogen ion is the only factor affecting the taste glands at the sides of the tongue. However, Richards[13] showed in 1898 that while qualitatively this is correct, from the quantitative point of view the hydrogen-ion concentration could not be the sole decisive factor concerned in the sour taste. It is true that mineral acids, whose degree of dissociation is much greater than that of the organic acids, are more sour to the palate. Furthermore, when the acid is neutralized, the sour flavor disappears; so complete is this disappearance that one could use the taste as an exact indicator for titrating an acid and for determining the end point with considerable precision.

However, in comparing the sour taste of acids with their dissociation constants quantitatively, one faces many inconsistencies. Kastle,[14] for instance, has shown that a solution of acetic acid will taste more sour than an HCl solution of the same molar concentration, although the hydrogen-ion concentration of the HCl is higher. In other words, various acid solutions of equally sour flavor will not possess equal concentrations of hydrogen ions, as would be expected. If, on the other hand, acid solutions of equal *p*H are compared, organic acids will generally taste more sour than inorganic acids. Such examples are numerous in actual practice in the kitchen or in the food industry.

It is a well-known fact that the juice of an orange at the beginning of the season tastes considerably more sour than that of a ripe orange, although the corresponding *p*H of both juices differs but little (at most from 2.9 to 3.2). Not only in this case, where acidity is masked by the increased sugar content, but also in the case of juices of different varieties with practically the same *p*H and similar percentages of sugar, the taste may be quite different if compared at the same time in the season. Harvey and Fulton[15] tested 30 varieties of tomatoes whose *p*H fell between 4.15 and 4.5 but whose taste differed enormously.

The fact that the addition of sugar to an acid solution changes its taste without changing its *p*H indicates that the hydrogen-ion concentration is not the only factor affecting the sour taste. Moreover, as shown above, the *p*H of fruit juice will not materially change even if considerably diluted with water, although it is obvious that such

[13] Richards, T. W., "The Relation of the Taste of Acids to Their Degree of Dissociation," *Amer. Chem. J.*, **20**, 121 (1898).

[14] Kastle, J. H., "On the Taste and Affinity of Acids," *Amer. Chem. J.*, **20**, 466 (1898).

[15] Harvey, R. B., and R. R. Fulton, "Relation of *p*H and Total Acidity to the Taste of Tomatoes," *Fruit Prod. J.*, **14**, 238 (April, 1935).

diluted juice will taste less sour. Richards tried to explain these discrepancies by suggesting that the very act of tasting removes the dissociated part of the acid and so leaves room for further dissociation. This might be the reason why weak organic acids taste more sour than mineral acids of the same *p*H.

Recently, other investigators have obtained additional data showing that the sour taste of acids is due also to factors other than free hydrogen ions. Bremond[16] has shown that the sour taste of wines is determined more by total acidity than by *p*H: on aging, wines deposit

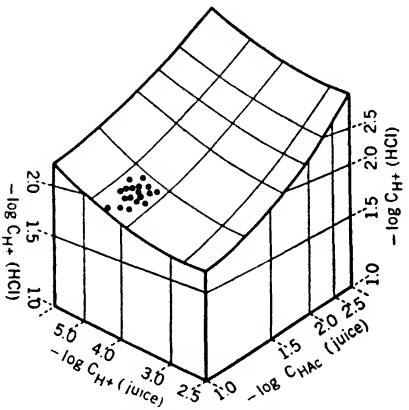


Fig. 20. Three-phase graph showing the relation of *p*H and total acidity to the taste of solutions (according to Harvey).

a precipitate of cream of tartar, thus reducing their total acidity and degree of sourness without materially changing the *p*H value. Other investigators have looked for a different manifestation of the sour taste. Renquist is of the opinion that a definite relation exists between the coefficients of diffusion of various acids, and that there is some connection between the sour taste and the surface tension of acid solutions. Apparently, surface tension plays a definite role in this respect in "aromatic" acids such as acetic or tartaric.

Harvey,[17] in making an extensive study of the acid taste in fruit juices, has chosen hydrochloric acid as a standard for comparison; in a concentration of 0.025 *N*, this acid is harmless to the taste and is

[16] Bremond, E., "Contribution a l'Étude Analytique et Physico-chimique de l'Acidité des Vins," Algeria (1937).

[17] Harvey, R. B., "The Relation between the Total Acidity, the Concentration of Hydrogen Ion and the Taste of Acid Solutions," *J. Amer. Chem. Soc.*, **42**, 712 (1920).

practically completely (97%) dissociated, i.e., the hydrogen-ion concentration practically corresponds to the total acidity.

By choosing various buffers, Harvey obtained solutions of equal *p*H although widely different in total acidity. Lastly, he prepared, from acetic acid and sodium acetate, solutions of equal total acidity and different *p*H. Comparing these solutions with one another and with the chosen standard, and plotting the results of these tests on tridimensional Cartesian coordinates, Harvey found that the sour taste is a function of the two variables, *p*H and total acidity, as shown by the accompanying graph (Fig. 20). Later, Harvey confirmed these results by comparing various fruit and vegetable juices.

Paul[18] and co-workers, using the methods of constants, also compared various acid solutions to HCl and found that on the basis of taste the acids should be arranged in the following series, which does not agree with their dissociation constants:

Compound	K
Carbonic acid [CO_2]	3.04×10^{-7}
Acetic acid [CH_3COOH]	1.82×10^{-5}
Lactic acid [$CH_3CH(OH)COOH$]	1.38×10^{-4}
Hydrochloric acid [HCl]	∞
Tartaric acid [COOH·CH (OH)·CH (OH)·COOH]	9.7×10^{-4}

In this table carbonic acid is the least and tartaric the most sour. Paul, therefore, came to the same conclusion as the workers previously cited —namely, that the sour taste is affected not only by the cations but also by the nature of the anions, as well as by the undissociated molecules of the acid in question.

B. SUGARS AND PECTINS IN CITRUS JUICES

1. Occurrence of Sugars in Citrus Juices

With the exception of the acids, the most important compounds of citrus juices are the sugars. In species such as lemons and limes, acids represent the major part of the solids (7 of the total 9 to 10%); in oranges, on the other hand, sugars constitute the main portion of the solids (approximately 8 or 9 of the total 11 to 12%).

There are wide variations in the sugar content of different varieties of the citrus juices. In some varieties of oranges, the juice is known to contain up to 15% of total sugars, but several varieties grown in the Philippines contain no sugar at all.

[18] Paul, Th., R. Dietzel, and K. Taufel, "Physikalische Chemie der Lebensmittel; VI, Physikalische-chemische Untersuchungen über die Säure Geschmacksempfindung," *Zeitschrift für Electrochemie*, Nos. 21, 22 (1922).

It has been generally acknowledged by many workers in this field that the juice of the stylar half of a ripe citrus fruit usually contains a higher concentration of sugars than that of the stem half. This stem-end to stylar-end sugar gradient becomes manifest as the fruit approaches maturity. The fact that in the peel the sugar gradient is reversed has already been mentioned. In this connection, it is interesting to note that the various segments of a single citrus fruit often differ considerably in their sugar content. Bartholomew and Sinclair[19] recently found that one segment of Valencia orange contained 16.1% soluble solids, while the two adjacent segments contained 13.4 and 15.4%, respectively.

As a rule, the concentration of sugars and other soluble solids is highest in fruits borne on the southern side, less in fruits borne on the northern side, and least in fruits borne on the inside of the tree.

The use of oil sprays and various fumigation materials for controlling citrus pests and diseases results in a noticeable decrease of the sugar content of citrus juices.

During ripening of the fruit the total sugars increase in quantity, as shown in Table XIII.

In a previous section on the sugars in citrus albedo, the chemical structure of the various carbohydrates and their interconnection have been described. The juicy part of citrus fruits contains sugars of the same constitution and more or less in the same proportions.

Table XI, compiled after Chatfield and McLaughlin,[20] presents data on the total sugar content of some varieties of *Citrus:*

TABLE XI

Sugar Content of Some Varieties of *Citrus*

Variety and origin	Min. %	Max. %	Average %
Oranges (U. S., all varieties)	4.5	11.8	8.8
Grapefruit, California	5.4	7.8	6.6
" Florida	5.0	8.4	6.5
Oranges, California	—	—	9.14
" Florida, Valencia	—	—	8.48
" " seedlings	—	—	9.34
Blood oranges, Florida	—	—	8.92
Limes, bitter *(C. aurantifolia)*	0.3	0.6	0.5
" sweet *(C. limetta)*	5.1	7.2	6.0
Grapefruit juice, Florida	4.54	9.66	6.65
" " California	3.38	9.51	7.03
" " Arizona	5.27	8.02	6.69
Lemon juice	1.1	3.6	2.3

[19] Bartholomew, E. T., and W. B. Sinclair, "Unequal Distribution of Soluble Solids in the Pulp of Citrus Fruits," *Plant Physiol.*, 16, 293 (1941).

[20] Chatfield, C., and L. I. McLaughlin, "Proximate Composition of Fresh Fruits," *U.S. Dept. Agr. Circ. 50,* Rev. (1931).

The proportion of sucrose to hexoses is shown in Table XII, compiled from tests made by Roberts and Gaddum.[21]

TABLE XII

Proportion of Sucrose to Hexoses in *Citrus*

Variety	Reducing Sugars %	Sucrose %	Total Sugars %
Grapefruit juice, Marsh seedless	3.44	1.34	4.78
" " seedy variety	3.96	2.24	6.20
Orange juice, seedling orange	4.48	4.86	9.34
" " blood orange	3.95	4.97	8.92
" " Valencia	3.46	5.02	8.48
" " Lue Gim Gong	4.32	4.89	9.21
Tangerine juice	3.09	7.89	10.98

Apparently, in grapefruit the content of reducing sugars (hexoses) is about twice as high as the content of sucrose, in oranges the proportion is about equal, and in tangerines the reducing sugars amount to less than one-half of the sucrose. An interesting feature, however, is that in all varieties mentioned in Table XII the percentage of reducing sugars is nearly always the same.

Other investigators have come to somewhat different conclusions. According to Collison,[22] sucrose usually constitutes little over one-half of the total sugar.

Following results have been obtained by Braverman and Carmi[23] in a preliminary report on the composition of Palestine oranges. Detailed tests on both small- and large-sized fruits picked throughout two seasons from the same trees gave, on the average, the results enumerated in Table XIII.

TABLE XIII

Proportion of Sucrose to Hexoses during the Citrus Season

Month	Small fruits			Large fruits		
	Reducing sugar %	Sucrose %	Total sugar %	Reducing sugar %	Sucrose %	Total sugar %
December	5.0	2.1	7.1	4.9	1.8	6.7
January	4.5	2.8	7.3	4.9	3.0	7.9
February	5.9	3.0	8.9	5.4	3.3	8.7
March	5.6	3.6	9.2	5.2	3.5	8.7

[21] Roberts, J. A., and L. W. Gaddum, "Composition of Citrus Fruits," *Ind. Eng. Chem.*, **29**, 574 (1937).

[22] Collison, S. E., "Sugar and Acid in Oranges and Grapefruit," *Fla. Agr. Expt. Sta. Bull.* **115** (1913).

[23] Braverman, J. S., and A. Carmi, "The Composition of Palestine Oranges," *Hadar*, **10**, 147 (1937).

Table XIII shows that the percentage of total sugars in juice naturally increases as the fruit ripens. It is, however, noteworthy that while the amount of reducing sugars in the juice remains practically unchanged (or increases only slightly), the increase in total sugars is accounted for largely by sucrose. Sucrose, which at the beginning of the season amounts to about 27% of the total sugars, reaches as high as 40% of the total by the end of the season.

It is known, however, that during storage of citrus juices the quantity of sucrose gradually decreases until, after a few months, there is finally no trace of sucrose, while the total quantity of sugars remains the same. This is due, of course, to the inversion of sucrose resulting from the presence of acid in the juice. The inversion depends largely on the *p*H of the juice, but sooner or later the total quantity of the sugars in any citrus juice undergoes inversion. Skillful tests and comparisons in this direction may give a fairly good indication of the age of a preserved or canned citrus juice.

2. Methods of Determination of Sugars in Juices

The quantitative estimation of pure sugar solutions may be accomplished by means of the polarimeter. However, this method is not conveniently employed with citrus juices, which contain a mixture of sucrose and reducing sugars as well as many other substances which may affect the reading of the polarimeter.

Total sugars may also be determined by fermentation with yeast in a specially constructed tube (Fig. 21), measuring volumetrically the carbon dioxide formed during fermentation.

Exact methods of estimation are based on the reaction of reducing sugars with alkaline solutions of metallic salts such as copper salts; oxygen is withdrawn and the metal is precipitated either as such or as a lower oxide. The metallic salt solution most commonly used is copper tartrate, known as Fehling's solution.

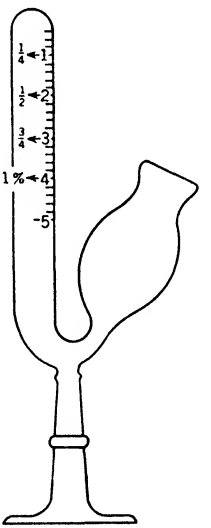


Fig. 21. Fermentation tube for sugar determination.

Since many substances interfere with this test, either by preventing the precipitation of cuprous oxide or by precipitating similar substances that might be mistaken for it, the interfering substances must be removed by clarification with neutral lead acetate.

The excess of lead acetate is thereafter precipitated with potassium oxalate or Na_2CO_3 and the clear filtrate is used for the determination.

Due to the difficulty of determining the exact endpoint, the original Fehling method has been replaced by the generally accepted volumetric estimation of Lane and Eynon,[24] in which methylene blue, an internal indicator, is rapidly decolorized by a slight excess of sugar. The number of mg of Cu reduced by a given quantity of reducing sugar varies, depending upon whether or not sucrose is present, as shown by Table XIV.

(a) Reducing Sugars (Lane-Eynon Method)[25]

REAGENTS. Soxhlet's modification of Fehling's solutions (a) and (b), to be mixed immediately before use:

(a) *Copper sulfate solution.* Dissolve exactly 34.639 g of $CuSO_4 \cdot 5H_2O$ in 500 cc of water and filter through asbestos.

(b) *Alkaline tartrate solution.* Dissolve 173 g of Rochelle salt (K-Na tartrate) and 50 g of NaOH in 500 cc of water, allow to stand two days, and filter through asbestos.

STANDARDIZATION AND METHOD OF TITRATION. Pipet accurately 5 cc of Soxhlet solution (a) and 5 cc of solution (b) into a 300 cc flask. Prepare a standard solution of pure invert sugar of such concentration that more than 15 cc and less than 50 cc will be required to reduce all the Cu. The titer may be calculated as follows:

$$\frac{\text{factor}}{\text{mg sugar in 1 cc}}$$

Add almost the whole of the sugar solution required to effect reduction of all the Cu, so that not more than 0.5 to 1 cc will be needed later to complete titration. Heat the mixture to boiling on a wire gauze, avoiding bumping, and maintain in moderate ebullition for 2 min. Without removing the flame, add 2 to 5 drops of 1% aqueous solution of methylene blue and complete titration by small additions of sugar solution to decolorization of the indicator, total boiling time being about 3 min. To obtain the factor, multiply the titer by the number of mg in 1 cc of the standard solution. Compare with the tabulated factor to determine the correction, if any, applicable to Table XIV.

[24] Lane, J. H., and L. Eynon, "Determination of Reducing Sugars by Fehling's Solution with Methylene Blue Indicator," *J. Soc. Chem. Ind.*, **42**, 32T, 463T (1923).

[25] Condensed from *Methods of Analysis, Association of Official Agricultural Chemists*, 1945.

TABLE XIV

Invert Sugar Table for Ten Cubic Centimeters of Fehling's Solution (Lane-Eynon Method)

Cc of sugar solution required	Solutions containing besides invert sugar:									
	No sucrose		1 g sucrose per 100 ml		5 g sucrose per 100 ml		10 g sucrose per 100 ml		25 g sucrose per 100 ml	
	Invert sugar factor*	Mg invert sugar per 100 cc	Invert sugar factor	Mg invert sugar per 100 cc	Invert sugar factor	Mg invert sugar per 100 cc	Invert sugar factor	Mg invert sugar per 100 cc	Invert sugar factor	Mg invert sugar per 100 cc
15	50.5	336	49.9	333	47.6	317	46.1	307	43.4	289
16	50.6	316	50.0	312	47.6	297	46.1	288	43.4	271
17	50.7	298	50.1	295	47.6	280	46.1	271	43.4	255
18	50.8	282	50.1	278	47.6	264	46.1	256	43.3	240
19	50.8	267	50.2	264	47.6	250	46.1	243	43.3	227
20	50.9	254.5	50.2	251.0	47.6	238.0	46.1	230.5	43.2	216
21	51.0	242.9	50.2	239.0	47.6	226.7	46.1	219.5	43.2	206
22	51.0	231.8	50.3	228.2	47.6	216.4	46.1	209.5	43.1	196
23	51.1	222.2	50.3	218.7	47.6	207.0	46.1	200.4	43.0	187
24	51.2	213.3	50.3	209.8	47.6	198.3	46.1	192.1	42.9	179
25	51.2	204.8	50.4	201.6	47.6	190.4	46.0	184.0	42.8	171
26	51.3	197.4	50.4	193.8	47.6	183.1	46.0	176.9	42.8	164
27	51.4	190.4	50.4	186.7	47.6	176.4	46.0	170.4	42.7	158
28	51.4	183.7	50.5	180.2	47.7	170.3	46.0	164.3	42.7	152
29	51.5	177.6	50.5	174.1	47.7	164.5	46.0	158.6	42.6	147
30	51.5	171.7	50.5	168.3	47.7	159.0	46.0	153.3	42.5	142
31	51.6	166.3	50.6	163.1	47.7	153.9	45.9	148.1	42.5	137
32	51.6	161.2	50.6	158.1	47.7	149.1	45.9	143.4	42.4	132
33	51.7	156.6	50.6	153.3	47.7	144.5	45.9	139.1	42.3	128
34	51.7	152.2	50.6	148.9	47.7	140.3	45.8	134.9	42.2	124
35	51.8	147.9	50.7	144.7	47.7	136.3	45.8	130.9	42.2	121
36	51.8	143.9	50.7	140.7	47.7	132.5	45.8	127.1	42.1	117
37	51.9	140.2	50.7	137.0	47.7	128.9	45.7	123.5	42.0	114
38	51.9	136.6	50.7	133.5	47.7	125.5	45.7	120.3	42.0	111
39	52.0	133.3	50.8	130.2	47.7	122.3	45.7	117.1	41.9	107
40	52.0	130.1	50.8	127.0	47.7	119.2	45.6	114.1	41.8	104
41	52.1	127.1	50.8	123.9	47.7	116.3	45.6	111.2	41.8	102
42	52.1	124.2	50.8	121.0	47.7	113.5	45.6	108.5	41.7	99
43	52.2	121.4	50.8	118.2	47.7	110.9	45.5	105.8	41.6	97
44	52.2	118.7	50.9	115.6	47.7	108.4	45.5	103.4	41.5	94
45	52.3	116.1	50.9	113.1	47.7	106.0	45.4	101.0	41.4	92
46	52.3	113.7	50.9	110.6	47.7	103.7	45.4	98.7	41.4	90
47	52.4	111.4	50.9	108.2	47.7	101.5	45.3	96.4	41.3	88
48	52.4	109.2	50.9	106.0	47.7	99.4	45.3	94.3	41.2	86
49	52.5	107.1	51.0	104.0	47.7	97.4	45.2	92.3	41.1	84
50	52.5	105.1	51.0	102.0	47.7	95.4	45.2	90.4	41.0	82

* Mg of invert sugar corresponding to 10 cc of Fehling's solution.

If the approximate concentration of the sugar in the sample is unknown, proceed by the incremental method of titration. Add to 5 cc of each of Soxhlet solutions (a) and (b) 15 cc of the clarified juice from a burette and heat to boiling over a wire gauze. Boil about 15 sec and add rapidly further quantities of the juice to test until

only the faintest perceptible color remains. Then add 2 to 5 drops of methylene blue and complete titration by adding the testing solution dropwise. Find the factor corresponding to the titer in the accompanying table and apply the correction previously determined. Estimate as follows:

$$\frac{\text{factor} \times 100}{\text{titer}} = \text{mg of reducing sugars in 100 cc juice.}$$

(b) Total Sugars

Since sucrose has no reducing power, it is, of course, necessary first to convert it to reducing sugars by means of inversion.

To do this, pipet 50 cc of the lead-free filtrate into a 100 cc graduated flask and add 25 cc of H_2O. Then add, little by little, while rotating the flask, 10 cc of HCl (sp. gr. 1.1); heat in a water bath to 70° C, cool to 20° C, and add water to the 100 cc mark. After the inversion, proceed to determine the total sugars by the Lane-Eynon method, as described above. In calculating the quantity of sucrose, it must be borne in mind that 95 parts of sucrose yield 100 parts of invert sugar, according to the ratio of their molecular weights:

$$\underset{\substack{\text{Sucrose}\\(\text{mol. wt. } 342)}}{C_{12}H_{22}O_{11}} + H_2O \longrightarrow \underset{\substack{\text{Two molecules of hexose}\\(\text{mol. wt. } 180 \times 2 = 360)}}{2\,C_6H_{12}O_6}$$

Therefore, only 342/360 or 0.95 of the resulting invert sugar determined by the above method will represent the corresponding weight of sucrose.

3. Pectins in Citrus Juices

In discussing the carbohydrates found in the endocarp, brief mention should be made of the pectins. The pectinous substances of the white albedo have been described in some detail. Some of the soluble pectins, however, are also present in small concentrations in the endocarp. Here they are dissolved in the juice and tend to give it a cloudy, colloidal appearance.

The soluble pectins in the juice can be easily demonstrated by adding over 50% of strong alcohol to a clearly filtered juice; a colorless, flocklike precipitate will appear on agitation.

It is generally known that the solid particles of the freshly expressed juice seem to be in suspension; the colloidal nature of the pectins permit the suspension of the solids to be maintained.

However, if chemical preservatives are added to the juice so that fermentation is inhibited, or if the juice is pasteurized at a low tem-

perature, the solid particles of the juice will settle in due course, leaving a supernatant clear liquid layer above. The settling of the pulp, usually called defecation, requires two to three weeks in freshly expressed juices. Except in special cases, such as the clarification of lemon juice for citric-acid manufacture, or in the preparation of cordials (clear juices, such as lime juice), this separation is very undesirable from the standpoint of marketing.

This effect undoubtedly results from the subsequent hydrolysis of the pectins into noncolloidal pectic acid, which, as has been previously mentioned, is due mainly to the action of special enzymes, pectinase or pectolase. To prevent the hydrolysis of pectins, the pectic enzymes should be destroyed by inactivating them through heating the juice for a short time to a minimum temperature of 190° F, although to prevent fermentation a temperature of 165° F is sufficient. Kertesz,[25a] however, reported that the pectin-methoxylase is completely inactivated by heating the juice to 80° C (176° F) for 45 seconds.

Such defecation of the juice appears also in squashes or other juice preparations, and even in frozen juices, if the pectic enzymes have not been previously destroyed in the original citrus juice. Moreover, packers of pasteurized grapefruit juice in tins are frequently troubled by the formation of a white curdlike deposit in the product after several months of storage. This curd formation and abnormal separation of the insoluble solids occurs if the canned juice has not been adequately treated by heat.[26] Sufficiently high temperatures at flash pasteurization or batch pasteurization will preclude such occurrences. If the pectolytic enzymes are not inactivated, the total soluble pectin will rapidly decrease. It has been shown[26a] that in orange juice it will decrease to about one-third of its original value in about 24 hours and then remain essentially constant. In comparing various citrus juices, the rate of hydrolysis of the natural pectins is greater in orange than in grapefruit and lowest in lemons.

The loss of cloud in citrus juices during storage after processing has been recently shown to be not entirely due to enzymatic hydrolysis of pectic substances; this is evident from the enzymatic changes which occur so rapidly after the reaming of fruit that at least a partial

[25a] Kertesz, Z. I., "Pectic Enzymes. III, Heat Inactivation of Tomato Pectin-Methoxylase (Pectase)," *Food Research*, **4**, 113 (1939).

[26] Parks, C. T., "Prevention of Curd in Grapefruit Juice," *Fruit Products J.*, **19**, 210 (1940).

[26a] Joslyn, M. A., and A. Sedky, "The Relative Rates of Destruction of Pectin in Macerates of Various Citrus Fruits," *Plant Physiol.*, **15**, 675 (1940).

coagulation of the cloud occurs before the juice can be screened, deaerated, and pasteurized, and from the appreciable loss in clouding of heavily clouded samples pasteurized at comparatively high temperatures. Loeffler,[27] in a comparative study of the quantitative "cloud index" values of citrus juices using a photoelectric colorimeter, showed that the cloud is not merely stabilized, but is actually increased, by flash pasteurization. The author suggests homogenization of citrus juices before pasteurization in order to prevent the unsightly separation in the bottled juice. This, he claims, not only increases the cloud but actually forms a stable suspension of the chromatophores in the supernatant liquid. However, the true explanation of this phenomenon of an increased "cloud index" of the juice after pasteurization is easily found if one takes into consideration the fact that some of the protopectin, contained in the small albedo particles floating in the juice, is hydrolyzed by the action of acid and heat into soluble pectin which passes into the juice, thus actually increasing the amount of soluble pectin in it.

C. SOME MINOR CONSTITUENTS OF CITRUS JUICES

1. Proteins

The bulk of the total soluble solids of citrus juices (85 to 90%) is composed of sugars and acids; the remainder consists of minor compounds such as pectin, mineral salts, vitamins, glucosides, and proteins. The proteins amount to about 1.75 to 2.05% (calculated as nitrogen on the dry-weight basis) in July and 1.04 to 1.32% at the end of the following March, as has been found by Cameron, Appleman, and Bialoglowski,[28] who investigated the seasonal changes in the total protein content of lemons, grapefruits, and oranges.

As the total amount of the naturally occurring proteins in citrus juices is very small, a systematic analysis is difficult. Hiwatari,[29] for instance, isolated from 27 kg of macerated grapefruit sacs only 2.2 g of glycocoll-betaine, 8.5 g of stachydrine, and 0.2 g of putrescine. Smith[30] isolated from the edible part of the orange an apparently soluble protein, not coagulated by heat in either neutral or alkaline solution, and having an isoelectric point of *p*H 4.7.

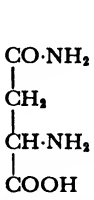
[27] Loeffler, H. J., "Maintenance of Cloud in Citrus Juices," *Proc. Inst. Food Technologists*, 1941, 29.

[28] Cameron, S. H., D. Appleman, and J. Bialoglowski, "Seasonal Changes in the Nitrogen Content of Citrus Fruits," *Amer. Soc. Hort. Sci. Proc.*, 33, 87 (1935).

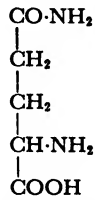
[29] Hiwatari, Y., "Ueber die Stickstoffhaltigen Bestandteile der Früchte von *Citrus grandis*, Osbeck, form, Buntan Hayat," *Jour. Biochem. (Tokyo)*, 7, 169 (1927).

[30] Smith, A. H., "A Protein of the Edible Portion of Orange," *J. Biolog. Chem.*, 63, 71 (1925).

Two basic amino acids (i.e., amino acids in which several basic groups and only one acid group are present)—namely, asparagine and glutamine—were found in orange juice by Scurti and de Plato:[31]

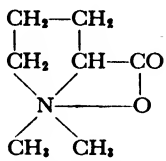


Asparagine
(m.p. = 230° C)

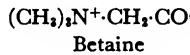


Glutamine

Two additional amino acids widely distributed in the plant kingdom were demonstrated in citrus juices by Yoshimura;[32] they are stachydrine and betaine, both exceedingly soluble in water:



Stachydrine
(m.p. = 235° C)

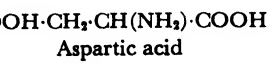


Betaine
(m.p. = 293° C)

Stachydrine appears to be the predominating amino base in orange juice (0.75 g per liter). Nelson, Mottern, and Eddy,[33] in a systematic study of the nitrogenous constituents of Florida Valencia orange juice, have reported also the following basic amino acids: arginine, choline, and aspartic acid.

Arginine
(m.p. = 112–114° C)

Choline



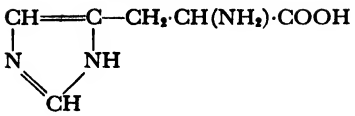
Aspartic acid

They also obtained evidence of the presence of histidine, and obtained a mixture of amino acids from which no definite pure compounds could be isolated.

[31] Scurti, F., and G. de Plato, "Sui Processi Chimici della Maturazione dei Frutti dell'Arancio," *Staz. Sper. Agric. Ital.*, **41**, 435 (1908).

[32] Yoshimura, K., *Bull. Kagoshima Imperial Coll. of Agr. and Forestry, Japan*, No. 3, 1–4 (1918).

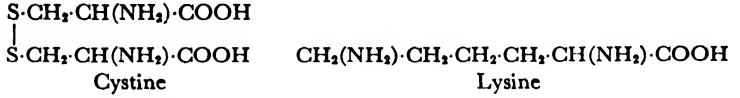
[33] Nelson, E. K., H. H. Mottern, and C. W. Eddy, "Nitrogenous Constituents of Florida Valencia Orange Juice," *Fruit Prod. J.*, **12**, 231 (1933).



Histidine

The authors describe in detail the methods used in the differentiation and isolation of the various proteins.

In another important study on the proteins of Valencia and Washington Navel oranges, Sinclair, Bartholomew, and Nedvidek[34] reported the presence of two additional proteins—namely, cystine and lysine:



Cystine Lysine

According to these investigators, the distribution of nitrogen in the proteins of the orange varieties tested is, on the average, as follows:

% of total N	Valencia	Washington navel
Ammonia	5.21	7.01
Total humin N	2.77	1.61
As amino bases	25.56	25.57
As amino acids	66.37	66.31
Total N, %	99.91	100.50

The presence in amino form of over half of the total nitrogen indicates the importance of this form in the physiological activity of citrus juices. The proteins found in the edible portion of the oranges are not common in other fleshy acid fruits, except in the endosperms of their seeds. In fact, the proportion of proteins in citrus seed-meal totals as high as 16% (see under "Seeds").

It has been suggested that among the factors influencing the deterioration of citrus juices in storage, even when pasteurized or otherwise preserved, the presence of proteins plays an important, so far not elucidated, role. This suggestion is based mainly on the fact that proteins, in general, are unstable compounds and that their breakdown is usually followed by a characteristically disagreeable flavor. The development of the disagreeable flavor, accompanied generally by the pronounced browning of the stored citrus juices, has been attributed by Wilson[35] to Maillard or melanoidin reaction between

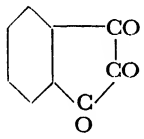
[34] Sinclair, W. B., E. T. Bartholomew, and R. D. Nedvidek, "The Isolation and Distribution of Nitrogen in Dilute Alkali-soluble Proteins of Healthy Valencia and Washington Navel Orange Fruits," *J. Agric. Research*, **50**, 173 (1935).

[35] Wilson, C. P., "Relation of Chemistry to the Citrus Products Industry," *Ind. Eng. Chem.*, **20**, 1302 (1928).

amino acids and sugars. Although this reaction takes place in a slightly alkaline medium (whereas citrus juices are acid), it may possibly arise under the influence of enzymatic or catalytic factors.

Although a nitrogen determination in deteriorated Florida orange juice made by Nelson, Mottern, and Eddy[36] showed that the soluble nitrogen was 120 mg per liter, or twice the amount present in fresh juice, no proteins were identified which have not been found in fresh juice; therefore, no direct evidence was obtained to indicate that proteins are definitely responsible for the deterioration of the flavor of citrus juices.

Amino acids, polypeptides, and peptones give a blue coloration on heating with an aqueous solution of triketohydrindine (ninhydrin):



The reaction is very sensitive.

Among other methods for the determination of amounts of citrus juices in various beverages, this reaction with ninhydrin showing the presence of amino acids in the juice of citrus fruit should be mentioned. When 10 cc of juice are neutralized with an excess of $CaCO_3$, filtered and heated on a water bath with some crystals of ninhydrin, a violet color appears after a few minutes. However, solutions containing less than 10% of lemon or 5% of orange juice do not give this reaction.[36a]

2. Enzymes

(a) Influence of Enzymes in Biochemical Reactions. Definition and Structure

Closely related to proteins are the ferments or enzymes, which are indispensable for the changes occurring in all plant and animal matter.

In previous sections this important class of substances has frequently been encountered: for instance, in the discussion of chlorophyll the enzyme chlorophyllase has been mentioned; some of the enzymes constituting the zymase complex have been cited in con-

[36] *Op. cit.*

[36a] Solarino, E., "A Color Reaction of Citrus Fruit Juice with Ninhydrin," *Citrus*, **18**, No. 5/7, 12 (1946).

nection with fermentation; the enzyme invertase, which is responsible for splitting the disaccharides, has been referred to; pectolytic enzymes have been discussed under pectin; etc. Enzymatic action will also be part of our further discussion. In fact, all biochemical reactions seem to be governed or influenced by specific enzymes.

Enzymes, according to the definition given by J. B. S. Haldane, are soluble colloidal organic catalysts synthesized, so far, only in the living cell, yet capable of acting independently of the life processes of the cell.

Only some of the many enzymes have been identified and isolated, and recently a number of them have been obtained in a crystalline state. Evidence to date indicates that enzymes have structures allied to, if not identical with, proteins. In aqueous solutions enzymes are themselves colloidal in nature or are bound to a colloidal carrier. The catalytic activities of the numerous enzymes, however, depend not only on their chemical structure but also on the nature of certain configurations (so-called prosthetic groups) specifically combined with the proteins, or on the special arrangement of the amino acids in the proteins. Only small chemical changes seem to be involved in the transformation of an active protein, or enzyme, into an inactive one, and vice versa.

(b) Properties of Enzymes

Contrary to simple chemical catalysts, most enzymes are thermolabile, i.e., they are destroyed or lose their activity when heated to temperatures between 70 and 90° C. For most enzymes, inactivation is effected by heating them to 70–80° C for 2–5 minutes. In citrus juices, the inactivation of pectolytic enzymes, responsible for the settling of the suspended solids, is achieved by flash pasteurizing the juices for only a few seconds at a temperature of 90–100° C. There is, however, some evidence that this property of the enzymes of becoming inactivated by heat is only temporary and that after an interval (in the case of citrus juices, a few months) the enzymes may regain their activity.

Probably the most important property of enzymes—no doubt responsible, at least in part, for the synthetic faculties of the living cell—is the reversibility of the reactions they control.

Another characteristic of enzymes is the specificity of their action. Contrary to inorganic catalysts, capable of affecting a number of quite different reactions, the catalytic action of enzymes is usually limited to a specific reaction or to a group of similar reactions. In the

second case the property, which is termed "group specificity," is applied to an enzyme attacking a series of molecules having a common chemical grouping.

Absolute specificity is shown, for instance, by invertase, which breaks down sucrose into glucose and fructose but does not affect lactose or maltose, although both lactose and maltose are also disaccharides and have the same empirical formula as sucrose. As another example, glucose can be attacked by one enzyme or a complex of enzymes to produce alcohol, citric acid, acetone, lactic acid, or the like; in each reaction specific enzymes take part.

Other enzymes, such as lipase, are capable of attacking all common vegetable oils, including the oil of citrus seeds.

The general feature of enzymes, therefore, is that they react only upon substrates containing a particular type of molecular structure.

(c) Classification of Enzymes

The many known enzymes may be classified either according to the nature of the substrate or according to the character of the reaction involved. Recent attempts have been made to classify them on the basis of their chemical structure, but since the composition of most of the enzymes is yet unknown, such methods are yet premature. The general scheme presented here mentions only the enzymes encountered in citrus products.

(1) HYDROLYTIC ENZYMES

ESTERASES are enzymes responsible for splitting esters. Their action consists in the hydrolysis and synthesis of esters according to the general equation:

$$R\cdot COOR' + H_2O \rightleftharpoons R\cdot COOH + R'\cdot OH$$

but they affect also esters of polyhydric alcohols, such as glycerides of fatty acids. The enzyme *lipase* is one of this class. It occurs in the pancreas and splits all vegetable and animal fats and oils into glycerol and free fatty acids. *Chlorophyllase* (page 27), which splits phytyl alcohol from chlorophyll (an ester), is a specific enzyme also in this group.

A detailed study[36b] has lately been made of the distribution and characteristics of a *phosphatase* enzyme newly found in the juice as well as in the flavedo, albedo, and the seeds of Valencia and Washington navel oranges, Eureka lemon, and Marsh seedless grapefruit.

[36b] Axelrod, B., "Citrus Fruit Phosphatase," *J. Biol. Chem.*, 167, 57 (1947).

This citrus phosphatase is capable of hydrolyzing monoesters of phosphoric acid in acid media. While enzymes found in citrus juices are generally suspended therein on the particles of solid matter, this phosphatase enzyme is the first enzyme to be found in true solution in citrus juices. In further studies by the same author small quantities of acetyl esterase and ribonucleinase have been found to exist in orange juice in true solution.

Axelrod[36c] suggests a simple and very rapid method of measurement of phosphatase activity as a useful indication of the extent of pasteurization of citrus juices similar to that made in the pasteurization of milk.

CARBOHYDRASES designate enzymes which can hydrolyze the bond between two simpler sugars in di- or polysaccharides, or the corresponding bond between sugar and aglucon in glucosides. Accordingly, they are subdivided into *polyases* and *glucosidases.*

Of special interest in the first subgroup are the pectolytic enzymes: *protopectinase,* which can convert protopectin into pectins: *pectase,* which hydrolyzes the ester groups in pectin splitting of methyl alcohol; and *pectinase* (or pectolase) which hydrolyzes pectins into pectic acid and finally into galacturonic acid, or its methyl ester. Pectinase, found in citrus juices, through the destruction of pectin causes the separation of the solid matter in preserved juices and should, therefore, be inactivated, as has been mentioned on page 266. (For further details on these enzymes, see pages 92–94.)

Of the second subgroup of carbohydrases, mention should be made of *invertase* (sucrase, invertin or *β-l*-fructosidase), which hydrolyzes cane sugar (sucrose) into glucose and fructose. This enzyme has been studied very extensively, and being of course present in citrus juices, inverts their entire sugar content after a few months of storage.

PROTEASES. This group includes all those enzymes concerned with the degradation of proteins into simpler amino acids. It is again subdivided into *proteinases* (pepsin, papain, and trypsin) and *peptidases.*

(2) OXIDIZING ENZYMES

OXIDASES (dehydrogenases or phenolases) are complete enzymes (consisting of peroxidase, organic peroxide, and oxygenase) which oxidize phenols and chromogens.

OXYGENASE oxidizes catechol, or compounds possessing an ortho-

[36c] Axelrod, B., "Phosphatase Activity as an Index of Pasteurization in Citrus Juices," *Fruit Prod. J.,* 26, 132 (1947).

dihydroxy grouping characteristic of catechol, with the formation of a peroxide.

PEROXIDASES set free active or atomic oxygen from hydrogen peroxide. Peroxidase is abundant in different tissues of orange, grapefruit, and lemon, and especially in the inner seed coat of tangerines.

During a detailed study on the distribution of peroxidase in different tissues of citrus fruits it was found that the inner seed coat of these fruit is of particularly high activity. Davis[36d] suggests a possibility to produce peroxidase enzyme on a large scale from the seeds of lemons, which are available in large quantities.

CATALASE, is not in itself an oxidizing enzyme but is closely related to this subgroup; it splits molecular oxygen from hydrogen peroxide only:

$$2\,H_2O_2 + \text{Catalase} \longrightarrow O_2 + 2\,H_2O$$

ASCORBINASE. To the oxidizing enzymes belongs also *ascorbinase* (or vitamin C oxidase) which is to some extent responsible for the destruction of vitamin C in citrus juices. In the juice of citrus, which are typical "peroxidase plants," oxygen reacts with the ascorbic acid oxidase, the oxygen oxidizes a molecule of ascorbic acid reversibly with the coformation of one molecule of hydrogen peroxide. The peroxide now oxidizes with the help of the peroxidase a benzopyran, and the latter again reversibly oxidizes a further molecule of ascorbic acid.[36e]

Using the Warburg technique in enzymology, an attempt was recently made[36f] to determine the type of oxygen-absorbing system present in the various tissues of the orange fruit, and the opinion is expressed that a system of indophenol oxidase, resembling cytochrome oxidase, is involved which is responsible for a major part of the oxygen uptake.

(3) FERMENTING ENZYMES

This group comprises a number of enzymes constituting the *zymase* complex (adenylic acid system including phosphorylase; cozymase; dehydrocozymase; carboxylase; cocarboxylase; etc.) which converts hexoses into ethyl alcohol and CO_2. It has been described in a preceding section. To this group belong also special enzymes occurring

[36d] Davis, W. B., "The Distribution and Preparation of Citrus Peroxidase," *Am. J. Botany*, **29**, 252 (1942).

[36e] Huszák, I., "Ueber die Funktion des Peroxydase-Systems der Pflanzen," *Z. physiol. Chem.*, **247**, 239 (1937).

[36f] Hussein, A. Aziz, "Respiration in the Orange. A Study of Systems Responsible for Oxygen Uptake," *J. Biol. Chem.*, **155**, 201 (1944).

in the fermentation of lactic acid, acetone-butanol, and other compounds.

(4) COAGULATING OR CLOTTING ENZYMES

To this group belong the coagulating enzymes, such as *rennin,* which curdles milk. Citrus juices probably also contain such enzymes, which cause the curdling of grapefruit and lemon juices in storage.

(d) Optimal Conditions for Enzyme Acitivity

The velocity of enzyme action is affected by temperature, by hydrogen ion concentration of the medium, by the ratio of substrate to enzyme, as well as by other factors.

The optimum temperature for most enzymes lies in the region of 40 to 50° C, although a number of enzymes act most effectively near 60° C. These optimum temperatures are affected by the *p*H of the substrate. The optimum *p*H value for invertase, for instance, is 4.2 at 22.3° C, for pectase 4.3, for zymase complex of yeast 4.5 to 6.5.

With a rise of temperature the reaction velocity of enzymes increases. For most enzymes, an elevation of 10° C in temperature causes the velocity of the reaction to double or treble. This increase in reaction velocity is designated by a coefficient $(K_{t+10})/K_t$, which is different for various enzymes:

Enzyme	Temp., °C (t)	$(K_{t+10})/K_t$
Invertase	0–20	2.0
Zymase	15–25	2.8
Catalase	0–10	1.5
Lipase	18–28	2.6

Below 40° C most enzymes are fairly stable, and although the reaction velocity decreases appreciably with a fall in temperature, activity is not checked even at very low temperatures. Balls and Lineweaver[87] have shown that enzyme action at low temperatures not only occurs but is also an important factor in food preservation. The velocity of some enzymes at low temperatures is quite surprising. Probably the first phase of enzymatic attack on the substrate is completed after the material has been stored for a long time at a low temperature; when the material is subsequently brought to ordinary temperature, the evident enzymatic action becomes more rapid.

Temperatures over 50° C cause rapid inactivation of enzymes. As mentioned above, most enzymes are completely inactivated at a

[87] Balls, A. K., and Hans Lineweaver, "Action of Enzymes at Low Temperatures," *Food Research,* **3,** 57 (1938).

temperature of 90° C, although some, such as pectolytic enzymes, invertase, diastase, and certain oxidizing enzymes, after having been thus inactivated, regain their activity on subsequent storage at ordinary or low temperatures.

The inactivation of enzymes can be attained also through the addition of certain compounds, so-called inactivators; their action, however, is specific to definite enzymes. Heavy metals and their salts, for instance, are toxic to many enzymes. Fermenting enzymes are inactivated by various chemical preservatives. Benzoic acid, benzoates, and formaldehyde inactivate these enzymes by forming insoluble protein compounds. Sulfites and sulfurous acid inhibit fermentation by preventing the activity of oxidase, and sodium fluoride by paralyzing the peroxidase.

(e) Mechanism of Enzyme Action

In the generally accepted theory of the mechanism of enzyme action, the first step is the formation of a definite compound between enzyme and substrate. This compound breaks down after a definite time interval to form the reaction products and the original enzyme. Thereafter a new molecule of the substrate takes the place of the combination which has decomposed, and so the cycle of combination and decomposition continues until the end of the reaction. There is ample evidence that compounds of enzyme and substrate do exist. An example of such evidence comes from the facts concerning the protection by substrates of their enzymes from inactivation by heat and other agencies: thus, invertase is less sensitive to the inactivating action of heat in the presence of its substrate, sucrose. Other evidence comes from spectroscopic observations on the color changes which take place under certain conditions when union occurs between catalase or peroxidase and hydrogen peroxide. The kinetics of enzymic change can also be easily interpreted on the basis of this theory.

However, not only the substrates combine reversibly with the enzyme. Molecules of other compounds which have special attachment-groupings similar in structure to the substrate may also combine with the enzyme, although they do not undergo any subsequent change. Such substances may affect physiological processes by reason of their possession of a structure allowing them to combine reversibly with the enzyme, thus competing with the substrate and preventing the enzyme from exercising its function of decomposing the substrate at a normal rate.

Similarly, there must be a special receptor-grouping in the enzyme

to receive and activate the substrate. Both basic and acid radicals form such receptor-groupings of enzymes. By the use of selective poisons the chemical nature of such receptor-groupings can be detected.

3. Fatty Constituents in Citrus Juices

It has not yet been definitely established whether fresh citrus juices naturally contain small amounts of fatty constituents or whether these are introduced through seeds crushed during extraction. The suspended matter of citrus juices contains variable amounts of lipides which consist approximately of two-thirds saponifiable fatty acid esters and one-third unsaponifiable matter. In comparing juices obtained from oranges by different methods of extraction, it was found[37a] that juices extracted with a burr reamer contained 25% more lipide material than those squeezed out by an automatic juice extractor.

In comparing petroleum ether extract from fresh and aged, canned orange juice, the fresh juice was found[37b] to contain: unidentified waxy bodies insoluble in petroleum ether; oleic, linoleic, cerotic, palmitic and stearic acids; unidentified aliphatic alcohols and resins; phytosterols; sterols and carotenoid pigments. The characteristics of the petroleum ether extract of aged, canned orange juice indicate that the fatty material has undergone oxidative changes with the creation of hydroxy acids and other decomposition products (such as higher ketones usually detected by their unpleasant taste) and had become rancid. It is reasonable to assume that such oxidation of the fatty material of the juice performs an important role (at least in part) in the development of off-flavors.

4. Mineral Constituents of Citrus Juices

All citrus juices contain, on the average, 0.4% of ash; lemon juice has a somewhat lower ash content, as shown by Table XV.[38]

The ash contains, in general, small amounts of calcium, sodium, potassium, magnesium, phosphorus, chlorine, sulfur, iron, copper, etc., as shown by Table XVI.

[37a] Swift, L. J. (U. S. Citrus Products Sta., Winter Haven, Fla.), "Determination of Crude Lipide in Citrus Juices," *J. Assoc. Official Agr. Chem.*, **29**, 389 (1946).

[37b] Nolte, A. J., and H. W. von Loesecke, "Chemical and Physical Characteristics of the Petroleum Ether Soluble Material in Fresh and Canned Florida Valencia Orange Juice," *Food Research*, **5**, No. 5 (1940).

[38] After Chatfield, C., and L. I. McLaughlin, "Proximate Composition of Fresh Fruits," *U.S. Dept. Agr. Circ. 50* (1931).

TABLE XV

Ash Contents of Citrus Fruits

Variety	Per cent of ash	
	In fruit	In juice
Oranges, all varieties	0.35–0.7	0.4
Grapefruit, Philippine Islands	0.63	
" California	0.39–0.4	0.4
" Florida	0.54	0.4
Tangerines		0.4
Limes, sweet	0.4 –1.0	
" bitter	0.7 –1.0	
Lemons, all varieties	0.5 –0.7	0.31–0.35

TABLE XVI

Ash Constituents in Citrus Juices (%)[39]

Component	Grapefruit (Florida)		Oranges (Florida)			Tangerine (Florida)
	Marsh seedless	Seedy var.	Valencia	Blood orange	Seedling	
K	0.089	0.171	0.172	0.185	0.187	0.177
P	0.010	0.030	0.032	0.028	0.030	0.015
Ca	0.008	0.012	0.009	0.011	0.016	0.014
S	0.002	0.004	0.004	0.005	0.003	0.006
Mg	0.005	0.009	0.005	0.006	0.010	0.007
Na	0.004	0.007	0.007	0.009	0.008	0.006
Fe	0.00009	0.00008	0.0001	0.0005	0.0002	0.00028
Al	0.0003	0.0007	0.0006	0.0008	0.0004	0.0003
C	0.019	0.037	0.032	0.035	0.039	0.036
O	0.093	0.11	0.17	0.19	0.20	—
Cl	0.001	0.002	0.003	0.003	0.003	0.003

It is noteworthy that all citrus juices contain, in addition to the elements enumerated in the tables, 0.3 to 0.9 mg per kg (fresh basis) of copper. This fact is of great importance as the cupric ion has an important bearing on the destruction of vitamin C in juice.

In a recent study on the composition of different varieties of citrus fruit, Harding and Fisher[40] found that the total ash content of the juice was generally highest in immature fruit and gradually decreased as maturity progressed.

Citrus juices, particularly orange juice, contain a surprisingly high amount of boric acid, i.e., 0.05 to 0.33 mg per liter.

The cations mentioned above occur in the juice as salts of organic acids, such as citrate, malates, tartrates, and lactates. When consumed by the human body, these acids are oxidized into carbon diox-

[39] Data from Roberts, J. A., and L. W. Gaddum, "Composition of Citrus Fruit Juices," *Ind. Eng. Chem.*, **29**, 574 (1937).

[40] Harding, P. L., and D. F. Fisher, "Seasonal Changes in Florida Grapefruit," *U.S. Dept. Agr. Tech. Bull. 886* (1945).

ide and, like most of the metals of the alkaline and alkaline-earth groups, they help to maintain the alkaline reserve of the body and exert an alkaline reaction on the urine.

5. Gaseous Constituents of Citrus Juices

Regardless of the method of extraction, citrus juices always contain a considerable amount of air. Air is administered to the juices during extraction mainly by means of rapidly revolving reamers; it is applied, as well, during passage of the juices through a finisher or through vibrating screens, where the coarse pulp is removed.

The oxygen of the dissolved air apparently reacts with some constituents of the juice, resulting in the disappearance of oxygen—a process which is accelerated by elevated temperatures.

Oxygen adversely affects the juice, primarily its vitamin C content; the ascorbic acid is oxidized into dehydroascorbic acid and subsequently the vitamin C potency of the juice is destroyed. Oxygen also reacts with the phenolic glucosides, such as naringin, causing the development of a bitter taste, especially in the early part of the season. Citrus juices allowed to stand for a short time rapidly consume the dissolved oxygen; thus, in determining the dissolved air content of the juices, reliable duplicate results on the same sample are practically impossible, because while one determination is being made, the oxygen of the second charge becomes rapidly consumed. A duplicate determination of a single juice sample has shown 3.4 cc and 2.8 cc of oxygen per liter, respectively.[41]

Table XVII gives some examples of the gas content of citrus juices extracted by different methods, as determined by Pulley and von Loesecke in grapefruit and orange juices.

Apparently the ratio of oxygen to nitrogen in the extracted juices differs from that in air; also there does not seem to be any consistency of this ratio in the juices. Pulley and von Loesecke found, furthermore, that this ratio, determined on citrus juices, was always less than that determined on plain water. This evidence indicates that the oxygen is combined with some constituents of the juice.

Since some of the oxygen is consumed by the juice during the process of withdrawing the gas from the sample, the value of oxygen shown by the analysis is not a true one, and, therefore, Clark[41a] suggested that one may calculate the amount of oxygen from the nitro-

[41] Pulley, G. N., and H. W. von Loesecke, "Gases in the Commercial Handling of Citrus Juices," *Ind. Eng. Chem.*, **31**, 1275 (Oct. 1939).

[41a] Clark, B. S., "Technology of Canned Juices," *Fruit Products J.*, p. 265 (May, 1941).

TABLE XVII

Gas Content of Citrus Juices

Method of extraction	Scale of operation	Gas content, cc/l*				
		Total Gas	CO_2	O_2	N_2	Ratio O_2/N_2
Rapid reaming (1750 rpm)	Laboratory scale (hand)	48.2	31.1	3.47	13.6	0.258
	Plant operation	28.5	14.7	4.02	9.7	0.415
	Plant operation	42.8	28.2	3.24	11.3	0.287
Rasping and pressing	Plant operation	36.7	22.3	4.17	10.2	0.409
Slow reaming (70 rpm)	Laboratory scale	54.9	38.7	2.52	13.7	0.184
Peeling and screw press	Laboratory scale	57.7	41.5	2.22	13.9	0.160
Mechanical	Plant operation	35.5	22.3	2.46	10.7	0.230

* Reduced to standard conditions: 0°C and 760 mm atmospheric pressure.

gen value by multiplying it by the factor 0.2625, which is the oxygen–inert gas ratio of the atmosphere. The maximum oxygen content which should, in the opinion of this author, be tolerated in processed citrus juices without significant influence on the quality of such products is as follows: 0.2 cc oxygen (observed) or 0.60 cc (calculated) for can size of 12 oz; and 2.00 cc oxygen (observed) or 5.5 cc (calculated) for No. 10 cans.

The author describes also a portable apparatus for determining the gas content in the plant of juices either before or after it is sealed in the container.

The rate of absorption of oxygen by orange juice has been found to be 35 γ per liter.[42] The oxygen uptake of the same juice when pasteurized was raised to 42 γ per liter. When extra ascorbic acid was added to juice the oxygen uptakes were 67 γ for unpasteurized and 110 γ per liter for pasteurized orange juice.

The effectiveness of the deaeration of citrus juices by various technical methods will be discussed later.

6. Determination of Air and of Dissolved Oxygen in Citrus Juices

In order to measure the degree of deaeration of citrus juices prior to pasteurization, several methods have been suggested. Apart from the above-mentioned apparatus, Loeffler[42a] developed a simple gas analysis apparatus in which air determination and differential analysis can be made in a short time (10 to 15 minutes).

Recently, quite a different method for determining dissolved

[42] Shillinglaw, C. A., and Max Levine, "Control of Oxidative Flavors in Beverages," *Food Research*, 8, 453 (1943).

[42a] Loeffler, H J., "Determination of Air in Citrus Juices," *Ind. Eng. Chem., Analyt. Ed.*, 12, 533 (1940).

oxygen in citrus juices has been suggested which is much simpler, and more rapid and accurate than the gasometric methods. This method is based on the polarographic procedure, using the dropping mercury electrode. An inexpensive polarizing apparatus of this nature used for grapefruit juice is described in detail by Lindquist.[42b] The use of the polarograph to determine oxygen in orange juice is also described in detail by Lewis and McKenzie.[42c]

D. VITAMINS

1. Definition

The nutritive value of citrus fruit is now largely measured by the vitamin content. The brilliant investigations by Hopkins have heralded a true revolution in the science of nutrition. Hopkins[43] showed in 1906 that the four main classes of food constituents—namely, carbohydrates, fats, proteins, and mineral salts—generally maintained at that time as the only essentials of a complete diet, are not sufficient for normal growth and upkeep of the body, and that "accessory food factors" are required. Indications are that some such "accessory food factors" were assumed as far back as 1747 when Lind, a surgeon in the British Navy, confirmed the theory that scurvy was due to a shortage of fresh fruit and vegetables in the diet. The British Navy subsequently made a ration of lemon or lime juice compulsory as a scurvy preventive. Since then, great achievements have been made in this new branch of science. Because citrus fruits represent an important source of vitamins C and P, and contain also some quantities of vitamins A, B, and G, each will be discussed separately.

Vitamins, chemically speaking, are not necessarily amines as had been supposed by Funk[44] who, in 1912, attempted to isolate the compound which prevents beriberi. Though he thought the compound was an amine, facts proved that it was not. Nevertheless, the name "vitamin" has been retained for these "accessory food factors," which are definite organic substances indispensable to the animal body, having a relatively simple structure and being comparatively unstable. Vitamins are powerful biological catalysts; in some respects they resemble enzymes and hormones. Unlike enzymes, they are rela-

[42b] Lindquist, J. R., "Rapid Dissolved Oxygen Test for Fresh Citrus Juices," *Food Ind.*, **19**, 182 (1947).

[42c] Lewis, V. M., and H. A. McKenzie, "Amperometric Determination of Dissolved Oxygen in Orange Juice," *Ind. Eng. Chem., Analyt. Ed.*, **19**, 643 (1947).

[43] Hopkins, F. G., *Analyst*, **31**, 385 (1906).

[44] Funk, C., *Die Vitamine*, Bergmann, Wiesbaden (1922).

tively heat-stable, for they withstand a temperature of 100° C in the absence of oxygen. Vitamins are generally not synthesized by the animal or human body; they do not furnish energy and are not utilized as building units for the structure of the organism. Although vitamins occur in relatively small concentrations, they perform in the human organism specific and vital functions and are essential for the transformation of energy and for the regulation of metabolism. Twenty-three vitamins have so far been identified as chemical entities to which specific functions have been assigned and, in addition, approximately fifteen nonidentified vitamins or factors are known.

Citrus fruits are an important source for vitamins C and P. The other common vitamins are present only in comparatively small amounts. Table XVIII gives the approximate distribution of vitamins in citrus fruits.

TABLE XVIII

Distribution of Vitamins in Citrus Fruits

Per 100 g of	Vitamin C (ascorbic acid), mg	Vitamin B_1 (thiamin), micrograms (γ)	Vitamin G (B_2) (riboflavin), micrograms	Vitamin A, I.U.
Orange.....	52–56	75–145	28– 90	50–400
Tangerine....	25–50	120	—	350
Grapefruit....	38–41	50–100	20–100	21
Lemon.....	52–60	30– 90	—	—

In addition to the vitamins mentioned in the table, citrus juices also contain vitamin P (or citrin) and inositol (see pages 152, 161).

2. Ascorbic Acid (Vitamin C)

Since all citrus fruits and their products are very important sources of vitamin C, this subject will be discussed at some length.

Both the albedo and the mesocarp of citrus fruits contain appreciable amounts of vitamin C. Citrus fruits were known for centuries to contain an antiscorbutic substance. Zilva[45] in England prepared from lemon juice a very potent concentrate—almost pure ascorbic acid—and established its molecular composition, resemblance to hexoses, and instability toward oxygen. At about the same time, Szent-Györgyi[46] succeeded in isolating this vitamin in crystalline form

[45] Zilva, S. S., "A Note on the Preservation of the Antiscorbutic Factor in Lemon Juice," *Biochem. J.*, **21**, 689 (1927).

[46] Szent-Györgyi, A., "Observations on the Function of Peroxidase Systems and the Chemistry of the Adrenal Cortex; A New Carbohydrate Derivative," *Biochem. J.*, **22**, 1387 (1928).

from the adrenal cortex, from oranges, and from paprika, and named it first hexuronic acid and later ascorbic acid ($C_6H_8O_6$). It crystallizes in white odorless plates, somewhat acid in taste, melting at 190° to 192° C. Ascorbic acid is exceedingly soluble in water (1 g in 3 cc) and is insoluble in typical fat solvents such as ether, benzene, and chloroform.

(a) Occurrence

Extensive research has been and is being continually carried out on the occurrence of vitamin C in fruits and vegetables and on its behavior. Although it is impossible in this work to embody the entire field, some phases of recent investigations will be mentioned.

It is probable that certain plant tissues contain vitamin C in combination with protein, the complex—called "ascorbigen"—readily yielding on hydrolysis vitamin C and protein. Using trichloracetic acid or metaphosphoric acid, Reedman and McHenry[47] have precipitated this protein-ascorbic acid complex. While certain vegetable tissues contain the complex, various fruits tested have so far shown no presence of it. The composition of "ascorbigen" is still unknown, and there is considerable controversy as to whether such a combined form actually exists.

Data collected by Fitzgerald and Fellers[48] indicate that climate, soil, and possibly other factors, besides maturity, have an important bearing upon the vitamin C content of fruits and vegetables. They also show that a locality which produces a very low vitamin C potency in one product may produce another with an exceptionally high value. Table XIX, compiled[49] from analyses of Palestine citrus fruits during three consecutive seasons—1941 to 1944—clearly shows that no regularity can be found in the ascorbic acid content throughout the season.

Similar inconsistency in vitamin C content has been found by Harris and Ray,[50] who tested different varieties of oranges (see Table XX on page 139).

There is much evidence to suggest that the vitamin C content of citrus fruits is decreased when the nutrient supply to plants is expanded. The theory has been advanced that a higher percentage con-

[47] Reedman, E. J., and E. W. McHenry, "Combined Ascorbic Acid in Plant Tissues," *Biochem. J.*, **32**, 85 (1938).

[48] Fitzgerald, G. A., and C. R. Fellers, "Carotene and Ascorbic Acid Content of Fresh Market and Commercially Frozen Fruits and Vegetables," *Food Research*, **3**, 109 (1938).

[49] Tests performed and tabulated by Dr. F. Stern of Jaf-Ora Ltd., Rehovoth, Israel.

[50] Harris, L. J., and S. N. Ray, *Biochem. J.*, **27**, 2006 (1933).

TABLE XIX

Seasonal Variations in Vitamin C Content (in Milligrams per 100 Cc) of Palestine Citrus Juices

Month	Oranges			Grapefruit			Lemons		
	Min.	Max.	Average	Min.	Max.	Average	Min.	Max.	Average
December	—	—	49.23	—	—	48.4	52.8	66.9	60.54
January	45.7	52.8	50.79	49.3	56.3	52.8	—	—	70.05
February	45.1	56.3	51.18	—	—	51.04	—	—	61.95
March	45.7	50.7	49.08	—	—	43.3	52.8	59.8	58.08
April	45.7	49.3	48.87	42.2	49.3	45.8	52.8	55.3	54.03
May	—	—	49.28	42.2	45.8	43.1	—	—	57.70

[1] Averages represent a mean value of daily tests during the month.

TABLE XX

Vitamin C Content in Different Varieties of Oranges

Variety	Minimum and maximum ascorbic acid (mg per 100 cc of juice)
Seville	18.9–45.5
Jaffa	33.3–53.7
Brazilian	52.6–62.5
Spanish Burriana	52.2–66.0
Spanish Valencia	50.0
Californian	63.2–70.6
South African	76.9

tent of vitamins is to be expected in crops which have to struggle for food in a poor soil. Recent investigations on citrus fruit in Arizona and Jamaica have shown that the vitamin C content falls as the soil's nitrogen and phosphate status is increased. Addition of potash, however, increases the value of vitamin C.

As far as varieties are concerned, it has been observed[50a] that, for oranges of equal size, the navel oranges contained much less vitamin C per orange, although the juice from the navel variety had the highest concentration of this vitamin—the reason being the smaller percentage of juice in these oranges.

Different parts of citrus fruit contain varied amounts of ascorbic acid; the gradient declines from flavedo toward endocarp. Several varieties of Italian oranges tested showed [51] the following vitamin C content in mg per 100 g:

Yellow peelings	175 -292
White peelings	86 -194
Fruit flesh	44.9- 73.2

[50a] Daniel, E. P., M. H. Kennedy, and H. E. Munsell, "Relative Vitamin C Content of Orange and Tomato Juices Determined Chemically and Biologically," *J. Home Econ.*, **28**, 470 (1936).

[51] Rauen, H. M., M. Devescovi, and N. Magnani, The Vitamin C Content of Italian Oranges and Orange Pulps, *Z. Untersuch. Lebensm.*, **85**, 257 (1943).

A similar distribution of vitamin C has been found[52] in the lime (*Citrus limetta* Risso) which contains, on the average: in epicarp (flavedo), 374; in mesocarp (albedo), 193; in endocarp (juice), 67 mg per 100 g.

It has been shown that in bruised or cut portions of fruits or vegetables, the amount of vitamin C is greatly augmented after a few days. In sliced potatoes, for instance, the amount of vitamin C rises from 10.8 mg to 24.1 mg per 100 g in three days. This traumatic formation of ascorbic acid, which is accompanied by an increase in the activity of the enzymes (ascorbinase, peroxidase, and catalase), is considered a defense mechanism[53]; it is probably the reason why the amount of vitamin C in the region between the flavedo and albedo of citrus fruit is greatly increased (sometimes doubled or trebled), compared with the amounts normally distributed throughout the fruit.

On the supposition that ascorbic acid in its phosphorylated form acts as an oxidation-reduction catalyst in the change of organic acids to sugars, the opinion has been expressed[54] that, during the ripening, the amount of vitamin C in citrus fruit decreases. However, this view is hardly acceptable, for no support has been found in numerous tests performed throughout the growing season. Moreover, the amount of ascorbic acid in the pulp and rind of Satsuma oranges has been found[55] to increase as the fruit ripens, while the quantity of acid in each segment of a given orange is the same.

Fruits and vegetables contain varied amounts of vitamin C. Szent-Györgyi found paprika to be very rich in vitamin C; rose hips are reported to contain as much as 1–2% of ascorbic acid. Recently, rather phenomenal quantities of ascorbic acid have been reported in South African guavas which, when dried into powder, had a content of 2,500 to 3,000 mg per 100 g.[56] These figures, however, represent the total reducing power, of which only a slight portion is ascorbic acid.

Some cultural methods evidently have an important effect on the chemical composition of fruits. As a result of an extensive study[56a] it

[52] Ribeiro, R. F., V. Bonoldi, and O. F. Ribeiro, Vitamin C Distribution in Different Parts of the Lime, *Rev. Faculdada Med. Vet., Univ. Sao Paulo (Brazil)*, 2, 23 (1942).

[53] Prokoshev, S. M., Traumatic Formation of Vitamin C in Sliced Potatoes, *Biokhimiya (U.S.S.R.)*, 9, 36 (1944).

[54] Ugon, N. A., and W. Bertullo, Degree of Maturity of Citrus Fruits Compared with Their Vitamin C Content, *Arch. Soc. Biol. Montevideo*, 11, 139 (1944).

[55] Iwasaki, Yasuo, and Tosio Komatu, Ascorbic Acid Content of Citrus Fruits, *J. Agr. Chem. Soc. Japan*, 17, 427 (1941).

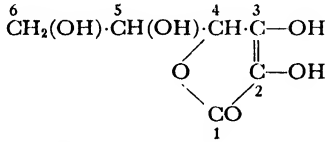
[56] *Science*, 98 (No. 2547) (Oct. 22, 1943).

[56a] Nelson, E. M., and H. H. Mottern, "Effect of Lead Arsenate Spray on the Composition and Vitamin Content of Oranges," *Am. J. Pub. Health*, 22, 587 (1932).

has been definitely established that the vitamin C content of oranges from trees sprayed with lead arsenate was found to be considerably lower than that of oranges from unsprayed trees of the same variety and the same degree of maturity.

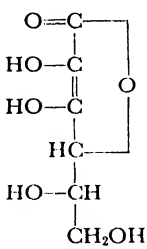
(b) Structure

The investigation of the structure of vitamin C, to which Haworth, Hirst, Reichstein, Cox, Karrer, Reynolds, von Euler, and Micheel have contributed, led to the establishment of the following formula:

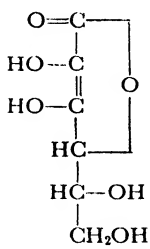


Vitamin C (*l*-ascorbic acid)

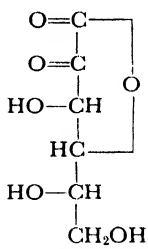
Carbon atoms 4 and 5 are asymmetric, and consequently ascorbic acid is optically active; its index of optical rotation $[\alpha]_D^{20°}$ equals $+23°$. It is a monobasic acid, giving well-defined salts of the type $C_6H_7O_6M$.



l-Ascorbic acid (vitamin C)



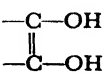
d-Ascorbic acid



d-Erythro-3-ketohexonic acid (isovitamin C)

d-Ascorbic acid shows very slight antiscorbutic properties (one-fifth to one-twentieth the potency of vitamin C); other hexuronic acids show none.

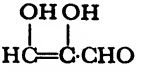
The foregoing structures are closely related to glucose; in fact, sugars are usually regarded as precursors of vitamin C. As can be seen from the structural formula, ascorbic acid does not contain a free carboxyl group but is a lactone forming a five-membered ring of the furan type. The acidic character of vitamin C is due to the presence of the enol configuration:



capable of giving, with alkalies, neutral water-soluble monometal salts without destroying the lactone ring.

(c) Oxidation

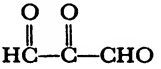
The enol configuration also renders ascorbic acid a very strong reducing agent. In this respect it is similar to a very closely related substance obtained by von Euler[57] in treating hexoses and pentoses with alkalies. To this substance, named *reducton* because it possesses unusually strong reducing power, von Euler assigned the following formula:



Hydroxyglycolaldehyde (reducton)

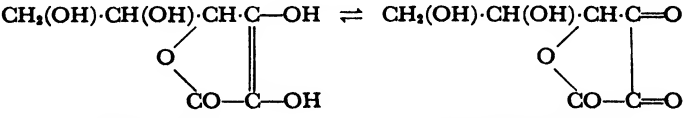
Here again, the same enol configuration may be noted. Not only do the hexoses react in this way with alkalies, but their products of decomposition, such as dioxyacetone, methylglyoxal, and glyceric aldehyde also do so. In fact, the ability of sugars to reduce Fehling's solution is ascribed to reducton, which is obviously formed during the action of the alkali of the Fehling solution upon the sugar tested.

When oxidized, reducton is transformed into:



Similarly, even weak oxidizing agents oxidize this enol group in the ascorbic acid quite easily into a dioxy group:

As a result, a dehydroascorbic acid is produced, which can, in turn, be easily reduced back to ascorbic acid—for instance, by means of H_2S.



Ascorbic acid Dehydroascorbic acid

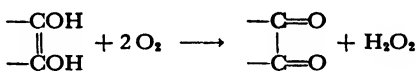
Both of these forms have been proved to exert antiscorbutic action in the animal body. The complex mechanism of vitamin C in the body apparently depends largely upon this reversible oxidation.

[57] Von Euler, H., and C. Martius, "Ueber Redukton (Enol-Tartronaldehyd) und Askorbinsäure," *Annalen der Chemie*, **505**, 73 (1933).

While numerous investigators have found varied amounts of dehydroascorbic acid in vegetables along with vitamin C, most of them deny its presence in fruits, especially in citrus fruits. Determination of the oxidation-reduction curve in orange juice made by Ball[58] has shown that dehydroascorbic acid is not a natural constituent. After many years of experiments in numerous laboratories, there is no evidence that dehydroascorbic acid or reducing substances other than ascorbic acid occurs in fresh or commercially canned citrus fruits.[59]

Oxidation of vitamin C takes place in the presence of molecular oxygen and is greatly accelerated even by traces of metals, especially copper and silver. This oxidation is accelerated also by the specific enzyme, ascorbinase (ascorbic acid oxidase). According to Somogyi,[60] citrus juices contain an active principle which inhibits the destruction of ascorbic acid and protects it from the action of ascorbinase. This substance is partially removed by filtration and is inactivated by heat. However, lemon juice seems to contain no such protective mechanism and is oxidized by atmospheric oxygen much more readily than orange juice that apparently contains more reducing components other than vitamin C.

According to recent investigations,[61] the oxidation of vitamin C involves the formation of hydrogen peroxide:



This explains why the oxidation of ascorbic acid proceeds slowly even if the juice, after extraction, is kept under conditions excluding oxygen; a small quantity of peroxide, once formed, continues to oxidize ascorbic acid, either through the decomposition of H_2O_2 into O_2 and H_2O or through direct reaction of H_2O_2 with ascorbic acid. Probably both reactions take place. Both the decomposition of H_2O_2 and the reaction of H_2O_2 with vitamin C are catalyzed by cupric ion.

The outstanding reducing power of ascorbic acid and its reversible oxidation have attracted the attention of numerous scientific workers in the field of biochemistry. An interesting connection has recently

[58] Ball, E. G., "Studies on Oxidation-Reduction," *J. Biolog. Chem.*, **118**, 219 (1937).

[59] Bessey, O. A., "Report on Ascorbic Acid in Citrus Fruits," *J. Assoc. Offic. Agr. Chem.*, **27**, 537 (1944).

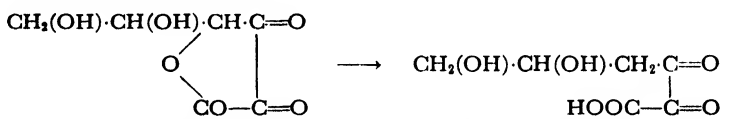
[60] Somogyi, J. C., Active Principles Which Inhibit the Destruction of Vitamin C in Vitro, *Helv. Physiol. Pharmacol. Acta*, **2**, 269 (1944).

[61] Weissberger, A., and J. E. LuValle, "Oxidation Processes; XVII, The Autoxidation of Ascorbic Acid in the Presence of Copper," *J. Amer. Chem. Soc.*, **66**, 700 (1944).

been established between the changes in the forms of ascorbic acid in the green leaves during the day and the changes in the intensity of photosynthesis; accordingly, a hypothesis has been put forward[62] that ascorbic acid takes direct part in plant photosynthesis. Furthermore, a supposition has been made[63] that vitamin C, being a substance with a preformed endiol group, catalyzes the condensation of sugar in the green leaf of the plant. In fact, it was possible for the first time to synthesize sugar from formaldehyde in the presence of ascorbic acid and calcium hydroxide at a temperature of 37° C and a *p*H of 8.2.

(d) Loss

Strong oxidizing agents transform the ascorbic acid irreversibly (breaking up the lactone ring) and thus permanently destroy its vitamin potency. Also, in neutral and alkaline solutions, vitamin C is very quickly destroyed and its potency lost. Thus:



Dehydroascorbic acid — 2-Keto-3-ketohexonic acid

Ascorbic acid is quite thermostable. Dehydroascorbic acid, on the other hand, is very thermolabile; in a neutral medium it is entirely destroyed if kept at 60° C for only 10 minutes. The destruction of dehydroascorbic acid is not an oxidative phenomenon and has been shown[64] to take place even under anaerobic conditions.

Extensive changes, especially in color and flavor, occurring in fruit juices during storage run parallel with the progressive decrease of the amount of ascorbic acid. The presence of air markedly increases the destruction of ascorbic acid and affects somewhat the change in color. Since ascorbic acid is oxidizable and pigments are reducible, the results suggest[65] that they may react with each other.

Darkening of citrus juices during storage occurs after ascorbic acid has been transformed into its dehydroascorbic form and when no

[62] Blagoveschenski, V. A., Jr., On the Significance of Oxidation-Reduction Systems in Photosynthesis, in *Synthesis of Organic Matter and the Role of Vitamins in Plants*, Academy of Sciences (U.S.S.R.), Moscow (1940).

[63] Kuzin, A. M., On the Role of Ascorbic Acid in the Synthesis of Carbon Chains, *ibid.*

[64] Engelhardt, V. A., and V. N. Bukin, Stability of Ascorbic Acid and Its Dehydro-form, in *Problems of Vitamins*, Vol 2, p. 269 (Leningrad, 1937).

[65] Beattie, H. G., K. A. Wheeler, and C. S. Pederson, "Changes Occurring in Fruit Juices During Storage," *Food Research*, 8, 395 (1943).

readily oxidizable substances are left in the juice. Hamburger and Joslyn[66] state that the principal reducing agents of these juices are ascorbic acid and flavanols.

The behavior of ascorbic acid in various citrus products during and after processing is of a very peculiar nature.

Immediately after processing by pasteurization, citrus juices retain from 92 to 98% of the ascorbic acid of the freshly reamed juice; however, storage temperature was found[66a] to be very important to vitamin C retention in such juices. Under refrigeration at 9° C, over 90% of the original vitamin C was retained after one year. Similar results were obtained in a survey made[66b] during commercial canning in twelve Florida canning plants showing that the average retention in unsweetened grapefruit juices was 97% of the original content.

However, at ordinary temperatures the loss of vitamin C in processed juices proceeds quickly.

A recent study[66c] made on the retention of ascorbic acid in canned juices stored for 12 months in 9 warehouses in different localities in the United States showed that the losses of ascorbic acid depend more on storage temperature than on length of the storage period. Whereas at 50° F (10° C) and at 65° F (18° C) no significant losses in ascorbic acid were indicated after 8 and 12 months, storage at 80° F showed a retention of only 73 to 77% after 12 months. Samples of grapefruit juice held at 98° F during 4, 8, and 12 months showed a retention of 75, 54, and 31%, respectively.

Orange juice which has been frozen quickly and permitted to liquefy at room temperature shows no significant loss of vitamin C.[66d]

Much work has been carried out in elucidating the mechanism of vitamin C destruction which proceeds as stated even in the absence of oxygen. At first many suggestions were made that this process is of an enzymatic nature. Szent-Györgyi[66e] suggested that, by the action of ascorbinase enzyme, ascorbic acid is oxidized thereby creating a

[66] Hamburger, J. J., and M. A. Joslyn, "Auto-oxidation of Filtered Citrus Juices," *Food Research*, 6, 599 (1941).

[66a] Ross, E., "Effect of Time and Temperature of Storage on Vitamin C Retention in Canned Citrus Juices," *Food Research*, 9, 27 (1944).

[66b] Moore, E. L., E. Wiederhold, C. D. Atkins, and L. G. MacDowell, "Ascorbic Acid Retention in Florida Grapefruit Juices," *Canner*, 98, 24 (1944).

[66c] Moschette, D. S., W. F. Hinman, and E. G. Halliday, "Effect of Time and Temperature of Storage on Vitamin Content of Commercially Canned Fruit and Fruit Juices," *Ind. Eng. Chem.*, 39, 994 (1947).

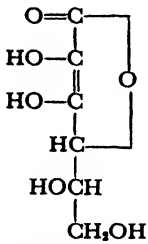
[66d] Nelson, E. M., and H. H. Mottern, "Vitamin C Content of Frozen Orange Juice," *Ind Eng. Chem.*, 25, 216 (1933).

[66e] Szent-Györgyi, A., *Studies on Biological Oxidation and Some of Its Catalysts*, Barth, Leipzig, 1937.

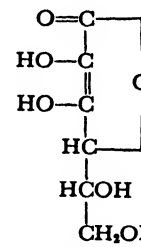
peroxide which in its turn, after being activated by peroxidase, oxidizes a flavonol (the aglucone of a glucoside) into the corresponding quinone. Enzymatic oxidation, however, is of minor importance here, because this usually occurs in the presence of oxygen while oxidation of stored citrus juices occurs even in vacuo. Furthermore, most of the oxidative enzymes are inactivated by the heat of pasteurization except perhaps the peroxidases which are more resistant to higher temperatures. On the other hand, the original, almost momentary, contact of citrus juices with air (before deaeration) may, as mentioned above (Weissberger and Lu Valle), form peroxides which supply oxygen for the subsequent deterioration of the ascorbic acid.

To prevent the loss of vitamin C in juices, a number of antioxidants have been suggested. Among the various chemical preservatives used, sulfur dioxide (SO_2) is one which exerts an antioxidant effect on vitamin C.

It has recently been reported,[67] as the result of a series of oxidation-reduction potential studies, that *d*-isoascorbic acid, the antiscorbutic activity of which is only one-twentieth that of *l*-ascorbic acid, is a strong antioxidant, for it oxidizes more rapidly than vitamin C in food products, thus protecting the latter from deterioration.



l-Ascorbic acid

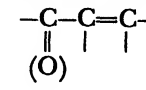


d-Isoascorbic acid

Six mg of *d*-isoascorbic acid added to 100 cc of tomato juice have been found sufficient to protect the natural *l*-ascorbic acid in the juice during storage, even under adverse conditions (at a high temperature of 49° C for 5 months); it was also effective in preventing deleterious changes in color and flavor.

Flavones such as quercetin, quercitrin, and rutin have been shown to be effective antioxidants for milk fat. While the flavanone glucoside, hesperidin, appears to have little or no antioxidant value, its chalcone is active. It is suggested[67a] that the

[67] Esselen, W. B., Jr., J. J. Powers, and R. Woodward, "*d*-Isoascorbic Acid as an Antioxidant," *Ind. Eng. Chem.*, 37, 295 (March, 1945).



group in the pyrone ring or in the open chalcone is responsible for antioxidant activity. Further research is necessary to find suitable antioxidants for processed citrus juices.

(e) Method of Preparing Ascorbic and Dehydroascorbic Acids

The original method of preparation of vitamin C from oranges described by Szent-Györgyi[68] is somewhat complicated. Many other methods have since been proposed. A patented process[69] for preparing vitamin C, starting with lemon juice, has been selected for description here.

After decitrating the juice, vitamin C is precipitated with a copper-free basic lead compound. From an acid solution of this precipitate the oily impurities are separated by means of butyl alcohol. Ethyl alcohol is then added to precipitate insoluble, physiologically inert lead salt. The solution is filtered, acetone is added to produce a further substantially inert precipitate, and the solution is filtered again. The filtrate is evaporated to dryness in vacuo adding enough $BaCO_3$ to neutralize the acid. Vitamin C is then extracted from the dry product and subsequently reprecipitated from a "solvent, of the nature of the group comprising methanol, ethanol, propanol, acetone ethyl acetate and combinations of these with petroleum ether and dioxane."

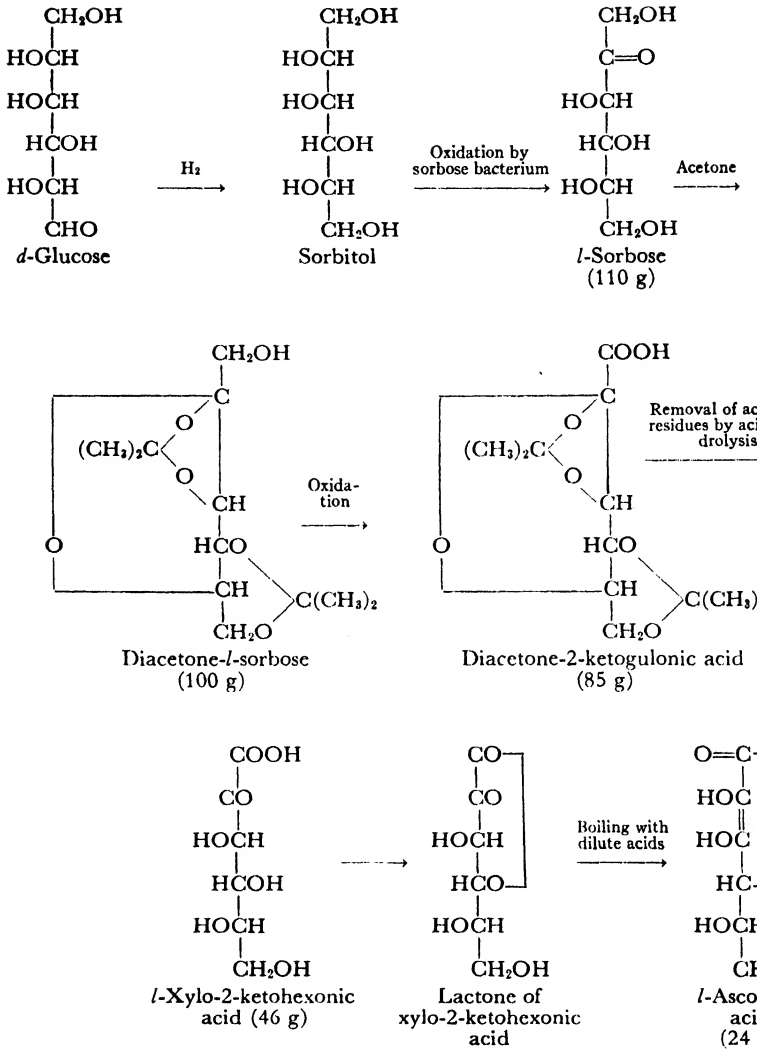
l-Ascorbic acid was the first vitamin to be prepared by total synthesis. Several different methods of synthesizing vitamin C exist but most of them have only a theoretical value, their yield being only about 0.01%. One technically possible and economically worth-while method, now largely used in industry and yielding some 15% of the original starting material, is that proposed by Reichstein *et al.*[70] Although theoretically the synthesis can begin with *d*-glucose, technically the process of artificial preparation of vitamin C starts from *l*-sorbose, according to the following scheme:

[67a] Richardson, G. A., M. S. El-Rafey, and M. L. Long, "Flavones and Flavone Derivatives as Antioxidants," *J. Dairy Sci.*, **30**, 397 (1947).

[68] Szent-Györgyi, A., "Preparation of Vitamin C from Oranges," *Biochem. J.*, **26**, 865 (1932).

[69] King, Charles G., and William A. Waugh, "Process of Preparing Vitamin C," U.S. Patent No. 2,233,417 (Sept. 18, 1933).

[70] Reichstein, T., A. Gruessner, and R. Oppenauer, "Synthese der *d*- und *l*-Ascorbinsaure (C-vitamin)," *Helv. Chim. Acta*, **16**, 1019 (1933).



A method of preparing dehydroascorbic acid is described by Fox and Levy,[71] who found that Norit charcoal rapidly converts ascorbic acid into dehydroascorbic acid apparently by direct transference of oxygen occluded on the surface of the charcoal. Very little loss occurs in this method by further destruction into the irreversible phase, and

[71] Fox, F. W., and L. F. Levy, "Reversible Oxidation of Ascorbic Acid by Means of Norite Charcoal," *Biochem. J.*, **30**, 208 (1936).

the dehydroascorbic acid prepared in this manner remains fairly stable for several days if saturated with CO_2 and kept on ice.

(f) Antiscorbutic Properties

Vitamin C deficiency in the human body causes the well-known disease scurvy, characterized mainly by hemorrhagic conditions: swollen and bleeding gums, changes in structure of the dentine in the teeth, and increased fragility of the bones. Even the hair in the coat of a shaved guinea pig ceases to grow when the animal is deprived of vitamin C. Harris[72] has briefly summarized the evidence concerning the modus operandi of vitamin C in the organism: (*1*) during infectious processes in human beings there is an increased consumption of vitamin C; (*2*) during infectious processes in experimental animals certain of the body tissues are depleted of vitamin C; (*3*) the leucocytes and certain other active cells are found to be exceptionally rich in vitamin C.

To explain the antiscorbutic action of vitamin C, Leibowitz and Guggenheim[73] have advanced the theory that the ascorbic acid acts as a detoxicant on poisons (analogous to phenol) resulting from metabolism. They have called attention to the chemical property of ascorbic acid of combining even in vitro with such toxic substances as KCN and phenol, and have shown that in all cases the detoxication resulted from a chemical combination between the poison and ascorbic acid.

The theory is supported by the well-known, but hitherto strange, fact that the biological functions of vitamin C depend on concentrations 100 to 1000 times that of other vitamins, which act only as catalysts (coferments).

However, not only scurvy, but numerous other diseases are greatly influenced by a deficiency of vitamin C. As a result of many hundreds of tests, Harris[74] and his co-workers found that groups of patients with such diseases as acute rheumatism, surgical and pulmonary tuberculosis, osteomyelitis, and rheumatoid arthritis excrete notably less vitamin C than noninfected control subjects.

The biological activity of vitamin C is measured in International Units. 1 I.U. equals 0.05 mg of pure ascorbic acid; or 1 g of vitamin C contains 20,000 I.U.

[72] Harris, Leslie J., "Vitamin C and Infection," in *Perspectives in Biochemistry,* Cambridge Univ. Press, 1937, p. 329.

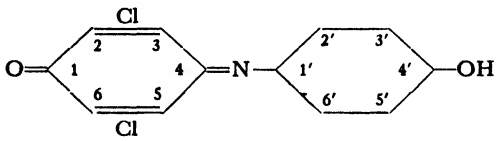
[73] Leibowitz, Dr. J., and Dr. K. Guggenheim, "On the Detoxicating Effect of Vitamin C," *Harefuah, J. Palestine Jewish Med. Assoc.,* **14,** 224 (May, 1938).

[74] *Op. cit.*

(g) Methods of Estimation

(1) THE 2,6-DICHLOROPHENOLINDOPHENOL METHOD

For a long time vitamin C was estimated only indirectly by its antiscorbutic action on guinea pigs. Tillmans and Hirsch[75] were the first to connect the reducing power of lemon juice with its antiscorbutic activity and to select a suitable specific indicator for the determination of vitamin C by direct titration. Such an indicator was 2,6-dichlorophenolindophenol:



The reaction with ascorbic acid may be thus indicated:

$$\underset{\text{Ascorbic acid}}{C_6H_8O_6} + \underset{\text{Dichlorophenolindophenol (blue)}}{O{:}C_6H_2Cl_2{:}N{\cdot}C_6H_4OH} \longrightarrow \underset{\text{Dehydroascorbic acid}}{C_6H_6O_6} + \underset{\text{Leucobase (colorless)}}{HO{\cdot}C_6H_2Cl_2{\cdot}NH{\cdot}C_6H_4{\cdot}OH}$$

To prepare a standard 0.001 *N* solution of 2,6-dichlorophenolindophenol, 242 mg of the dye are dissolved in a liter of water, as follows: the dye is placed in a small beaker and treated with successive portions of hot water, each portion being decanted and filtered into a one-liter volumetric flask; a total of about 650 cc of hot water should be thus used, washing the filter until entirely colorless. 100 cc of solution containing 0.908 g of KH_2PO_4 (primary potassium phosphate, *M*/15 solution), and 200 cc containing 2.372 g of $Na_2HPO_4{\cdot}2H_2O$ (secondary sodium phosphate, *M*/15 solution), should be added as a buffer to the dye solution; the whole is brought up to 1000 cc.

The dye solution is standardized against pure crystalline vitamin C or is titrated with sodium thiosulfate, as follows: 15 cc of the dye solution are pipetted into a 50-cc Erlenmeyer flask, 0.5 to 1.0 g of KI and 0.5 to 1.0 cc of dilute H_2SO_4 (1:4) are added, and after shaking to facilitate oxidation of KI, the liberated iodine is titrated with 0.10 *N* sodium thiosulfate, using the usual starch indicator. Each cc of 0.01 *N* iodine solution or 0.01 *N* sodium thiosulfate solution is equivalent to 0.88 mg of ascorbic acid. The factor of the dye solution is thus established. The dye solution must be kept on ice, and it is advisable to prepare it fresh very often.

The estimation of vitamin C in citrus juices is then carried out as follows: 10 cc of strained citrus juice are somewhat diluted with distilled water and titrated directly with the standardized 0.001 *N* solution of 2,6-dichlorophenolindophenol until the blue color of the dye remains constant for a few minutes. Instead of 2,6-dichlorophenolindophenol, methylene blue dye, which reacts quantitatively with ascorbic acid under the influence of strong light (300-watt lamp), can be used.[76]

[75] Tillmans, T., P. Hirsch, *et al.*, "Das Reduktionsvermoegen pflanzlicher Lebensmittel und seine Beziehung zum Vitamin C," *Zeits. f. Untersuchung der Lebensmittel*, **63**, 1, 21, 241, 267, 276 (1932); **65**, 145 (1933).

[76] Effern, J., The Estimation of Vitamin C in Milk and Milk Products, *Vorratspflege u. Lebensmittelforsch.*, **5**, 306 (1942).

(2) OTHER CHEMICAL METHODS OF ESTIMATION

Numerous methods have more recently been proposed; some, in fact, are more convenient from the standpoint of preparing the standard solution as well as of ease in reading the end point. Some of them are used with citrus products.

Ascorbic acid produces, with silicomolybdic acid,[77] colored reduction products which can be conveniently measured colorimetrically in Nessler tubes (with standards of pure ascorbic acid) or in a photoelectric colorimeter. The reagent, silicomolybdic acid, is prepared by the action of ammonium molybdate upon sodium silicate in acid solution. This reagent keeps indefinitely. The reaction is very sensitive: 0.01 mg of ascorbic acid in 50 cc of solution produces a discernible color.

A quite simple method of estimating the ascorbic acid in citrus juices is suggested by Stevens,[78] using 0.01 *N* solution of iodine. To 20 cc of juice, 4 cc of 12 *N* sulfuric acid is added to lower the *p*H of the sample to about 0.02–0.08 (lack of acid causes sluggish titration). A measured excess of the iodine solution is added together with some starch. After half a minute, the whole is titrated back with a 0.01 *N* thiosulfate solution.

A potentiometric determination of ascorbic acid in citrus juices, using a combination of I and iodate, is suggested by Ramsey and Colichman.[79] This determination is based upon a stable potassium iodate solution as the only standard oxidant and, at the same time, makes use of the specific oxidation of ascorbic acid by iodine. The standardization of the unstable 2:6-dichlorophenolindophenol is thereby eliminated.

In the presence of SO_2, vitamin C can be determined in citrus juices by the addition of acetone,[80] which combines with SO_2. The end point in this method is, however, poorly defined, since the combination of acetone with SO_2 is not very stable. Instead of acetone, formaldehyde, which is more stable, can be used. The amount of formaldehyde, however, must be varied according to the SO_2 content of the product because formaldehyde condenses with ascorbic acid, except at *p*H 0.6, and it is rather difficult to find a convenient buffer at such a low *p*H.[80a] Oxidation of SO_2 in citrus juices prior to

[77] Isaacs, M. L., "Colorimetric Determination of Vitamin C," *Ind. Eng. Chem., Analyt. Ed.*, **14**, 948 (1942).

[78] Stevens, I. W., *ibid.*, **10**, 269 (1938).

[79] Ramsey, J. B., and E. L. Colichman, "Potentiometric Determination of Vitamin C," *ibid.*, **14**, 319 (1942).

[80] Mapson, L. W., and Wakes, *J. Assoc. Chem. Ind.*, **62** (1943).

[80a] Monselise, G. G., and B. Salinger, "On the Stability of the Bisulfite-Formaldehyde and Bisulfite-Acetone Compounds at Different *p*H Values," *J. Assoc. Eng. Arch., Palestine*, **8**, 9 (Sept., 1947).

determination of ascorbic acid can be made by H_2O_2 in the presence of phosphoric acid.[81]

A survey of various other methods used for ascorbic acid determination has been made by King.[82]

3. Vitamin P

Various chemical and clinical observations made by Rusznyák and Szent-Györgyi[83] led them, in 1936, to assume that ascorbic acid is accompanied in the cell by another important and active factor. Their earlier experience showed that pure synthetic ascorbic acid was ineffective in certain pathological conditions characterized by an increased permeability or fragility of the capillary wall, such as in cases of purpura sickness, while this condition can readily be cured by the administration of paprika extracts or lemon juice. The active substance was found to be a fraction isolated from lemon juice and consisting of practically pure flavone or flavonol glucoside (citrin). Intravenous administration of 40 mg of this fraction daily to man restored in a fortnight the normal capillary resistance: spontaneous bleeding ceased, and the capillary walls lost their fragility toward pressure differences. These results suggested that this class of vegetable dyes, the flavones or flavonols, may play an important role in animal life and the authors proposed, therefore, to give the citrin the name "vitamin P" or permeability vitamin.[84]

As has already been discussed in the section on glucosides, citrin, found in citrus fruits, is a mixture of two glucosides, hesperidin and eriodictin. These are flavanone glucosides yielding upon hydrolysis hesperitin and eriodictyol, the former being a 4′-methyl ester of the latter.

Hesperidin is apparently in equilibrium with its naturally occurring isomer, an open-chain chalcone. Hesperidin chalcone forms very small yellow crystals, while hesperidin is colorless.

Further work[85] in this direction has indicated that not all members of the phenylbenzo-γ-pyran pigments are active in this respect. The flavanol quercitrin, for instance, which differs essentially from hes-

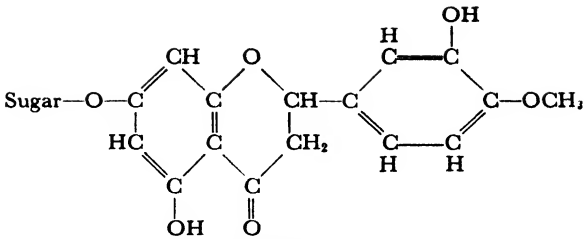
[81] Levy, *Biochem. J.*, **37** (1943).

[82] King, C. G., "Chemical Methods for the Determination of Vitamin C," *Ind. Eng. Chem., Analyt. Ed.*, **13**, 225 (1941).

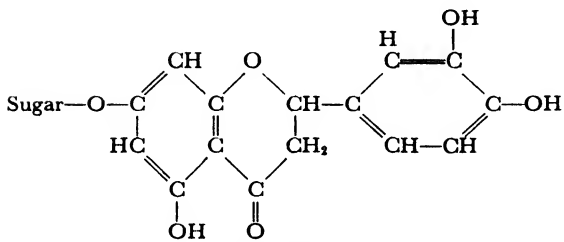
[83] Rusznyák, St., and A. Szent-Györgyi, "Vitamin P: Flavanols as Vitamins," *Nature* (London), **138**, 27 (July 4, 1936).

[84] Armentanó, L., A. Bentsáth, T. Béres, St. Rusznyák, and A. Szent-Györgyi, "Ueber den Einfluss von Substanzen der Flavongruppe auf die Permeabilität der Kapillaren. Vitamin P," *Deut. med. Wochenschr.*, **62**, No. 33, 1325 (1936).

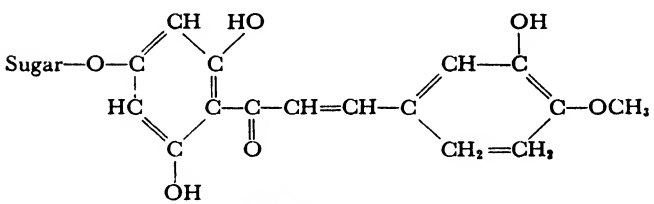
[85] Bentsáth, A., St. Rusznyák, and A. Szent-Györgyi, "Vitamin P," *Nature* (London), **139**, 326 (Feb. 20, 1937).



Hesperidin
(m.p. = 261–262° C)



Eriodictin



Hesperidin chalcone
(m.p. = 251–252° C)

peridin as far as C-2 and C-3 atoms are concerned, exerts no vitamin P activity. Moreover, the investigators came to the conclusion that experimental scurvy is the symptom of a mixed C and P avitaminosis, the pure P avitaminosis having no clinical symptoms. Lajos[86] reported that vitamin P not only increases the capillary resistance, decreases the permeability of the blood vessels, and is valuable in the treatment of vascular purpura, but is also beneficial in the treatment of hemorrhagic nephritis of different origins. Armentano[87] studied the effect of the flavones in reducing blood pressure in cats and dogs. According to their activity, he grouped the flavones in the following order: quercitrin was the most active; citrin, naringenin, quercetin,

[86] Lajos, S., "Klinische Erfahrungen mit Citrin (Vitamin P)," *Klinische Wochenschr.*, **16**, 1615 (1937).

[87] Armentanó, L., The Effect of Flavone Dyes on Blood Pressure, *Zeit. ges. Experimentelle Medizin*, **102**, 219 (1938).

6,8-oxyflavone, and rhamnetin were less active; hesperidin, hesperetin, and eriodictyol were inactive.

The many clinical successes in treatment of cases of pathologically increased permeability and fragility of the capillary walls with vitamin P permitted the vitamin nature of the flavones to appear as quite probable. However, the conclusive evidence could be arrived at only through bioassay. This attempt met with great difficulties, for there is yet no criterion for compounding a completely flavone-free diet, and the methods of the chemical determination of flavones are not sensitive enough and not specific.

The work of Szent-Györgyi and co-workers on guinea pigs has been repeated by Zilva,[88] who found that the administration of a daily dose of 1 mg of citrin, of a mixture of ⅔ mg of hesperidin and ⅓ mg of eriodictyol, or of 1 mg of purified hesperidin did *not* delay the onset of scurvy or the fatal termination of the disease in guinea pigs on a scorbutic diet.

The administration of vitamin P does not control the large subcutaneous hemorrhages characteristic of the scorbutic state, which can be arrested by a large dose of ascorbic acid alone. However, vitamin P can produce an increased capillary resistance in the scorbutic subject. A deficiency of this vitamin may exist in man even when he has been taking large doses of ascorbic acid for prolonged periods.[89]

Summarizing the more recent studies, it has been indicated that the plant pigment contained in the peel and juice of citrus fruit and christened vitamin P, is a food factor important in some cases to maintenance of normal capillary strength and permeability. It is believed that this substance, *the precise chemical nature of which remains to be determined,* acts in conjunction with vitamin C as a part of an oxidation–reduction system. The therapeutic value of vitamin P in increasing capillary resistance has now been demonstrated in man, guinea pig, rat, and mouse.[90] Scarborough is of the opinion that neither hesperidin nor hesperitin fulfill the requirements of a vitamin. They may, however, be precursors of a more active substance, possibly the chalcone, and could then be regarded as provitamins, as in the case of carotene.

[88] Zilva, S. S., "Vitamin P," *Biochem. J.*, **31**, 915, 1488 (1937); *Nature*, **140**, 588 (1937).

[89] Scarborough, H., "Deficiency of Vitamin C and Vitamin P in Man," *The Lancet*, 644 (Nov. 23, 1940).

[90] Scarborough, H., "Observations on the Nature of Vitamin P and the Vitamin P Potency of Certain Foodstuffs," *Biochem. J.*, **39**, 271 (1945).

The discrepancy between the results obtained by various investigators must still be elucidated by further research, but whatever the explanation of the activity of vitamin P may be, the fact remains that under certain conditions vitamin P derived from natural sources has a striking therapeutic effect.

The vitamin P content does not seem to be correlated with the ascorbic acid content.[91] During World War II, a process was patented in Germany for the preparation of vitamin P from citrus fruit residues by fractional extraction, first with an organic solvent for the ballast material and then with a suitable solvent for the citrin.[92]

4. Vitamin A (Axerophthol)

The orange and yellow tints of citrus peels as well as of citrus juices are due to the carotenoid pigments, which have been discussed in another section. For a long time it has been known that these pigments, found also in abundance in carrots and other vegetables and fruits, as well as in fish oils, exert a distinct effect in animal and human bodies.

In fact, an "accessory food factor," named by McCollum and Davis vitamin A, was discovered in 1913, and its relation to the carotenoid pigments was solved by Moor (1930) of Cambridge and Karrer (1931) of Zurich. It appeared that the unsaturated hydrocarbon, β-carotene, containing two β-ionone ring structures, is split by the organism to form two molecules of vitamin A which is, chemically speaking, an unsaturated alcohol. This and other carotenoids containing at least one β-ionone ring and capable of producing one vitamin A molecule are called, therefore, *provitamins* or precursors of vitamin A.

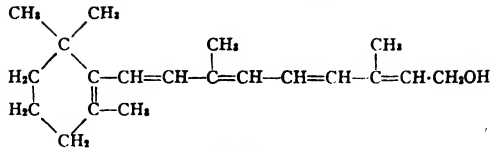
While its precursors are abundantly found in the plant kingdom, vitamin A has not been demonstrated in plants. It occurs only in the animal organism. Its structural formula has been elucidated by Karrer and is given on page 156 with the formula of β-carotene.

It is now known that vitamin A is, in fact, at least two closely related substances—vitamin A_1, predominating in the livers of salt-water fish, and vitamin A_2, in the livers of fresh-water fish. The discovery of the latter vitamin has thrown into debate some of the details of the structural formula of vitamin A.[93]

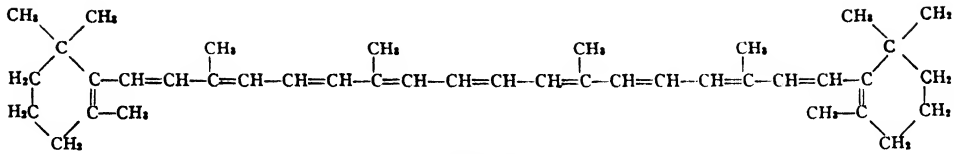
[91] Bacharach, A. L., and M. E. Coates, "The Vitamin P Activities of Citrus Fruits, etc.," *J. Soc. Chem. Ind.*, **63**, 198 (1944).

[92] Lautenschläger, C. L., F. Lindner, A. Mayer, and E. Bartholomäus, Flavanone Glucoside Preparation from Citrus Fruit, German Patent 723,225 (June 18, 1942).

[93] Embree, N. D., and E. M. Shantz, "Cyclization of Vitamin A_2," *J. Biolog. Chem.*, **132**, 619 (1940).



Vitamin A



β-Carotene

While, theoretically, β-carotene should yield two molecules of vitamin A, experience has shown that even under optimum conditions β-carotene yields at most only one molecule of vitamin A, whereas at least two molecules of other provitamins are required for the formation of one molecule of vitamin A. There apparently occurs an asymmetrical fission of the provitamin molecules.[94]

The conversion of carotenoids into vitamin A is a specific physiological process which has not yet been duplicated in vitro. It is an extraordinary reaction and is probably performed in the liver. Olcott and McCamm[95] report an enzyme, carotenase, isolated from liver extract and capable of performing this transformation even in vitro; this has, however, not been definitely confirmed.

Recently, simultaneous announcement of vitamin A synthesis has been made from both sides of the Atlantic—by Distillation Products, Inc., Rochester, N. Y., and by two Dutch chemists, J. F. Arens and D. A. van Dorp, in the laboratories of N. V. Organon Ltd., Oss, The Netherlands.

Vitamin A crystallizes in pale yellow plates; m.p. = 63–64° C. It is insoluble in water and soluble in most organic solvents. It can be distilled in a molecular vacuum still (10^{-3} mm pressure) at a temperature of 137–139° C. Carotene and vitamin A are, therefore, quite resistant to heat and are consequently not destroyed by pasteurization. However, this vitamin is very susceptible to oxidation, which can be prevented by the addition of SO_2 to the preserved juices.

Biologically, vitamin A is of importance as a growth promoter and in preventing xerophthalmia. In recent years, especially in World

[94] Underhill, S. W. F., and K. H. Coward, "Crystalline Esters of Vitamin A; I, Preparation and Properties," *Biochem. J.*, 33, 589 (1939).

[95] Olcott, H. S., and D. C. McCamm, "Carotenase; Transformation of Carotene to Vitamin A *in Vitro*," *J. Biolog. Chem.*, 94, 185 (1931).

War II, vitamin A was widely used to remedy night blindness. Deficiency of vitamin A is accompanied by a lowered resistance to infection, but this is a usual occurrence when there is a deficiency of any essential vitamin.

The International Unit of vitamin A is fixed at 0.6 gamma (γ) or micrograms ($= 0.0006$ mg) of pure β-carotene, i.e., one gram of crystallized vitamin A has a biological activity of 4,500,000 I.U. The optimum daily requirements of an average adult are about 2,000 to 4,000 I.U. of vitamin A or 3,000 to 7,000 I.U. of carotene.

Citrus juices, of course, having, on the average, only 50 to 400 I.U. of carotene, are not a very important source of vitamin A in comparison with such sources as carrots with about 4,500 I.U. per 100 g, or spinach with 20,000 I.U. A method of determination of carotene in orange juice has been described in the discussion of the color of citrus juices (see page 103).

5. Vitamin B_1 (Thiamin, Aneurin)

Chronologically, this "accessory food factor" is the first to which the name vitamin was accorded. About fifty years ago (in 1897), it was observed by Eijkman,[96] a Dutch physician working in the East Indies, that persons fed largely on rice "commercially polished," from which the embryo or germ had been removed, often contracted the disease beri-beri, long familiar to the Orient and very common among the poorer classes. It was then acknowledged that this disease originates from a dietary deficiency and is not due to infection.

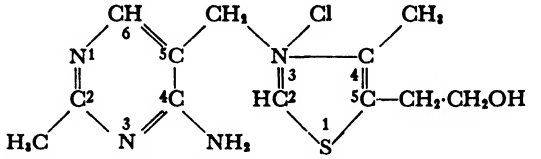
Funk[97] in 1911 was the first to give this substance, contained also in yeast, the term *vitamin,* believing it to be chemically a "vital" amine. Further investigations have shown that this factor is a complex of several vitamins, comprising at least six or seven different substances with distinct curative properties. The vitamin B complex comprises two already completely identified substances, vitamin B_1 or thiamin, and B_2 or riboflavin, both very important to the human body. The other factors, designated B_3, B_4, B_5, and B_6, have been shown to be needed for the satisfactory growth of rats and pigeons, but nothing certain is known about their effects on human beings.

Vitamin B_1 is known in Europe under the name *aneurin,* in the United States as *thiamin,* and in Japan as *orysanine.* It has been isolated as a pure crystalline compound from rice husks by Jansen and

[96] Eijkman, C., *Arch. Path. Anat. (Virchow's)*, **148**, 523, (1897).

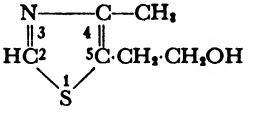
[97] Funk, C., "The Preparation from Yeast and Certain Foodstuffs of the Substances the Deficiency of Which in Diet Occasions Polyneuritis in Birds," *J. Physiol.*, **45**, 75 (1912).

Donath[98] and from yeast by Windaus,[99] and was finally synthesized by Williams[100] and his co-workers in 1934. The empirical formula of vitamin B_1 is $C_{12}H_{17}ON_4SCl$; its structural formula shows that the molecule consists of a substituted pyrimidine nucleus and a substituted thiazole nucleus linked by a $-CH_2-$ group:

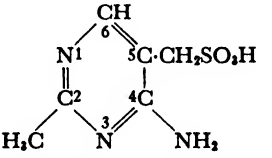


It is thus a quaternary thiazolinium salt.

By the action of sodium sulfite, vitamin B_1 is split into two compounds, a water-soluble base and an acid:

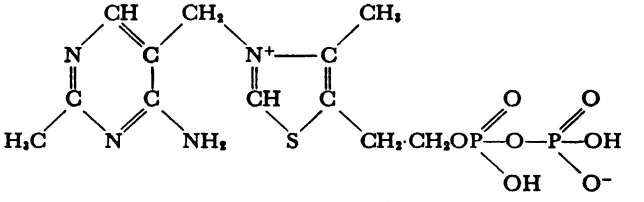


4-Methyl-5-hydroxyethyl-thiazole



Sulfonic acid of 2,5-dimethyl-4-aminopyrimidine

It is of much interest to note that the pyrophosphoric ester of vitamin B_1 is the effective group of the enzyme carboxylase, the so-called cocarboxylase, which, as has been mentioned, plays an important part in alcoholic fermentation:



Cocarboxylase

Vitamin B_1 crystallizes in colorless needles, melting at 248–250° C with decomposition. Synthetically, vitamin B_1 is prepared as its chloride-hydrochloride salt, which is soluble in water to the extent of 1 g in 1 cc and is insoluble in the usual organic solvents. It is thermo-

[98] Jansen, B. C. P., and W. F. Donath, Isolation of Anti-beriberi Vitamin, *Verslagen Koninkl. Akad. Wetenschappen, Amsterdam Wisk. natk. afd.*, **35**, 923 (1926).

[99] Windaus, A., *et al.*, "Darstellung von Krystallisiertem Anti-neuritischem Vitamin aus Hefe," *Z. physiol. Chem.*, **204**, 123 (1932).

[100] Williams, R. R., and co-workers, "Studies of Crystalline Vitamin B_1; Studies of Pyrimidine Portion," *J. Amer. Chem. Soc.*, **57**, 536, 1093, 1751, 1849, 1856, 1876 (1935); **59**, 530 (1937).

stable in strongly acid medium and can be heated to 120° C at *p*H 3.5 without decomposition. In neutral and alkaline solutions, however, vitamin B_1 is extremely thermolabile; its molecule undergoes a cleavage and its biological activity disappears. This is the reason why juices of acid fruits preserve their full vitamin B_1 potency after pasteurization, while sterilized nonacid juices and vegetables lose practically all their vitamin B_1. On the other hand, owing to the cleavage of the molecule by sulfites, as described above, preservation of juices with SO_2 entirely destroys the vitamin B_1 in contrast to vitamin A and vitamin C which, by the reducing action of SO_2, are prevented from oxidation. However, as already pointed out early in this section citrus juices are not important sources of vitamin B_1.

The vitamin B_1 content of citrus fruits measured recently showed the following averages in γ per 100 cc of juice:[101]

Valencia oranges	70
Pineapple "	65
Seeded grapefruit	35
Seedless "	32
Tangerines	69

Oranges (size 176 per case) contain 80–91γ, grapefruit (size 70) 81–85 γ, and tangerines (size 150) 45 γ of vitamin B_1 per fruit.

This vitamin is widely distributed in the plant kingdom. Most leaves contain about 25 I.U. per 100 g. It is found in abundance in the husks of rice, in yeast, in the embryo of wheat, etc.

Serious deficiency in vitamin B_1 results in beri-beri; it also causes peripheral neuritis of the feet and legs, polyneuritis, and a total lack of appetite, and seriously hinders normal growth. The absolute necessity of vitamin B_1 for the organism apparently depends on the fact that it is indispensable for the decomposition of carbohydrates.

The International Unit of vitamin B_1 is accepted at 3 γ of the pure crystalline compound. The hydrochloride derivative, now synthetically produced in very large quantities, has a biological activity of 330,000 I.U. per gram.

The average daily requirement of vitamin B_1 for adults is about 500 I.U.; however, this depends not only on the age and weight of the person but also on the energy value of his food.

6. Vitamin G (B_2), Riboflavin or Lactoflavin

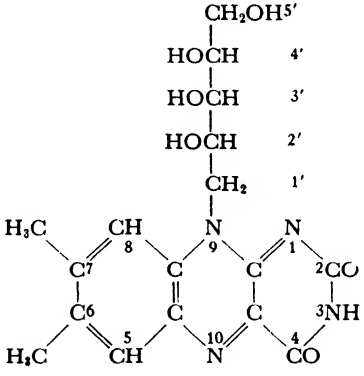
The second important constituent of the vitamin B complex is B_2, or the antipellagra vitamin. However, it has recently been shown

[101] Bailey, I., and A. W. Thomas, "Thiamine and Riboflavin Contents of Citrus Fruits," *J. Nutrition*, **24**, 85 (1942).

to contain at least two different vitamins: the one has been christened a new vitamin G and is known in Europe as *lactoflavin;* the second is nicotinic acid, which is actually the factor that cures or prevents pellagara and has, therefore, been given the name of vitamin PP (pellagra-preventing).

Vitamin G was first discovered by Warburg and Christian[102] as a constituent of the so-called "yellow oxidation enzyme." It was obtained in a crystalline state by Kuhn,[103] who, together with György, established its nature as a vitamin. The structural formula of this vitamin was elucidated by Karrer,[104] who also succeeded in preparing it synthetically.

Chemically, vitamin G ($C_{17}H_{20}N_4O_6$) is a dimethyl derivative of isoalloxazine and has a five-carbon sugar, *d*-ribose, attached as a side chain to the nitrogen in the 9-position.



Pure vitamin G crystallizes in fine orange-yellow needles melting at 282° C with decomposition. It is soluble in water to the extent of 12 mg in 100 cc. It is only slightly soluble in a few, and practically insoluble in most, of the organic solvents. Vitamin G is very soluble in alkaline solutions, and in this form it shows considerable optical rotation ($[a]_D^{20°} = -114°$), while in neutral or acid media its optical activity is exceedingly small.

One of the most striking properties of riboflavin is its sensitiveness to light: when illuminated in neutral solution, the ribose (sugar)

[102] Warburg, O., and W. Christian, "Über ein neues Oxydationsferment und sein Absorptionsspektrum," *Biochem. Z.*, **254**, 438 (1932); "Über das gelbe Oxydationsferment," **257**, 492 (1933).

[103] Kuhn, R., P. György, and T. Wagner Jauregg, "Über Lactoflavin den Farbstoff der Molke," *Ber.*, **66**, 1034, 1950 (1933); "Flavine und Flavoproteine als Vitamin B_2," *Z. Physiol. Chem.*, **223**, 241 (1934).

[104] Karrer, P., and co-workers, "Synthesen von Flavinen IV," *Helv. Chim. Acta*, **17**, 426, 522, 1435 (1935).

residue is completely split off. Riboflavin is essential in the metabolism of carbohydrates. Its main activity is attributed to its close relationship to the yellow enzyme system of Warburg. In this enzyme system (which comprises also adenylic acid) the riboflavin apparently participates as a hydrogen carrier (dehydrogenase), reversibly accepting and donating two atoms of hydrogen, which are added to its 9 and 10 positions. This behavior, as in the case of cocarboxylase (see under vitamin B_1) has proved a relationship between enzymes and vitamins.

Serious deficiency in vitamin G results in impaired growth and may cause inflammation of the digestive tract, with marked diarrhea. Subsequent disturbances occur in the brain and other parts of the central nervous system.

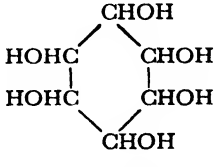
The proposed International Unit for G is 5 γ (0.005 mg) of the pure crystalline vitamin. The daily requirement of an adult is 2 to 3 mg of vitamin G. The best sources of this vitamin are green, leafy vegetables such as cabbage and spinach, also tomatoes and other vegetables. Citrus fruits contain only 20 to 100 γ (micrograms) of riboflavin per 100 g of fresh fruit. Recent measurements have shown the average contents of vitamin G in γ per 100 cc of Florida citrus fruits to be as follows:

Valencia oranges	15
Pineapple "	16
Seeded grapefruit	12
Seedless "	11

Oranges have 20 γ and grapefruit about 27 γ of vitamin G per average fruit.

7. Inositol ($C_6H_{12}O_6$)

Although it has not yet been given the name of vitamin, inositol has long been known as a very active growth-promoting factor, under the name biose I. Inositol has already been mentioned under "Photo-



synthesis." It is a hexahydroxycyclohexane, widely distributed in plants, partly in the free state and partly esterified with phosphoric acid as phytic acid.

Inositol can theoretically exist in eight *cis-trans* configurations, but only one of them can be optically active.

Inositol has a sweet taste. It crystallizes from water at above 50° C in anhydrous form (m.p. = 225° C) but below 50° C as dihydrate (m.p. = 215° C). It is insoluble in absolute alcohol or in ether, and has been found in small quantities in the juices of all citrus fruits.

Nelson and Keenan[105] have isolated *i*-inositol in the juice of oranges, lemons, and grapefruit and have found 0.0047, 0.0124, and 0.0028%, respectively.

Its biological action on human beings is yet unknown. Inositol deficiency in mice and rats brings about a complete denudation of skin.

E. SEEDS

The last component of the citrus fruit, the seeds, are usually situated in two rows inside the endocarp just around the central axis. Each segment contains several seeds in the part of the carpel wall adjacent to the white core.

There are, however, citrus varieties which have no seeds at all, such as the Marsh seedless grapefruit, the Jaffa (Shamouti) orange, and the navel orange. The pollen and many of the ovules being imperfect in these varieties, fertilization does not take place, and the undeveloped ovules may appear as two rows of dark flecks in lieu of the seeds.

Citrus seed contains a considerable amount of proteins which sometimes totals as high as 16%. Some indications show[106] that citrus seed meal contains only one type of protein—namely, a definite globulin. The interesting feature of these seeds is their comparatively high content of an oil which is of some commercial interest.

Analysis of fresh seeds shows, on the average:

Analysis	Per cent
Water	44.67–39.06
Oil	19.52–25.50
Nitrogen	1.71– 1.38
Crude protein	10.68– 8.66
Fiber	4.68– 7.23
Non-nitrogenous substances	18.70–17.25
Ash	1.75– 2.30

[105] Nelson, E. K., and G. L. Keenan, "*i*-Inositol in Citrus Fruit," *Science*, **77**, 561 (1933).

[106] Saunders, F., "Studies in Proteins. II, Concerning the Uniformity of the Protein Fraction Extracted from Orange Seed Meal by Salt Solutions," *J. Amer. Chem. Soc.*, **53**, 693 (1931). Saunders, F., and L. K. Rotha, "Studies in Proteins. III, Uniform Solubility of the Protein Fraction of Orange Seed Meal in Solutions of Various Sodium Salts," *ibid.*, **54**, 342 (1932).

However, when dried, the seeds contain[107] between 30 and 35% of oil, and occasionally as much as 50 to 57%.[108]

The oil can be expressed or extracted by solvents in the usual way, and although it has a somewhat sharp and bitter taste it is acceptable as food after appropriate purification and bleaching. Some authors designate citrus seed oil as a semidrying oil with composition and properties approximately similar to those of cottonseed oil. It is suggested that this oil contains an excessive percentage of liquid glycerides, while the solid glyceride content is below 28%.

Table XXI presents a collection of comparative data made by several investigators.

TABLE XXI

Constants of Seed Oils from Different Citrus Varieties

	Lemon seed oil 1920[109]	Lime seed oil 1920[110]	Citron seed oil 1921[110]	Spanish orange seed oil 1943[111]	Valencia orange seed oil 1944[112]	Orange seed oil (Japan) 1918[113]	Kumquat seed oil (Japan) 1918[113]
Specific gravity, $d_{20°}$	0.916–0.918	0.930	0.930	0.9203	0.9153	0.9200	0.9223
Refractive index, $n_D^{25°}$			1.4757	1.4666	1 4686	1.422	1.473
Relative viscosity $\frac{\text{oil } 60°}{\text{water } 20°}$				17.2			
Acid no.				1.254		0.90	1.05
Saponification no.	190–191	177.3	177.3	191.15	197.5	192.7	193.4
Iodine no.	103–108	83.8	83.8	103.5	101.7	105.27	113.03
Unsaponifiable matter %				0.45	0.95	1.22	1.22
Hehner no. (iodine no. of fixed acids)	94			95.27			
Freezing point	−5° to −6°	−2.05° to −3°	−2.05°				

The fatty acids of citrus seed oil have been found to consist of:

Saturated acids:		Unsaturated acids:	
palmitic	20.7%	linolenic	0.6%
stearic	4.7%	linoleic	36.5%
arachidic	0.9%	oleic	36.6%

Citrus seeds are easily collected in abundance from the residues of

[107] Bertolo, P., Lemon Seed-Oil, *Giom. chim. applicata*, **1**, 54 (1920).
[108] Diedrichs, A., Orange Seed-Oil, *Z. Nahr. Genussm.*, **27**, 133 (1914).
[109] Bertolo, P., *op. cit.*
[110] Fish and Gattefossé, "L'huile de Graine Citron de l'Afrique Equatorial," *Mat. grasses*, **12**, 5371 (1920); *Parf. Moderne*, **13**, 185 (1920).
[111] de Muigo, M., O. Ferñández, and A. Toledono, Chemico-analytical Study of Orange Seed Oil, *Anales fis. quim.* (Spain), **39**, 181 (1943).
[112] Van Atta, G. R., and W. C. Dietrich, "Valencia Orange Seed Oil," *Oil and Soap*, **21**, 19 (1944).
[113] Kobayashi, I. S., *J. Soc. Chem. Ind.* (Japan), **21**, 1235 (1918).

washing in the preparation of citrate of lime from lemons, or from the pulp of filtered-off juices (in the finisher), and can be separated from the adhering pulp by washing in a stream of water. The seeds should be dried immediately; otherwise they become moldy. Processes of extracting the oil from citrus seeds are not described in this book, for they can be found in any treatise on the manufacture of vegetable oils. Oil from lime seed is used in Trinidad for the manufacture of soap; formulas and practical methods for the saponification of this oil have been worked out by Collens and Warneford.[114]

It has already been mentioned that the inner seed coat of citrus fruits possesses a high activity of peroxidase. Lemon seeds, which are available in quantity, may present a good source for the large-scale production of this enzyme.[115]

The crude oil, which is now commercially produced in Florida, has an extremely bitter taste. The bitter principle, identified as limonin ($C_{20}H_{30}O_8$), has been isolated[115a] from the soapstock obtained in refining the crude oil.

[114] Collens, A. E., and F. H. S. Warneford, "The Manufacture of Soap from Lime-Seed Oil," *Tropical Agr.*, **61**, 306 (1923).

[115] Davis, W. B., "Distribution and Preparation of Citrus Peroxidase," *Am. J. Botany*, **29**, 252 (1942).

[115a] Nolte, A. J., and H. W. von Loesecke, Grapefruit Seed Oil—Manufacture and Physical Properties, *Ind. Eng. Chem.*, **32**, 1244 (1940).

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PART II

TECHNOLOGY

CHAPTER V

PREPARATION OF THE FRUIT AND EXTRACTION OF CITRUS OILS

A. PREPARATION OF FRUIT FOR PROCESSING

1. Flow Sheet for the Manufacture of Citrus Products

While citrus fruits have been known for many generations, products made from them, in contrast with many other fruits, are of only recent development. Grape and apple products, wines, cider, etc., have, on the other hand, been known since ancient times.

The reason for the belated popularity of citrus products should be sought in two main factors: First, the change in the nutritional habits of the consuming public, coupled with the general development of new chemurgical methods of utilization, has diverted public interest from fermented products to natural ones and has, in general, encouraged the consumer to become more fruit conscious. Secondly, while all other fruits are more or less homogeneous, having usually a very thin skin and sometimes a stone or pip (such as apples, pears, stone fruits, and deciduous fruits), citrus fruits are very complex in structure, having a series of comparatively thick skins and envelopes; the flavedo, the albedo, the locular walls of the segments, the pulp consisting of the juice sacs, and the pips comprising cotyledons and germ, each having a different composition and structure.

Therefore, while practically all other fruits can be easily worked in a pulper, hasher, or hydraulic or continuous press, etc., to obtain the final products, the utilization of the citrus fruit presents great technical problems, due to the necessity of first separating the widely different components.

Moreover, when an apple or a pear, for instance, is mature, the entire fruit is fit for industrial utilization. In this respect citrus fruits are quite different: the best time for extracting the essential oil, for example, is when the fruit is still unripe, whereas the juice is not yet suitable for consumption; or again, pectin is best worked from green fruit, while the same peel, when the season is well advanced, will

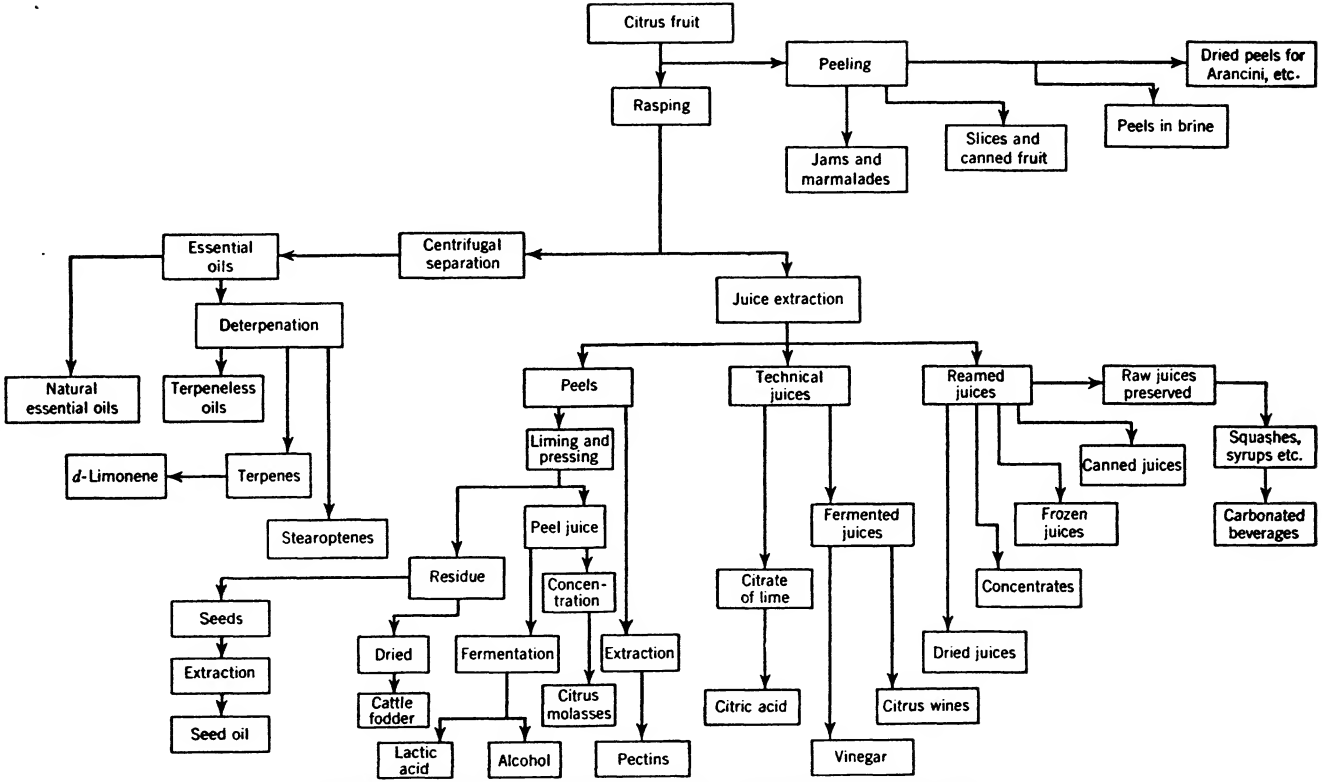


Fig. 22. Flow sheet showing a complete utilization of citrus fruits.

deliver much more alcohol on fermentation. These factors have an important bearing on the economic side of production: nature has stipulated fixed quantities of oil, juice, peel, alcohol from sugars, etc.; the requirements of the consuming markets, however, do not coincide with the proportions ordained by nature. A glut on the market of essential oils, for instance, does not parallel the demand for concentrated juices, etc.

Many factories utilize the fruit only partly. For instance, citrus jams and marmalades are produced in factories handling numerous other fruits. Some fruit and vegetable canning plants also can grapefruit slices. In such cases, the citrus fruits are not completely utilized. All components are fully utilized only by comparatively large enterprises. Citrus products manufacture, being in most parts of the world only a seasonal industry, is often allied with the production of other fruit or vegetable products, which fill in the gaps formed by the "dead" season. This factor is especially important in localities where seasonal labor is not available.

When a citrus products factory is planned, it is important, therefore, to decide first upon this major production policy. The selection of products is, of course, conditioned by the market possibilities and many other economic factors.

The accompanying flow sheet (Fig. 22) shows the general lines followed in complete utilization of citrus fruits. Some deviations from this general scheme, as well as comprehensive flow sheets showing details in the production of individual substances, will be presented in the discussion of the technology of each product. Although the following review covers the entire field, the reader may select for himself those products of practical interest to him.

While, as previously mentioned (page 19), culled fruits are utilized chiefly in the production of by-products, World War II has taught us that conversion of a considerable part of the total crop into easily preserved products occupying less shipping space is sometimes necessary. Due to recent developments in chemurgy, the trend of agriculture today is to select crops especially adapted for industrial utilization, and although citrus fruits are best grown primarily for direct consumption, future development can bring about a considerable change in this respect: it may be found more appropriate to convert greater proportions of the total crop into concentrated juices, for instance, or similar products.

The main principle in the organization of a citrus products industry is the utilization of the entire fruit. The extraction of only one

product does not prove remunerative. Experiments in the manufacture of citrus products conducted in California during the past 45 years have led only recently to the same conclusion. The first plant to utilize citrus fruit as raw material was erected in National City, near San Diego, in 1899; it has since closed down and reopened five or six times, each time under new proprietors, until finally it was recognized that this industry could succeed only if the entire fruit, from peel to seed, was completely exploited.

The citrus products industry in the United States benefits greatly from the numerous chemical laboratories and research institutes, either private and governmental, or by belonging to cooperative societies of citrus growers. These institutions have prepared the ground for the development of a huge industry and continue to search for new possibilities.

2. Maturity Test

Before the exact date for starting the processing of certain citrus fruits is determined, it is most important to ascertain whether the fruit is sufficiently mature. Even then, it will perhaps be necessary in the course of the season's production to blend some of the products obtained in the early stages of the working season with those from the end of the season, to insure a standardized product. It is certain, however, that unripe fruit gives poor results, especially as far as juice is concerned. Periodical maturity tests are, therefore, an important requirement of every factory.

As previously mentioned (page 34), the color of the skin alone is not a dependable guide to ripeness or palatability of citrus fruit. The sugar-acid ratio is, therefore, customarily accepted in the citrus industry as the standard of maturity. The juice of citrus fruit contains in solution mainly sugars (both sucrose and glucose) with smaller amounts of organic acids (chiefly citric), and, in addition, some soluble mineral matter or ash, with negligible quantities of other organic substances, such as vitamins, glucosides, enzymes, etc. Collectively, these substances are referred to as "total soluble solids."

The total soluble solids are estimated by floating a Brix hydrometer in the juice, and, since sugars are the most important of these soluble substances (at least in oranges, grapefruits, and tangerines), this test is popularly spoken of as the "sugar test." Acidity, on the other hand, is determined by titration with 0.1*N* NaOH in terms of citric acid. The proportion of total soluble solids to acidity gives the so-called "maturity ratio." For example, if a sample of freshly ex-

pressed orange juice shows 10.4% total solids by Brix and 1.3% citric acid, the maturity ratio will be 10.4/1.3 or 8:1.

In the existing regulations concerning citrus maturity standards, the stage of maturity legally required for picking is often thus expressed. California, for instance, requires a minimum ratio of 8:1 for oranges and 7:1 for grapefruit, whereas the South African standards are 5:1 for seedling oranges, 5.5:1 for Valencias, and 6:1 for navels; the South African ratios are evidently very low. In Palestine, the ratio of 8:1 is adhered to.

A convenient means for a quick determination of total solids is the portable pocket refractometer, such as is used in the sugar industry

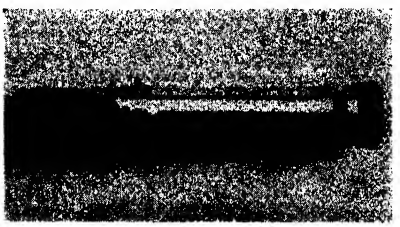


Fig. 23. New model of pocket refractometer by E. Goldberg (Tel-Aviv).

for estimating the ripeness of beets, or in the grape and tomato industries. It has been found that solutions of practically all sugars in equal percentage composition by weight give the same refractive indices. A determination of the index serves, therefore, as a measure of the dissolved sugars, even though the solution may contain a mixture of several different sugars. The usefulness of the refractometer has been extended by the discovery that the refractive index of impure sugar solutions—even though the impurities may consist of mineral salts—is a reliable measure of total solid substances and much superior to the method of analysis by density measurements.

A new model of a pocket refractometer is shown in Figure 23. It was developed in 1944 by Professor E. Goldberg, formerly of Zeiss and now in Tel-Aviv (Israel), and is specially adapted to fruit juices and concentrates. One drop of the juice spread over the polished face of the fixed prism will show the refractive index immediately, or, using the minute scale inside the ocular, the concentration of the total solids. The refractometer is easily cleaned, practically unbreakable, and very convenient to use in the citrus grove, in the laboratory, and in routine work in the plant.

The refractometer described above can, of course, be used freely as

an immersion instrument, the reading being taken as the apparatus is dipped in a small beaker containing the juice. This method is much preferred when the thin film of substance smeared over the prism may evaporate too quickly, thus changing the refractive index.

Since the refractive index varies inversely with the temperature, it is very important to maintain uniform conditions during measurements and to apply the temperature corrections if necessary.

It has been shown previously (page 96) that citrus juices have a tendency to develop a bitter taste on aging—a tendency which decreases with the maturity of the fruit, due to the fact that the glucoside content of citrus fruit gradually decreases on ripening. For lack of a better method of testing the ripeness of citrus, the sugar-acid ratio must be accepted by the producer of citrus products. However, standards should be fixed for every variety and for each locality. They should, moreover, be verified every season, for climatic or other conditions may require some deviations from the accepted standard.

The acidity test alone is considered by some a sufficient basis for determining the maturity of citrus, for, while the sugar fraction increases during ripening, it is subject to some fluctuations, whereas the acid content decreases very uniformly throughout the season. In Australia, for instance, the stage at which oranges are usually considered no longer sour to the taste is when not more than 23 cc of 0.1*N* NaOH are required for neutralizing 10 cc of juice, i.e., a total acidity of about 1.60%. However, while this may be true in particular regions, it is very doubtful that this system of maturity testing is generally applicable.

On the other hand, measurements for strongly acid citrus fruit, such as lemons and limes, are, of course, of a considerably different nature: these fruits contain very little sugar and are valued mainly for their acid content. Therefore, the tests for acidity and probably for percentage of juice are those of chief interest to the manufacturer. The content of acid as well as of essential oil is always greater at the beginning of the season. Lemons for citric acid and oil manufacture are, therefore, more valuable at the early stages of their ripeness, while lemons and limes should be processed for juice later in the season.

The foregoing are examples of factors which the manufacturer should consider for a final decision on the working plan for a particular season in a given locality.

Table XXII shows, as an example, the composition of Valencia

oranges of various sizes. These fruits have been picked simultaneously from a single grove.[1]

TABLE XXII

Composition of Valencia Oranges of Various Sizes

Size	% by weight			Composition of juice				Wt. per orange, g	Volume of juice, cc/orange	Gallons (U.S.) of juice/box
	Peel	Pulp	Juice	Sp. gr., 20/20	Balling degree	Acidity calcd. as citric, %	Ratio solids to acid			
100	45.2	8.4	46.4	1.0414	10.35	0.857	12.1	308	137.5	3.6
126	43.2	7.2	49.6	1.0425	10.60	0.869	12.2	280	133.0	4.4
150	41.9	7.1	51.0	1.0479	11.90	0.892	13.35	230	111.5	4.4
176	43.2	6.7	50.1	1.0510	12.65	0.893	14.15	201	95.8	4.4
200	40.6	8.3	51.1	1.0527	13.05	0.908	14.3	178	86.4	4.6
216	43.2	6.5	50.3	1.0551	13.60	0.898	15.3	157	75.0	4.3
252	44.6	7.0	48.4	1.0572	14.10	0.965	14.65	143	65.7	4.4
288	42.1	8.2	49.7	1.0576	14.20	0.972	14.6	116	59.4	4.5
344	40.0	9.0	51.0	1.0591	14.55	1.012	14.35	103	49.6	4.5

It can probably be assumed that certain variations with time occur in the composition of citrus fruit in a given locailty. Measuring a limited number of samples of Valencia oranges in the interval from 1936 to 1941, MacDowell[2] found that the total solids increased from 11.32 to 12.37% and the ascorbic acid content from 48.9 to 53.1 mg per 100 cc. In mid-season oranges, the increase was from 56.3 to 64.5 mg.

3. Receiving and Storing of Fruit

Although rather expensive, the best method of delivering fruit to the factory is, indisputably, in field boxes. Delivery in bags should not be permitted, as some of the fruit is always crushed and deformed. Moreover, storing fruit in bags, even for comparatively short periods, is detrimental, owing to the respiratory activity of the fruit, and causes a considerable amount of waste. Under the best conditions, if work is carried out in shifts and delivery is effected only during the day, the fruit is kept at the plant for at least six hours before processing. Usually, fruit allowed to stand over the weekend, in addition to the time elapsing after picking, remains in storage a total of forty more hours following its arrival from the packing house.

The field boxes should be stacked at a convenient height, and pro-

[1] Joslyn, M. A., and G. L. Marsh, *Calif. Citrograph*, **23**, 240 (March, 1938).

[2] MacDowell, L. G., "Changes in Florida Fruit in Recent Years," *Citrus Industry*, **26**, 8 (1945).

tected from the sun if storage is outside the main building. In the absence of field boxes, the best procedure is to deliver the fruit loose

Fig. 24. Open fruit bins and roller conveyor.

in large trucks and to store it in bins or tanks, in which the fruit must in no case be piled more than one meter high. The objection to such

Fig. 25. Fruit receiving and storage bins.

open tanks lies in the difficulty of unloading them in order to "feed the line." (See Fig. 24.)

The best method for storing very large quantities of fruit consists

of a series of high rectangular bins with sloping partitions to minimize the pressure caused by upper layers of fruit on the lower layers. The bottom of these bins is an inclined plane, permitting the fruit to roll without difficulty to the feeding conveyor. Such bins are usually built of wooden planks with slits to provide ample ventilation. Figure 26 shows diagrammatically the best arrangement for storage bins, for purposes of unloading and returning of waste.

After going through the weighbridge, the loaded trucks arrive at a specially built concrete platform, inclined to permit the quick empty-

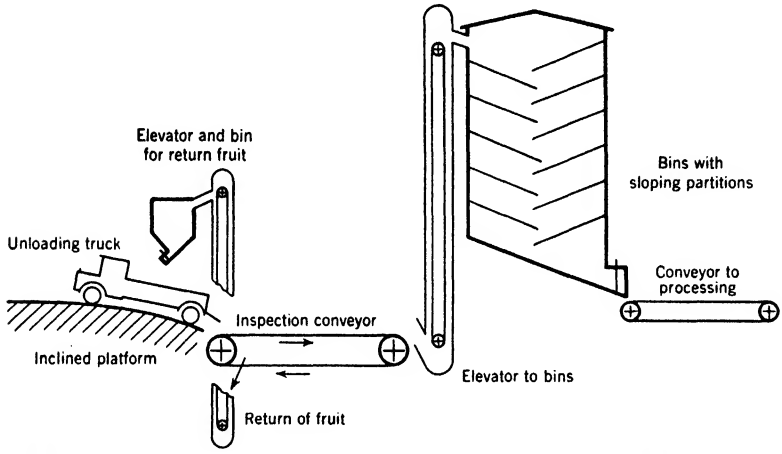


Fig. 26. Diagram showing arrangement for receiving and storage of fruit.

ing of the fruit through the back of the truck (Fig. 25). The fruit so dumped is carried by a roller conveyor, where sand, leaves, and trash fall to the ground. The fruit is caused to rotate continuously on the roller conveyor and the operators can easily cull out all rotten or undesirable fruit. The discarded fruit can be carried back by the returning side of the conveyor and subsequently elevated to a bin situated above the discharging car or truck, as shown in Figure 26, to be eventually returned to the same truck before it leaves the factory yard. At the other end of the roller conveyor, sound fruit is discharged into a second elevator and carried to the top of the bins, which are gradually filled. Each bin is provided with a sliding door which permits its gradual emptying into the "line."

Whatever method is used, the fruit should be received and stored apart from the extracting and processing rooms, to reduce the danger of infection.

4. Feeding the Line and Inspection

Whether brought to the inspection table, to the washer, or into the oil extracting machines, the fruit must be conveyed by some means. Conveyors must be carefully chosen to prevent injury to the outer skin (flavedo) of the fruit and to avoid loss of essential oil. Belt conveyors made of canvas or balata usually serve the purpose. They should be strongly built and made to suit the required capacity. If the fruit is first inspected, a roller conveyor is preferable. The rotating rolls turn the fruit during conveyance, permitting the inspectors to see the fruit from all sides.

Fig. 27. Fruit-conveying water channel and inspection wire netting.

In factories where oil extraction precedes the burring of the juice and is worked on whole fruit, the fruit is preferably fed into a water channel built of concrete or metal, in which a strong flow of water carries the fruit to the inspection belt and from there into the elevator supplying the oil machines. The inspection belt in this case may consist of a woven wire net, permitting the water of the channel to run through while the fruit is carried along. An arrangement of such a water-conveying channel and inspection wire netting is shown in Figure 27.

Citrus fruits used for the manufacture of by-products are mainly culls and usually come from the packing house. As already stated, culls, in general, are not an inferior quality of fruit; they are mostly

oversized, undersized, or otherwise unfit for packing due to slight injury or any defect in the outer skin. Inspection is, therefore, necessary on the fruit intended for processing in order to eliminate specimens that may impair the quality of the product. Fruits showing any signs of blue or green mold, brown rot, infection by the Mediterranean fruit fly, or any other disease must in no case be permitted into the line.

Defective fruit must thus be removed by a few specially trained workers and thrown into boxes assigned for the purpose, which should be emptied periodically and not be allowed to decay in the vicinity of the sound fruit. This first general inspection for quality is usually effected before washing the fruit or extracting the oil. However, further along the line, additional sorting is done. In the first place, some of the fruit is bruised or crushed during the process of extraction, and such fruit is eliminated after leaving the washing equipment. Unsound fruit should again be looked for on the belt carrying the cut halves, as occasionally fruit infected with brown rot inside the pith becomes evident, although, before cutting, such fruit has externally shown no signs of defect.

5. Washing and Sizing

The next operation is generally washing the fruit, although extraction of essential oils, if carried out on whole fruit, is usually effected before the washing. The process of extracting oil from whole fruit is described on pages 184–190, but it should be mentioned here that this method offers the best opportunity for a thorough washing and scrubbing of the fruit, since water jets are employed to carry away the extracted oil. After such procedure, an efficient rinsing of the fruit is sufficient.

However, if the oil is not extracted prior to juice reaming, the fruit must be thoroughly washed before cutting into halves, for it often arrives at the factory dusty and contaminated with foreign matter, such as dirt, smut, or remains of the spray adhering to the skin. Washing the fruit is essential not only to improve the flavor and appearance of the product, but also to facilitate preservation by removing mold spores, yeast, and other harmful microorganisms.

Washing is done in specially designed citrus washing machines consisting of a soaking tank, provided with suitable revolving paddles continuously dipping the fruit into the water without injuring it, and an immersed inclined conveyor carrying the fruit to the cutter. In some cases compressed air is blown into the water tank to

create agitation for efficient cleaning. Sometimes revolving brushes are used, but if they are tough they may injure the oil cells; soft brushes, on the other hand, are not very efficient when immersed in water.

An efficient washer with good brushes is made by Food Machinery Corporation (Fig. 28). The fruit passes from the soaking tank into a series of transverse brushes to which a regular flow of soap solution or a suitable detergent is supplied. As it passes from one set of rotat-

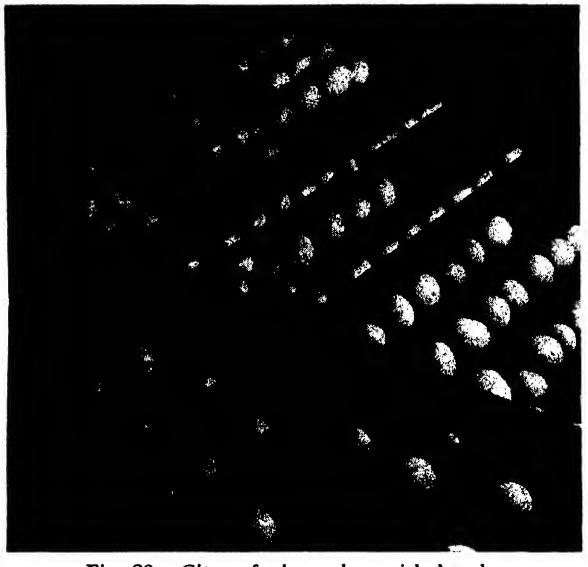


Fig. 28. Citrus fruit washer with brushes (courtesy Food Machinery Corporation, Dunedin, Florida).

ing brushes to the next, the fruit is thoroughly scrubbed and cleansed; it finally receives a spray of chlorinated water and is then rinsed with jets of pure cold water. Brushes with a continuous spiral groove agitate the fruit during rotation.

The water in any washer must be changed continuously, fresh water coming in while dirty water is carried away by the overflow. To minimize the microflora, washing is best done by adding to the water some germicidal and detergent preparation. Of these the chlorine compounds—hypochlorites or chloramine—in a solution containing 100 to 200 ppm of available chlorine, are most effective.

A process has recently been patented[3] by which whole citrus fruit

[3] Ducker, L. F., and G. A. Little, "Fruit Juices such as Orange and Grapefruit Juice," U.S. Patent No. 2,328,265 (Aug. 31, 1944).

is subjected to a 12.5–18.5% aqueous borax solution at 150–170° F for so short a time that the heat is claimed not to penetrate to the interior of the fruit. The fruit is then rinsed with cold water and the peel surface dried prior to juice extraction. Before such a process is adopted, it should be ascertained that the elevated temperature does not affect the peel oil.

To remove excess moisture after the washing process, slowly revolving brass roll eliminators take the place of the brushes at the end of the washing line. Brass has been found to have a greater affinity

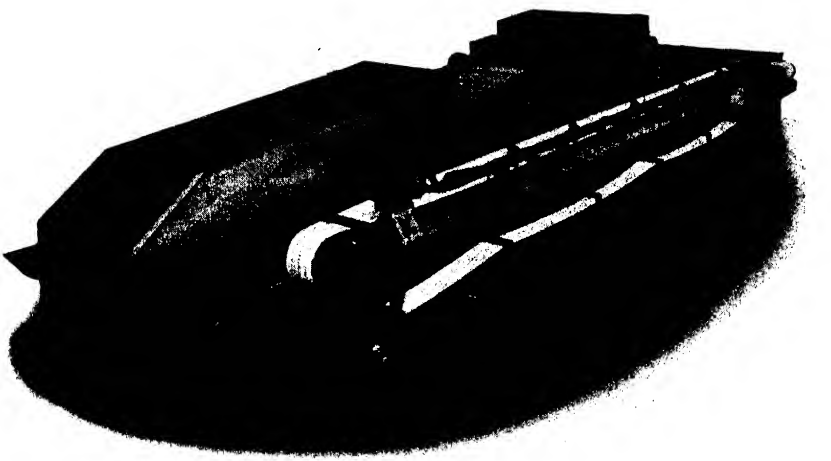


Fig. 29. Citrus fruit sizer
(courtesy Food Machinery Corporation, Dunedin, Florida).

for moisture than do the skins of fruit, and the moisture thus separated is removed by squeegees beneath the rollers.

Final inspection is recommended at this stage before the fruit is halved. If automatic juice extractors are used, the fruit should be sorted according to size. At least three sizes are usually required: small, medium, and large. The simplest method is to let the fruit run through two slats, so placed with relation to each other as to form a V—with a gradually widening slit between them. Special machines are available for this purpose, consisting, in the main, of a transversely inclined belt on one side and a revolving metal roller on the other (Fig. 29). When the fruit arrives at a spacing permitting it to drop through, it rolls into a separate bin for each size. For fruits which are not perfectly spherical (as, for instance, grapefruit, the

Spanish ovals, or the Jaffas—varieties oval in shape), exact sizing presents considerable difficulties. A convenient apparatus for measuring the diameters of citrus fruit (Fructuometer) is shown in Figure 30.

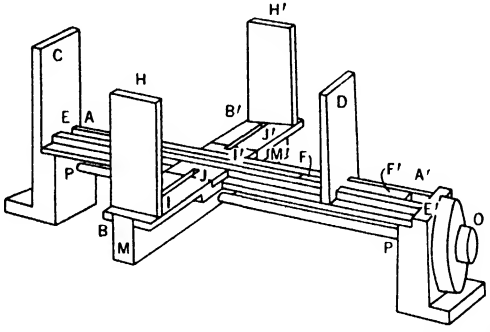


Fig. 30. Fructuometer, apparatus for measuring the diameters of fruit (after J. D. Oppenheim, *Hadar*, 8, 255 [1935]).

B. EXTRACTION OF CITRUS OILS

1. Methods of Extraction

Before proceeding further with the utilization of citrus fruits, the manufacturer must decide at what stage the extraction of essential oil is to be attempted. Essentially, there are two methods: first, to operate on whole fruit *prior* to the extraction of juice; secondly, to cut the fruit in halves and to obtain the oil from the exhausted peel *after* the extraction of juice. Each method offers advantages, which the manufacturer must weigh before installations are made and machinery chosen. Simultaneous extraction of both juice and oil is possible only by means of a new machine (see page 239).

Those who prefer to extract the oil from exhausted peels claim that sanitary conditions during squeezing of the juice can be better maintained if the fruit, after washing, is immediately subjected to juice processing, while during oil extraction the water sprayed over the fruit to create the oil emulsion is continually returned, thus creating a danger of contamination with yeasts and other microorganisms.

These two objections can be easily overcome: first, by adding an antiseptic to the continuously circulating water in the oil extracting machinery; secondly, by rinsing afresh the fruit, which has lost most of its oil, with strong water jets before being subjected to juice squeezing.

However, oil extraction applied to whole fruit has many advantages: (*1*) treatment of whole fruit for oil is in itself the most efficient method of properly washing the fruit; (*2*) with properly chosen machinery, a higher output of oil can be obtained; (*3*) the procedure is more rapid and lends itself to better automatic processing and to a possibility of a continuous line flow; (*4*) the fruit remains uncut, but weak or slightly bruised fruit having escaped previous inspection becomes crushed in the oil machines and can thus be easily eliminated by additional selection before proceeding to the circular knife; (*5*) whether the juice is hand pressed on rotating rosettes or squeezed mechanically, the oil, if previously extracted, is prevented from mixing with the juice—admixture detrimental to a good quality product; (*6*) the separation and clarification of the oil when extracted from whole fruit is easier and more complete.

All manual and mechanical methods used in oil extraction are based on the fact (see page 41) that, as long as the fruit is fresh and not flabby, the oil secreted in the glands or sacs of the flavedo is maintained under a certain turgor pressure. When the glands are ruptured, the oil will eject with considerable force and to a relatively great distance. This effect can be easily demonstrated by bending a piece of fresh citrus peel against a candle flame: the ejected oil will catch fire at a distance. This crude test is used by the operator to estimate the amount of residual oil available.

All extraction methods deal, therefore, in the main, with the particular means of piercing or rupturing the oil glands and collecting the oil quickly to prevent the remaining spongy peel from reabsorbing it. Thus, practically all oil machines have some arrangement whereby the surface of the fruit is kept constantly wet by sprays of water. These sprays (the structure of which will be described later—see page 187) have, therefore, the following objects: (*1*) to carry away as quickly as possible the ejected oil by forming an emulsion; (*2*) to prevent loss of oil by spurting; (*3*) to fill the spongy cells of the flavedo and albedo surrounding the oil sacs and to prevent them from reabsorbing the liberated oil; and (*4*) to increase the turgor pressure of the oil glands by augmenting the osmotic pressure of the surrounding cells.

It should be borne in mind, however, that only fresh fruits have this faculty of ejecting their oil contents when rasped or triturated. The force of ejection is greater when the fruit is less ripe and gradually falls off as maturity progresses. Further, this turgor greatly varies with the degree of freshness of the fruit and is nearly lost in

fruits which have been stored for a long time. The actual amount of oil present in the peel is of little value; it often happens that fruit containing its full amount of oil will yield practically none through the machines because the stale and flabby condition of the fruit with tough, elastic peel renders laceration of the oil glands very difficult.

2. Machines Rasping Whole Fruit

(a) Écuelle

The écuelle (Fig. 31) can be regarded as the prototype of all machines rasping whole fruit. It is a very old process, originating prob-

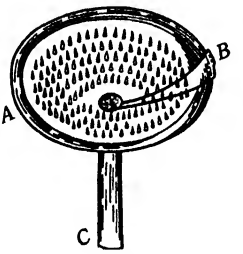


Fig. 31. The écuelle.

ably in southern France, but is still used to some extent for lime oil in the British West Indies and for orange oil in French Guinea, where abundant, cheap labor is readily available. It consists of a shallow copper funnel, the narrow end of which is sealed. The slopes of the funnel are covered on the inside with brass spikes upon which the fruit is rolled over as the hand exerts a small amount of pressure. While the spikes are piercing the oil glands, the ejected oil, together with the cell sap, collect slowly in the narrow tube of the écuelle; the oil is then decanted and finally separated from the aqueous portion and the detritus consisting of small fragments of abraded peel. No water is sprayed upon the fruit during this process. The écuelle method is, of course, a very primitive way of oil extraction and requires even more manual labor than the well-known "sponge-method," which will be described later (page 191).

It is interesting to note that the slight pressure of the hand exerted upon the fruit in the écuelle involves a very important principle underlying oil recovery by all mechanical means. As will be seen in the discussion immediately below, every oil machine has been designed to incorporate this factor in a different way. The problem could be

solved mechanically in the following manner: the fruit passes in series through a continuous screw conveyor, the walls of which are rendered rough either by covering them with spikes similar to those of the écuelle or by sharp extrusions made in the channel of the conveyor. Theoretically, such an arrangement should solve the problem. However, in practice, this procedure will give no results because the slight force pressing the fruit against the prickly surface is lacking. This delicate pressure is exerted in the "Cannavo" and the "Avena" models of Italy and in the modern American drum extractor by the aid of centrifugal force, in other types, such as "Speciale" and "Jaf-Ora," by the natural pressure of several layers of fruit bumping against one another.

(b) Bergamot "Machinette"

The first attempt to translate the action of the écuelle into mechanical design was made in Italy by Nicola Barillà in 1840. This "machinette" consists of two round plates, both having a rough abrasive surface. Put together, they form a "life saver." Upon the lower plate, which is stationary, a number of sized, spherical bergamot fruits are placed and covered with the second, similarly constructed, plate which rotates at a speed of 120 to 240 rpm. The rasping of the fruit is rather delicate, and no water sprays are used, the worked fruits being wiped carefully with a sponge to remove residual oil. This machine is not adapted to work lemons or, in fact, any citrus fruits which are not nearly spherical. It is still used in Calabria (Italy) for the production of bergamot oil, and is sometimes called the "Calabrese or Gangeri machine" (Fig. 32).

Extraction machinery has passed through a natural evolution of rasping machines—each more or less successful and each designed to perfect the mechanical extraction of oil. We shall not endeavor here to discuss the complete series, for all of them have been ably described by Donovan (1937)[4] and have at present only an historical significance. We shall concentrate on the latest models currently used in most industrial regions.

(c) "Avena" Machine

The "Avena" machine, which utilizes the centrifugal principle, was originally patented in 1924 by Giuseppe and Placido Avena of Pistunina (Sicily). It resembles a large size sugar centrifuge about 1.20 meters in diameter, in which the rotating portion is not the

[4] See bibliography at end of this chapter.

usual basket but a flat plate at the bottom, while the surrounding walls are stationary (Fig. 33). The periphery of both the rotating plate and the walls is fitted with abrasive pyramidal projections, some made of glass and others of stainless steel. The disk, rotating at approximately 60 rpm, hurls the fruit repeatedly around and against the walls, thus causing a uniform rasping of the peel. After the rasping is completed, a door in the wall of the centrifuge opens automatically and the fruit is thrown out by the same centrifugal force.

Fig. 32. The bergamot "machinette" as worked in Calabria.

The principle of operation is similar to that of a potato peeler. To increase its capacity, the Avena machine is now built in two "floors"—two rotating disks running one on top of the other, with an opening between them allowing the fruit to distribute itself evenly in each compartment. During the rotation, water is continuously sprayed from tubes leading to each compartment. The mixture of oil, water, and abraded raspings is drained away into a circular channel at the bottom and through a central pipe to the separation vessels. The entrance of fruit from the hopper, the discharge of the rasped fruit, and the water sprays are regulated automatically and work intermittently every 2 to 4 minutes, according to the degree of freshness of the fruit.

The largest Avena has a capacity of 1 ton of fruit per hour and requires a 1 HP motor.

The Avena machine, being very flexible in operation and capable of handling fruit in various stages of ripeness, is probably the best of its type. Equally suitable for different citrus varieties, it is widely used in Italy, Spain, and Brazil, where a local type has been evolved.

(d) "Speciale" Machine

The "Speciale" machine, patented in 1928 and manufactured by Francesco Speciale of Giarre (Sicily), makes use of centrifugal force

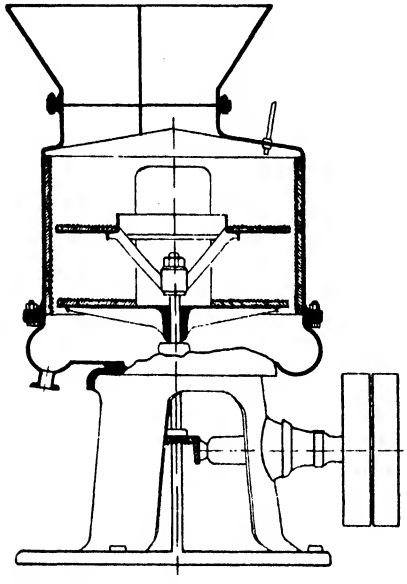


Fig. 33. The Avena rasping machine, vertical cross section.

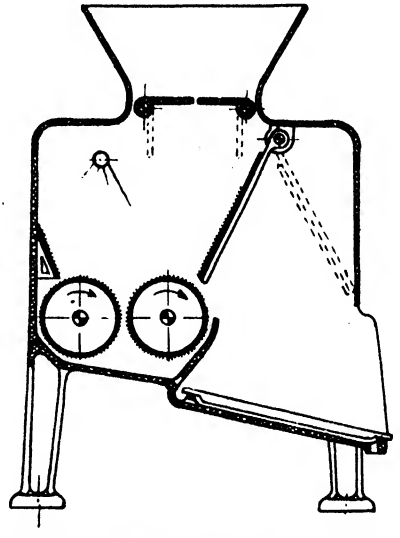


Fig. 34. The Speciale rasping machine, vertical cross section.

in a quite different way. It consists in principle of two parallel cylinders covered with abrasive pyramidal points made of brass and rotating in the same direction against a similarly abrasive plane (Fig. 34). The fruits thrown upon these cylinders in considerable quantity are hurled and bumped against the stationary plane, and are then thrown back to the rotating cylinders until they are completely rasped on the surface. Water sprays carried by a perforated tube running alongside the cylinders continually wash away both oil and detritus. The stationary plane swings on hinges and opens automatically when the fruit is sufficiently rasped to allow it to be discharged through the

lower chamber. This chamber consists of an inclined slope with a false wooden bottom which permits the fruit to run down while the liquids and raspings pass through and gather in the lowest box to be carried away for separation. All moving and abrasive parts are made of brass; the outer shell is made of wood and lined with tinned copper sheets.

The Speciale, usually constructed with two parallel working chambers, has a capacity of 2 tons of fruit per hour and is very well adapted to lemons, although in some regions it is also used for oranges. In the

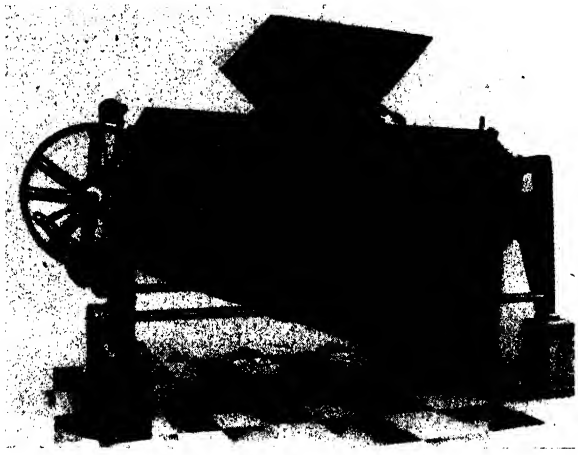


Fig. 35. The Speciale rasping machine.

writer's opinion, the Speciale machine gives the highest yields of oil although its action, being somewhat drastic, results in crushing many fruits. It is widely used by manufacturers in Italy, Palestine, and other regions (Fig. 35).

(e) American Drum Extractor

Due to the high cost of labor, the producers in the United States could not easily use the primitive machinery described above; they, therefore, have sought totally different methods, which will be discussed later in this chapter. Quite recently, however, there has come into use in America the drum extractor, which also rasps the fruit superficially and uses the centrifugal principle in a continuous flow. The extractor consists chiefly of a hollow cylindrical drum, made of stainless steel, about 1 meter in diameter and about 6 to 9 meters in length. The surface of the entire drum is made rough by small ex-

trusions projecting towards the interior. Inside the drum, a continuous spiral guide directing the fruit permits the fruit to have a longer run. The drum rotates either on a central shaft or on outside rails guided by fringed rollers. A continuous water spray is delivered alongside the rotating drum. The fruit enters at one end of the drum, rotates and bumps against the protruding spikes, and, following the spiral guides, leaves the drum at the other end. Except for the rough surface and the spiral guide, the operation is based on a principle similar to that of a vegetable washer. The scrapings, together with the oil emulsion, escape through the holes in the drum created by the extrusions.

This simple arrangement, although entirely inflexible, permits the processing of huge quantities of fruit. However, the yield of the oil is rather small.

An apparatus for extraction and recovery of oils on similar lines from whole citrus fruits has been patented[5] in the United States. This apparatus consists of a rotatable cylinder having an abrasive lining and containing a "liquid such as water employed as a spray," flowing in a direction opposite to the fruit.

(f) Jaf-Ora Model

Quite recently, a novel apparatus for the extraction and recovery of citrus oils has been developed in Palestine.[6] The new extraction machine utilizes the principle of the "Speciale" machine in which the fruit is rasped on a pair of rollers turning in the same direction. However, to make the rasping more effective and to make the whole operation automatic and continuous, a number of such pairs of rollers are placed in parallel succession, the fruit passing from one such pair of rollers to the second, then to the third, and so on, in a zigzag succession, as shown in Figure 36. The pairs of rollers can be placed in one plane or in a cascade manner. The fruits push their way through an opening in the stationary wall, against which they bump while en route to the next pair of rollers. Contrary to all known machines, this arrangement permits the fruit to flow separately from the oil-containing liquid, not permitting any lengthy contact with the extracted oil which is thus prevented from being sucked back by the spongy surface of the rasped fruit. The continuous movement of the fruit through the aisles created by the partitions subjects the fruits, by

[5] Platt, Wm. C. (assigned to California Fruit Growers Exchange), U.S. Patent No. 2,354,878 (Aug. 1, 1944).

[6] Braverman, J. B. S., A. Katz, and Jaf-Ora Ltd., "Machine for Extracting Essential Oils," Palestine Patent No. 3316 (March 14, 1947).

continuously changing their position, to thorough scraping and rasping.

The capacity of this machine is very great; with six pairs of rollers it works 5 to 6 tons of fruit per hour.

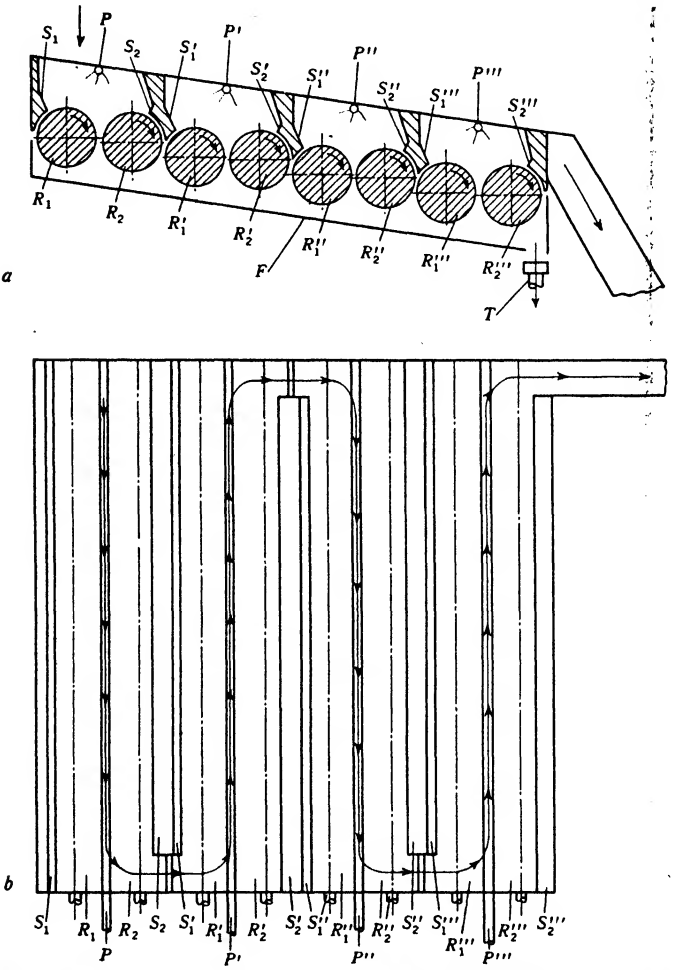


Fig. 36. The Jaf-Ora oil extraction machine: *(a)* side view; *(b)* top view. *R'*, *R''*, etc., rollers with rough surface. *S'*, *S''*, etc., abrasive surfaces against which the fruit bumps during rotation of the rollers. *P'*, *P''*, etc., water jets. *T*, outlet tube. *F*, inclined floor to receive emulsion.

3. Methods Involving Treatment of Peels

All methods under this heading, including the hand-press process, involve the preliminary separation of the peel from the flesh of the

fruit. Originally, this required the halving of the fruit crosswise or longitudinally and the cutting-out of the pulp from the halves by means of a special sharp-edged spoon ("rastrello"); the hemispheres of the rind, consisting of the entire flavedo and only part of the albedo, were used for oil extraction. The remaining pulp was pressed (especially in the case of lemons and limes) into juice mainly for technical purposes, such as the manufacture of citrate of lime and citric acid.

However, since citrus juices have acquired an important position in the food industry, the crosswise-halved citrus fruits are now squeezed for juice on rotating rosettes; the remaining peel contains not only the flavedo and the entire albedo but also shreds of tissues of the endocarp. Such peels cannot very well be used for oil extraction by the hand-press method and should be worked only in the so-called "sfumatrice" machines.

(a) "Sponge" Processes

The oldest hand process is the "sponge" process or "processo alla spugna," as traditionally called in Italy, where it is still in use to the extent of about 50% of the entire citrus-oil production of that country.

In former times, the fruit was cut longitudinally into three pieces (the so-called "scorzetta" method); this practice, however, was abandoned about forty years ago, and now the "sponge" method consists generally in halving the fruit crosswise with an appropriate knife, removing the pulp by means of a sharp spoon (Fig. 37), using a jerklike movement of the hand.

The rinds so obtained are moistened with water for a few hours, which is claimed to facilitate the expression of the oil. In Palestine, where hand-pressed oil was produced to a considerable extent during World War II, the peels were soaked for several hours in a dilute solution of milk of lime, and afterwards taken out and left to dry overnight in the air. This procedure gave the peels a stronger and tougher texture which not only facilitated the extraction of the oil but produced a better yield. The principle underlying the lime treatment is based on the binding of soluble pectinous substances of the peel to the lime, creating insoluble calcium pectates which do not interfere with the oil on expression. Furthermore, as explained by Donovan (1937), the turgor pressure of the oil in the glands apparently increases owing to the increased osmotic pressure of the neighboring spongy cells after they have absorbed considerable quantities of water.

The next step in the hand process is to squeeze the rinds into a cuplike sponge using the pressure of the hand. This procedure, as used in Italy, requires much dexterity, skill, and maintained effort. The operators—usually men—sit on low stools, before earthenware jars provided with a lip. The workmen press the rinds into a cuplike sponge hanging from a string on a bamboo stick which rests across the middle of the jar. The rind is turned several times and the pressing operation repeated until the peels are exhausted. Slowly the sponge is saturated with oil and is from time to time pressed into the jar. The oil is then decanted; a specially made depression in the jar just below

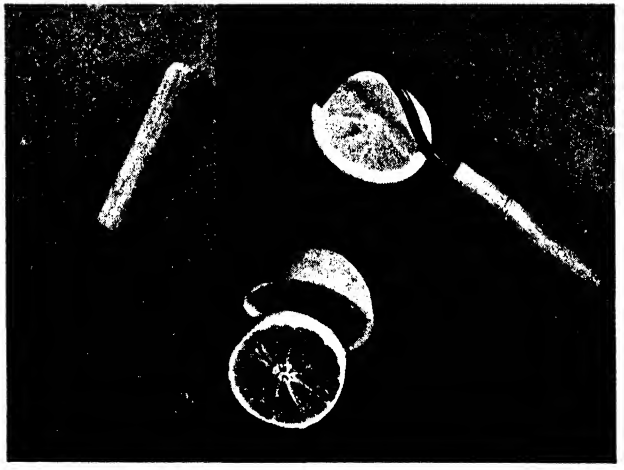


Fig. 37. Rastrello, the spoonlike knife, and the mode of using it.

the lip is intended for holding back water and impurities that collect at the bottom of the jar. Thus, the entire process requires much labor and is very expensive. Also, the yield of oil obtained by this process is in many cases considerably lower than with good mechanical extraction. La Face (1930) has shown that at least 25 to 30% of total oil is left in the worked peels, while in using the Italian machines (Avena, Speciale, Cannavo) working whole fruit, only 10 to 14% of the oil was found to remain in the worked fruit.

To alleviate the continuous manual effort required by the operator in squeezing the peel, numerous simple machines have been devised in Italy, all of them based on the lever principle; they are fully described by Rodanò (1930). They have all been early attempts to mechanize the hand-press method. Recently an interesting deviation from this type of hand machines was made in Palestine. When the

extraction of citrus oils was undertaken on a large scale during the recent world war, no machines existed, nor were there any skilled operators to be found in Palestine. A small press-like apparatus was soon designed (by Mrs. Z. Samish of the Agricultural Experiment Station at Rehovoth) which proved very successful (see Figure 38). Two peels at a time are placed between two round disks, each lined with flat round sponges. The lower disk is perforated and under it an earthenware or glass bowl is placed. The upper disk is attached

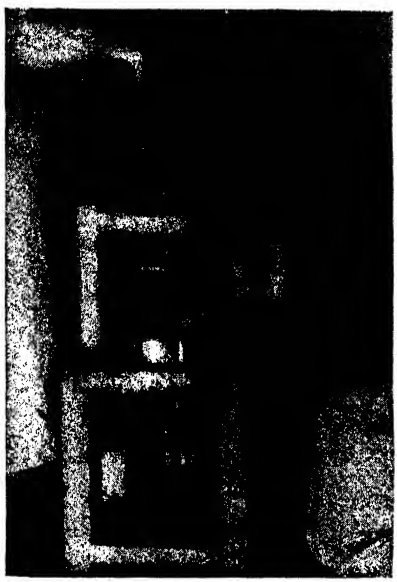


Fig. 38. The improved sponge process (Palestine).

to a movable shaft and is provided with a convenient grip for both hands of the operator. The peels are inserted between the disks; the operator lowers the upper disk and rotates it in either direction several times; the disk is then lifted and the two exhausted peels removed and replaced by two fresh ones. From time to time the sponges are pressed against the lower base to express the accumulated oil into the jar placed underneath. Any workman can learn to operate this simple apparatus in a very short time.

(b) "Sfumatrici" Machines

All of the hand processes which handle the individual peels are very expensive, especially by reason of the fact that they

demand preliminary removal of the pulp by hand, using the spoon-like "rastrello." Further developments in this field are to be found in the so-called "sfumatrici" machines. Contrary to the machines rasping whole fruit, the "sfumatrice" makes use of the natural turgor pressure of the oil by bending the peel in many directions but without the use of a sponge as an absorbing medium. Instead, the ejected oil is washed away by streams of water, thus creating an emulsion as do the rasping machines.

There are various types of "sfumatrici" and all of them are described in detail by the authors already mentioned. All of them are based more or less on the same principles and, therefore, only the very

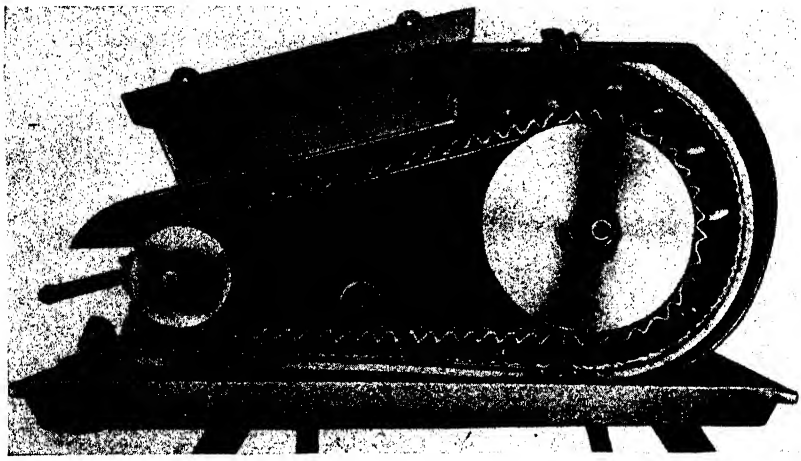


Fig. 39. The Speciale sfumatrice (front cover removed).

latest models will be mentioned here. They all have a moving member mounted within or against a stationary part, the peels being dragged, bent, and consequently compressed in the narrow space between these two parts.

(1) "SPECIALE" SFUMATRICE

As shown in Figure 39, the peels, in this machine, are drawn in from the hopper by an endless belt carrying ribbed units of resistant metal into the narrow space between the belt and an equally ribbed stationary guider. Between the two surfaces, the peels are bent and compressed. The distance between the two ribbed surfaces is adjustable by screws, and gradually becomes less as the end is approached. Water sprays to wash down the ejected oil are provided within the

working chamber at points of entrance and discharge of the peels. This sfumatrice is entirely automatic and requires only a 1-HP motor; it is capable of expressing the oil from peels of about 1.5 tons of fruit per hour. It was patented in Italy in 1932 by Francesco Speciale of Giarre (Sicily).

(2) "RAMINI" SFUMATRICE

G. Ramini of Palermo gave the same principle a different mechanical interpretation. In his machine, patented in 1934, the peels travel between two horizontally placed disks, the upper stationary, the lower rotating. Both disks, which are made of a strong wire cloth, are placed at a slight angle to each other, so that the distance between them is 8 cm at the widest and 3 cm at the narrowest point. These distances can be adjusted by lowering or raising the rotating disk, in

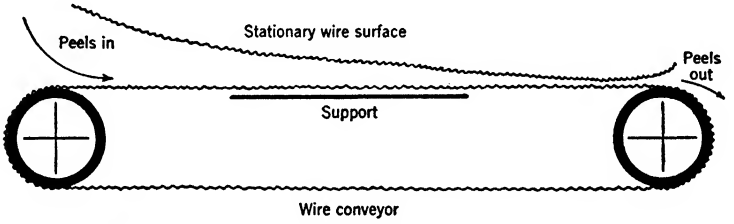


Fig. 40. Proposed simplification of Ramini principle.

accordance with the thickness of the peels or the desired pressure. The peels entering from the hopper are distributed through an aperture in the upper stationary disk and are carried around through a semicircle, being bent and rolled on their way to the narrowest point, where they are discharged. Water is sprayed over the peels on their path between the disks, but this machine is claimed to work also without water. It has a capacity of about 1 ton per hour and requires only a 1-HP motor.

The writer takes this opportunity to suggest a simplification of the Ramini principle in extracting orange oil from peels. It consists in an endless wire conveyor over which another stationary wire cloth is placed so that the distance between the two wire cloths at the beginning of the conveyor differs from the distance between them at the end (see Fig. 40). The peels fed at one end of the conveyor will be dragged, bent, and squeezed until they are discharged at the other end. The wire cloth of the conveyor should, of course, be supported underneath by strong flat guiders. This simple arrangement should, in the writer's opinion, be capable of working continuously much

greater quantities of peel than the previously described models. Furthermore, a much longer working path would be possible.

(3) PIPKIN MACHINE

For the recovery of essential oils from peels, W. A. Pipkin has patented in the United States (U.S. Patent No. 1,798,555, March 31, 1931) the so-called Pipkin oil machine, consisting of knurled rolls through which the peels pass under pressure; the resulting emulsion is then separated in a high-speed centrifuge.

The bulk of oil produced in Florida is made by a modified machine by the same author[6a] consisting of two stainless steel drums which are adjustable to control the pressure on the peel. The pressure maintained is merely sufficient to puncture the oil sacs without actually crushing the peel. The drums are grooved, the grooves running around the circumference of the drums are deep enough to receive and keep the oil from contact with the peel as the drums revolve. Some of the oil is, however, reabsorbed by the albedo which may act as a sort of sponge. The Pipkin machine is claimed to yield about 6 pounds of orange oil per ton of fruit.

4. Methods of Separation and Centrifuging

(a) Separation

With the exception of the "écuelle" and the "sponge" processes, in all oil-extracting processes the oil must be separated from the emulsion and its accompanying detritus.

First, for the necessary removal of coarse particles, scrapings of peel, etc., from the liquid phase, efficient screening is essential. This was originally accomplished with the aid of a series of fine copper sieves; but recently there have been placed on the market very efficient continuous rotating screens with a very fine mesh, adapted to an Archimedean screw press to squeeze the scrapings so that when discharged they are nearly dry and contain practically no oil. In another device, the screen is built like a small stationary channel, while a continuous screw, with brushes fixed at its edges, rotates and facilitates the screening; the scrapings, moving along the channel sieve, are collected at the end of the screw and are automatically pressed and discharged (Fig. 41). Rasping machines yield a considerably greater amount of these scrapings than the sfumatrice machines. But, whatever the degree of fineness of the filter press

[6a] Pipkin, W. A., U. S. Patent No. 2,004,056 (June 4, 1935).

screens, a certain amount of very fine detritus always remains in the mixture of oil and water.

The modern method of separating these solid particles consists in the use of special continuous centrifuges constructed to permit the solids to be discharged continuously. Such are the Sharples-Nozljector and the Quiros continuous centrifugal separators. The first is constructed by the American firm of Sharples, Inc., and is somewhat similar in construction to a large-sized Alpha-Laval separator; it consists of a number of conical disks inside the revolving bowl, with an additional arrangement for continuous discharge of the solid phase, as soon as accumulated, by the centrifugal force at the inside wall of

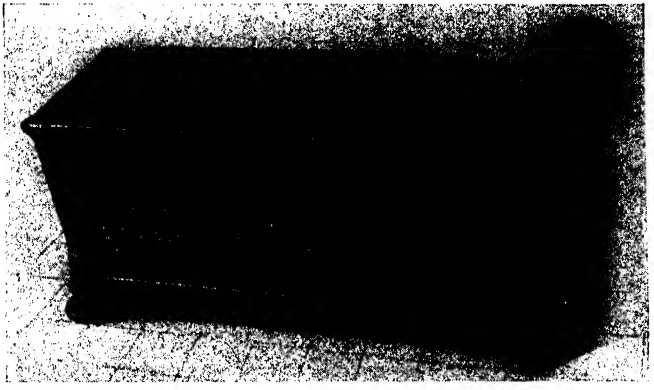


Fig. 41. Continuous screw press with brushes to screen the raspings.

the bowl (Fig. 42). The Quiros is constructed by Centrifugals, Inc., of New York, and consists of a solid bowl 72 inches in diameter, without disks but divided into ten pockets, each having a discharge opening fitted with special valves for an intermittent ejection (every one to two seconds) of solids or sludges (Fig. 43). Both separators are heavily built, have a very large capacity (1 to 4 tons of cake per hour), and are rather expensive.

The mixture of oil and water must now be separated. With the introduction of machine-pressed oils, this step constituted the most serious difficulty. The primitive method used in the early days, although sufficiently simple, had numerous drawbacks affecting the quality of the oil obtained. This method of separation, still used by many small producers in Italy, consists in collecting the oil emulsion in a series of Florentine vessels, allowing the oil with the remaining detritus to collect at the top. Wool is then impregnated with the oil

phase and subsequently pressed in an ordinary screw press which breaks up the emulsion, water appearing first and oil last, while the detritus is retained by the wool (Fig. 44).

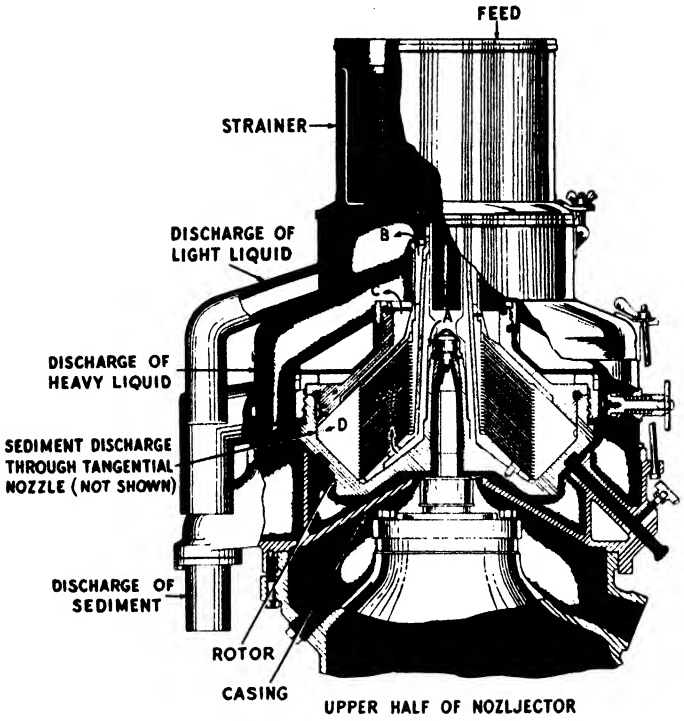


Fig. 42. Nozljector.

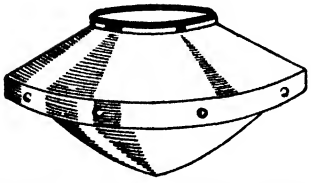


Fig. 43. Bowl of the Quiros centrifugal separator.

Continuous addition of fresh water to the peel has been found to extract important ingredients from the oil, namely, some of the oxygenated compounds, which are slightly soluble in water. Apparently, the best procedure would be to recirculate the water after being separated from the emulsion; thus, a series of decantation Florentine

vessels are set up in which the first contains the largest amount of floating oil phase, the last vessel containing only a thin film. The

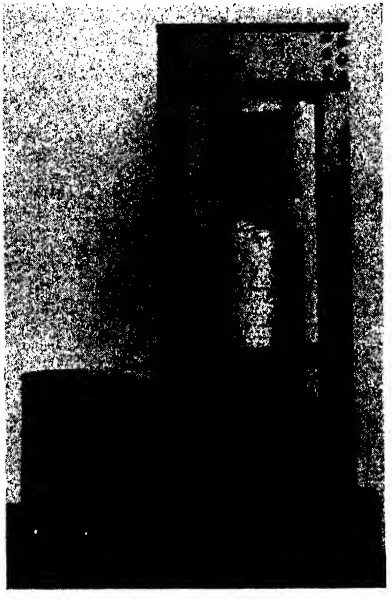


Fig. 44. Hydraulic press for separation of oil emulsion.

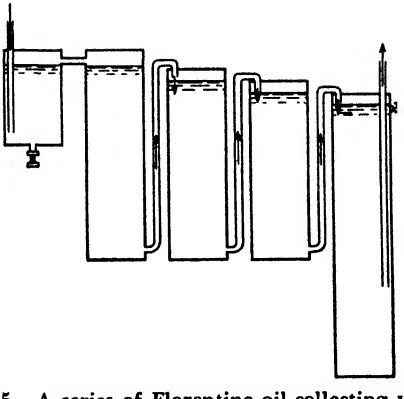


Fig. 45. A series of Florentine oil-collecting vessels.

water from the last vessel is returned to the oil extraction machine for recirculation (Fig. 45).

It is not the water alone, but also the pectin of the peel accumulat-

ing in the water, that dissolves the oxygenated compounds. Pectin easily undergoes hydrolysis, splitting off the methyl alcohol, which has a high solvent action upon citral. This loss of citral or other aldehydes can be decreased by rapid centrifuging.

(b) Centrifuging

Although the primitive method of settling and decantation described above is still in use to a large extent in Italy and Spain, machine-pressed oils thus obtained are of low quality. A rather long time is required to complete the flotation of the oil in the Florentine ves-

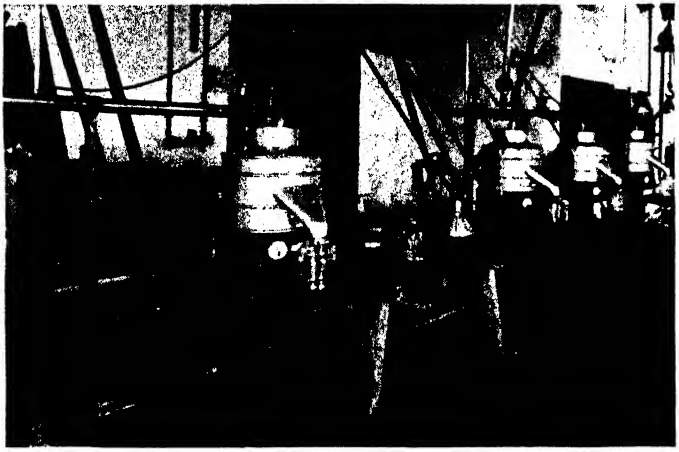


Fig. 46. A battery of Alpha-Laval centrifugal separators. (Note the disk on the bench.)

sels, during which time a marked deterioration of the oil takes place: (*1*) the oil dissolves the pigments of the ruptured chromoplasts and becomes exceedingly dark; (*2*) prolonged contact with tissue fluids of the peel, which are acid, and sometimes also with juice of broken fruit, has a pronounced adverse effect on some constituents of the oil and is particularly destructive to citral; (*3*) allowed to stand for a long time, the oil phase containing detritus is likely to ferment, which irreparably spoils the oil; (*4*) a considerable proportion of oil remains in the wool residues after pressing, and its recovery is possible only by distillation, which yields an oil of very poor quality.

To overcome these shortcomings, modern industry freely uses centrifugal separators, which were first applied for this purpose in Messina (Italy) by Bennett in 1914. The liquid oil emulsion emerging from the filter presses is now fed immediately into centrifugals such

as the Alpha-Laval or Sharples Super. These separators yield, from one spout, a clear, purified oil, while from the second spout the water is discharged, which can be recirculated continuously through the line.

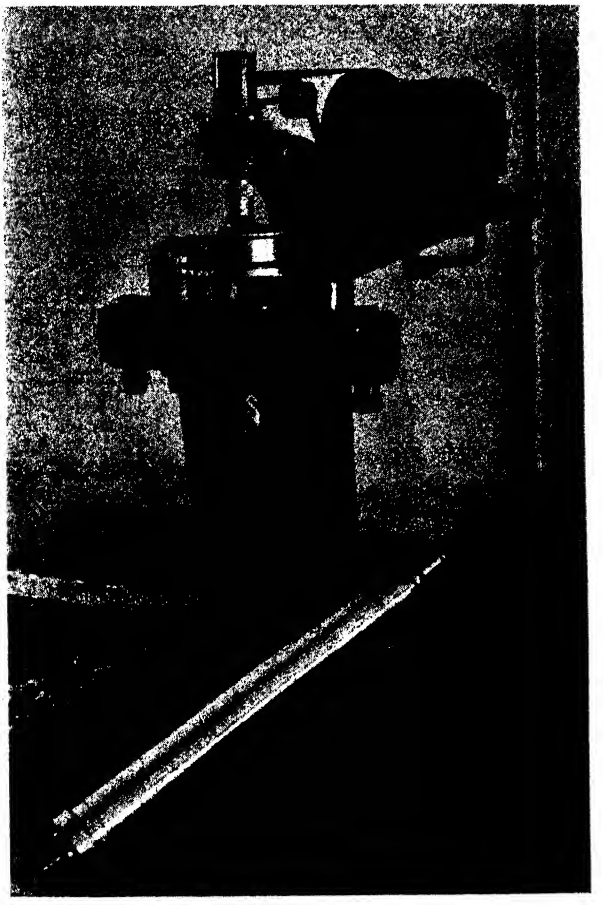


Fig. 47. Sharples Super-Centrifuge, with bowl shown in front.

The Alpha-Laval separators (Fig. 46) consist of about 52 conical plates, fitted into a strong bowl and provided with special gravity rings selected according to the density of the oil. The seals are equipped with replaceable rubber rings. The bowl and all the plates are heavily tinned. These particular types of separators are widely used in the clarification of transformer and other oils.

The Sharples Super-Centrifuge is built of a long cylindrical bowl of stainless steel or Monel metal and has no plates (Fig. 47). This separator has a through-put capacity of over 500 liters per hour and can be very easily cleaned in about 10 minutes. It is obvious that any of these centrifugals must be periodically cleaned when the suspended solids (detritus) accumulate and clog the bowl space. In continuous work, this occurs about every 2 to 3 hours.

Both separators can be easily adjusted for clarification of the oil after the stearoptenes and other waxy substances have settled.

(c) Bennett Process

The process by which the centrifuge is used alone is not yet sufficient to obtain, in the case of the citrus oils, the complete and easy breaking up of the oil emulsion. In a comprehensive study of the action of various agents on aqueous dispersions, Bennett found that the presence of small proportions of sodium bicarbonate ($NaHCO_3$) promoted the separation of the oil from the emulsion. This can be further assisted by the addition of sodium sulfate (Na_2SO_4) or similar salts. Bennett patented this very useful process in 1930.[7] It consists in adding to the water which circulates in the oil-extraction machines—both rasping and "sfumatrice"—about 2% of sodium bicarbonate with some sodium sulfate.

The principles underlying this method are manifold. First, by adding these easily soluble salts, the specific gravity of the water is raised, thereby creating a greater difference between the density of the water and that of the oil, which itself is very near unity (usually 0.850). Thus, easier separation is effected in the centrifuges. The same applies to the surface tensions of the two components of the emulsion. In addition, sodium sulfate or similar salts tend to neutralize the electric charge of the colloidal micelles. Finally, $NaHCO_3$ neutralizes the acidity of the tissue fluids of the peels as well as that caused by any broken fruit, increasing the *p*H of the circulating liquid to somewhat above 7. By neutralizing the acidity, any possible adverse effect of the acid medium on the quality of the oil in suspension can be avoided. Furthermore, the activity of various enzymes present in the emulsion, which are normally active in acid medium, is inhibited at the higher *p*H. Also, if the liquid remains acid, the life of the separators, if not made of resistant materials, is considerably shortened.

[7] Bennett, A. H., "Process for the Recovery of Essential Oil from Lemons and Other Fruits of Trees of the Genus *Citrus*," U.S. Patent No. 1,814,888 (July 14, 1931); Palestine Patent No. 195 (Sept. 18, 1931).

As previously discussed (page 88), the pectin present in the peel is a very strong emulsifier. In this case the action of $NaHCO_3$ can be explained as breaking up the emulsifying effect of pectin by creating sodium pectates which are less gelatinous in nature. The bicarbonate solution, which is substituted for water, gradually becomes loaded with various dissolved substances, such as pectic salts of sodium, coloring matter, etc., and, when these reach too high a proportion, should be discarded.

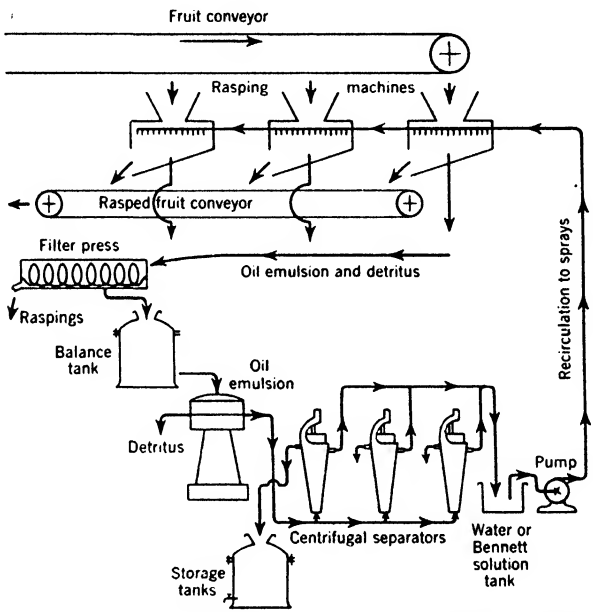


Fig. 48. Flow sheet presenting a scheme for a modern installation for citrus oil extraction.

In some factories in Italy, milk of lime has been used—with less success—in lieu of sodium bicarbonate.

Citrus oils recovered by the Bennett process are fully equal in quality to "sponge" oils, which are considered the nearest approximation to the natural state in which the oils are secreted in the peel. They have the same composition, odor, flavor, and aldehyde (citral) content; they have, however, a slightly higher nonvolatile residue than the hand-pressed oil. While machine-pressed oils, especially those produced by the rasping machines, have a more pronounced color, in the writer's opinion this factor is a good indication of their keeping qualities.

The flow sheet (Fig. 48) presents a scheme for a modern installation for oil extraction.

5. Oil Recovery from Crushed Fruit

Designed primarily for large-scale operation and to reduce labor requirements, the method of oil recovery from crushed fruit was used in the United States largely before the introduction of the latest drum extractor for the superficial treatment of whole fruit. The method is grouped separately because it treats the fruit simultaneously for both oil and juice.

The washed fruit is crushed in a crusher consisting of three cast-iron rolls covered with a 1/16-inch sheet of Monel metal over the face and sides. The fruit first tumbles through the rolls, which are set approximately 1/2 inch apart. The fruits are broken open and about half of their juice is expelled. The flattened fruit is then put between the top of the second roll and the bottom of the third (the separation is approximately 1/8 inch), where the remaining juice is expressed. Due to the rolling action exerted by the crusher, most of the oil, together with all tissue fluids, is ejected from the skin at the same time the juice is pressed out of the fruit.

The mixture of pulp, oil, and juice is then screened, first coarsely over a 1/8-inch mesh, slightly inclined, rotating screen, and then through a second similarly arranged screen with very fine 100-mesh perforations. After the pulp has been largely removed the mixture of juice and oil is centrifuged, as described above. In this case the separation of the oil is obviously not complete: there is always a phase which lends itself with great difficulty to separation. Furthermore, in this operation the oil discharged from the centrifugals is not clear and must be clarified again. Finally, the juices so obtained usually contain considerable quantities of oil and in the course of time cause deterioration of the flavor in the juice. Such juices are, however, used for concentration in vacuo or for technical purposes.

6. Various Other Methods of Oil Recovery

(a) Distillation

With the same purpose in mind—namely, economical production—several ways of easy and quick recovery of citrus oils have been sought. Distillation, and especially distillation in vacuo, is one of the processes developed.

One of the most striking features of essential oils is their property to distill over with steam, although their boiling point is usually much above 100° C. Naturally, this property holds also in vacuo where a much lower temperature can be maintained with boiling water. However, steam will not carry the oil away effectively unless the peel is thoroughly minced and the oil glands ruptured and disintegrated.

This method, known in the literature as the Peratoner method (patented in Italy by Peratoner and Scarlata of Palermo), consists in milling the whole fruit or the peels, after the extraction of the juice, in a suitable disintegrator and subjecting the ground product to steam distillation under reduced pressure at a temperature usually about 50–60° C. The lower the temperature under which distillation takes place the better the quality of the oil, but generally it has a characteristic odor and flavor and its quality is decidedly inferior to that of citrus oils obtained by hand- or machine-pressing. Distilled citrus oils are, of course, colorless; they have practically no dry residue (on evaporation) and a lower content of aldehydes and esters. Their keeping qualities also are very poor.

Oils from blossoms (Neroli oil) and from twigs (petitgrain oils) are principally produced by the distillation method. The production of these oils is confined mainly to the sour orange—the bigaradier—and is practiced on a large commercial scale principally in southern France. During blossoming time (May to middle June) the flowers are picked by women on ladders, each blossom being pinched off with the finger nails and dropped on to cloths spread underneath the tree. The petals of the flowers are then separated from the sepals, covered with water in the still, and steam-distilled at ordinary atmospheric pressure, either by means of coils with superheated steam or directly by live steam. The yield of Neroli oil is very much influenced by weather conditions prevailing during harvest and, under exceptionally good atmospheric conditions, may run as high as 1700 g of oil per ton of flowers. The extracted Neroli oil is separated from the water, which is also a valuable product of commerce (orange-flower water). Upon cooling, the oil becomes turbid and a heavy paraffin-like deposit is separated, occasionally solidifying to a butterlike mass. This particular branch of the citrus-products industry has a direct bearing on production only in those centers where the bigaradier is extensively cultivated or where other citrus varieties grow wild. The sour-orange, in general, is not palatable and there is no danger in reducing the subsequent crop by picking the blosssoms.

In a very similar way petitgrain oils are steam-distilled from the young leaves and twigs and the immature fruit of the bigaradier, or from other varieties. The process is practiced mainly in southern France and Italy and recently also in Paraguay.

(b) Extraction by Solvents

A number of methods have been suggested to recover essential oils from the citrus peel by means of various solvents, such as alcohol, petroleum ether, etc., and several patents have been obtained for such processes. However, none of them has succeeded commercially. Organic solvents extract from the peel a great amount of impurities together with the oil. Furthermore, it is extremely difficult to separate the solvent completely from the oil; the remaining solvents, of course, render the oils worthless as a flavoring substance. Some oils of this type have been produced in the United States under the name of "oleo resins" and have been used in scenting soaps.

The method of extraction by solvents is commonly used in the recovery of neroli oil from orange blossoms, besides the distillation method already mentioned. Extraction is effected in either of two ways:

1. *Enfleurage.* A specially prepared fat is enriched with the oil of the flowers. Purified lard or any other pure fat is spread over glass trays (so-called "chassis") and the blossoms laid on the fat for a period of time. The blossoms are later removed and replaced by fresh flowers until the fat becomes saturated with the oil. The enriched fat is sold as such for pomades or other cream preparations in perfumery, or treated with alcohol to extract the oil. The method is applied also to certain other flowers.

2. *Countercurrent extraction with a solvent.* A battery of extractors is employed, into which wire baskets carrying the flowers are immersed. Through these, previously purified petroleum ether (of specific gravity 0.650) is circulated in countercurrent, i.e., the fresh solvent meets the nearly exhausted flowers. Each batch of flowers is usually extracted three or more times, depending on the nature of the flowers. The correct number of extractions, as well as the number of times the same solvent can be used, must be established by experiment. (In some manufacturing plants this kind of extraction is performed with warm solvent.) The saturated solvent is separated from the oil by distillation. As a rule, the bulk of the solvent is distilled over in a large still under ordinary atmospheric pressure; when the temperature reaches a point which may be detrimental to the

fragrance of the oil the remaining solvent is distilled under vacuum in a smaller still. The last traces of the solvent are removed by passing small amounts of alcohol into the molten wax, thus causing a violent ebullition. Such extracts of orange blossoms are known to the trade as "concrètes" because they contain, besides the oil of neroli, a considerable quantity of the waxy components of the flowers.

7. Deterpenation. Preparation of Terpeneless Oil

The chemical composition of citrus oils has been previously discussed at considerable length. The principal constituents of citrus oils are the terpenes, the sesquiterpenes, and the oxygenated compounds. Of these, only the last are largely responsible for the characteristic odor of citrus oils, although they are present in very small proportions. It has, therefore, always been the aim of the perfumer and the manufacturer of flavoring extracts to recover this valuable portion of citrus oils in some sort of concentrated form. In fact, the greater part of citrus oils manufactured are "concentrated" in this way either by the citrus-products manufacturer himself or by special perfumery and essential-oil plants. This process, consisting primarily in the elimination of the less odorous terpenes and sesquiterpenes and obtaining, so to say, the quintessence of the oils, is therefore called *deterpenation* or the preparation of *terpeneless oils.*

The terpeneless oils have been shown to constitute but a slight proportion of the natural oil: probably 5 to 6% in lemon oil and only 2% in orange oil. Thus, in the market, one often encounters such denominations as "terpeneless oil of orange, concentrated 50:1" or "terpeneless lemon oil, concentrated 20:1," and so on, according to the specific methods of preparation. The only exception in this respect is the oil of bergamot, with only 50% of terpenes.

Although the process of deterpenation is based mainly on fractional vacuum distillation, some methods are based on selective solubility of the various constituents of the oils and still others on a combination of both principles. Most plants working in this field are keeping the details of their procedures secret, although, in the writer's opinion, little secrecy actually exists; the work evidently requires much skill and perseverance.

Only the general lines of the process of deterpenation will be described here. An operator must vary the details in accordance with the specific oil and the particular apparatus with which he is working. Most citrus oils contain over 90% of *d*-limonene, which can be readily driven off by distillation in a specially constructed vacuum

still provided with a very high reflux column, a cooler, and two receivers, as shown in Figure 49. The object of the reflux column, usually filled with rashing rings or broken porcelain pieces, is to prevent the escape of the oxygenated compounds, which boil at a much higher temperature than the terpenes. The most important precaution to be observed at this first stage is that the citrus oil and the entire still should be *absolutely dry*, otherwise, as already explained, the steam will carry away with it the precious oxygenated compounds.

Fig. 49. Apparatus for deterpenation of citrus oils.

Remaining in the still are the oxygenated flavoring constituents, some sesquiterpenes, and a considerable amount of a waxy mass consisting of stearoptenes and coloring matter. In some cases the sesquiterpenes have also been eliminated; they may best be removed by a similar distillation in vacuo, preferably after transferring the remaining mass into a smaller still. In order to obtain the oxygenated compounds, the residue is steam-distilled (without vacuum) and collected in a small receiver, while the stearoptenes and other impurities are left behind in the still. Some manufacturers claim that the waxy residue has a tendency to withhold desirable fractions of the oxygenated constituents. Nelson and Mottern (1934) have de-

signed for recovery of the oxygenated compounds a small laboratory still (Fig. 50) which can be easily translated into similar apparatus on a commercial scale. In their still the residue is admitted dropwise at the top of a tower packed with baffle plates, where it meets a rising current of steam. The steam deprives the residue of all its volatile constituents, which are then condensed together with the steam and caught in the trap.

Other methods are based on the difference in solubility of the terpenes and the oxygenated compounds in dilute alcohol. If very dilute alcohol is used, these methods are long and tedious; stronger alcohol, on the other hand, tends to dissolve some of the waxy and coloring matters. Recently, however, a new countercurrent extraction process has received much attention and has already been introduced in commercial practice. This novel deterpenation process, which has been patented by van Dijck and Ruys,[8] consists in using two solvents at the same time: pentane to dissolve the terpenes, and dilute methyl alcohol as solvent for the oxygenated compounds. The process is carried out in a very simple glass apparatus consisting of a cylindrical glass tube, about 2 meters long and 4 cm in diameter. The citrus oil is introduced in the middle of the tube, which is slightly inclined, while the two solvents enter the tube at opposite ends, passing each other in countercurrent fashion: the heavier phase (methyl alcohol) flowing by gravitation, the lighter phase (pentane) running in the opposite direction under a slight pressure. The tube is separated by fixed gauzes into a number of mixing and settling spaces at regular intervals. In the mixing spaces, which are provided with agitators fitted to a central shaft and driven by a small electric motor and a reduction gear, the two phases are thoroughly mixed, while in the settling intervals the two phases are allowed to separate into two

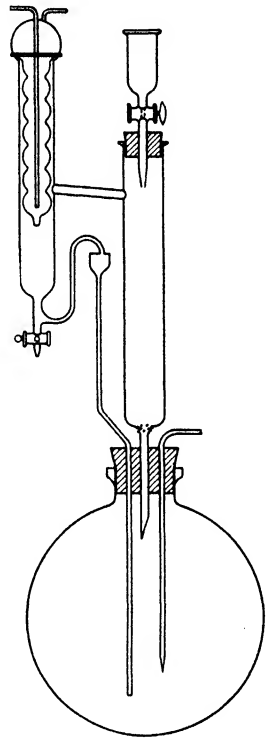


Fig. 50. Laboratory still for deterpenation of citrus oils (after Nelson and Mottern).

[8] Van Dijck, W. J. D., and A. H. Ruys, British Patent No. 355,294; U.S. Patent No. 2,023,109; D.R. Patent No. 539,831.

layers by gravitational action. After passing the whole length of the tube the pentane, containing the terpenes, is discharged at one end and recovered by distillation at atmospheric pressure, while *d*-limonene remains in the still. The methyl alcohol phase, containing the oxygenated compounds, is discharged at the other end of the tube and is freed from the solvent by distillation in vacuo (about 10 cm Hg); to prevent deterioration of the valuable oxygenated compounds the temperature of the liquid must invariably remain below 55° C. When about 80% of the alcohol has been recovered the distillation is stopped and the remaining liquid treated with 5 parts by volume of salt brine. After shaking, the oxygenated compounds separate out as an oily top layer.

Since such widely differing methods are being used in the process of deterpenation, it is not surprising that the resulting terpeneless oils differ greatly in constitution. A decided difference is observed in the angle of rotation, for instance, if only the terpenes, or both the terpenes and sesquiterpenes, are removed.

8. Seasonal Variations in the Yields of Oils

Numerous data collected by various investigators have shown that the yield of citrus essential oils from different countries of origin—indeed, even from different localities within a single country—vary considerably. The yields vary also from season to season. It is difficult to say whether climatic conditions or cultivation methods, or both, are responsible for the differences.

Moreover, the fruits of the same tree picked at the same time show wide discrepancies. In fact, the yield of the oil cannot with certainty be related to the weight of the fruit or to its surface, for both are variable and undergo appreciable changes during the months of growth.

These difficulties, which are universal in dealing with plant products, are, in the opinion of the writer, responsible for the general inconsistency in various analytical data regarding seasonal and other variations in the yield of oils.

There is, for instance, a divergence of opinion whether the amount of oil in the peel diminishes as the season progresses. The general view is that the amount of oil decreases. However, there are indications that analytically the amount of oil does not diminish but that it is difficult to extract technically, for, as already mentioned, the turgor under which the essential-oil glands are held at the beginning of the season is considerably lessened when the fruit is ripe and is

even more greatly reduced when it is overripe. In the absence of this turgor, the oil does not spurt easily and is retained by the peel.

Bennett and Donovan[9] found that the oil content of overripe lemons in June is equal to that of lemons industrially worked during the normal season (from November to March). Their conclusions can be supported by the results of a systematic study made by A. Carmi and the writer during the entire season of 1937 on Palestine oranges, as shown by the following table, in which each figure represents an average of at least five fruits picked from a single tree of a 25-year-old grove. Variety, Shamouti (Jaffa) grafted on a sour orange.

Essential Oil, Per Cent by Weight of Fruit

Date	Small Fruit	Large Fruit
Dec. 5, 1935	0.325	0.436
Dec. 20, "	0.290	0.341
Jan. 9, 1936	0.210	0.115(?)
Jan. 24, "	0.320	0.420
Feb. 6, "	0.373	0.506
Feb. 21, "	0.333	0.348
Mar. 6, "	0.416	0.583
Mar. 20, "	0.450	0.466

In further work in collaboration with Dr. F. Stern in 1944 on lemons, the results showed a steady diminution in the amount of oil, while similar tests with oranges showed little change.

Date	Oil (Lemons) per mille	Oil (Oranges) per mille
Oct. 1943	6.70	3.86
Nov. "	5.30	3.55
Dec. "	4.55	3.85
Jan. 1944	3.99	3.40
Feb. "	4.30	3.56
Mar. "	3.47	3.05
Apr. "	3.75	3.45
May "	3.80	4.42

9. Keeping Qualities of Citrus Oils

The citrus essential oils, being mixtures of a number of different substances—including, also, unsaturated compounds—tend naturally to change with time. Thus, for instance, unsaturated hydrocarbons (the terpenes) easily polymerize and resinify, creating gums, to the detriment of the aroma and fragrance.

The three major enemies of the essential oils are air, light, and heat. The oxygen of the air has a direct oxidizing effect upon the stored oils, while light also affects the color and the fragrance of the

[9] Bennett, A. H., and F. K. Donovan, "Peels in Brine, Essential Oil Content, Detection of Extraction," *Perfum. and Essent. Oil Rec.*, **29**, 12 (1938).

oils, due most probably to polymerization. Heat naturally accelerates these undesirable processes. It is of the utmost importance, therefore, that when essential oils are stored, these three detrimental factors be carefully excluded. The containers must accordingly be as full as possible and hermetically sealed (Fig. 51).

The usual containers are copper vessels, tinned on the inside, of sizes between 7 and 28 lbs. Lately, these have been replaced more and more frequently by bottle-shaped aluminum vessels of various sizes and forms. If citrus oils are kept in glass bottles, these should be of dark glass, filled to the neck, and well corked and sealed.

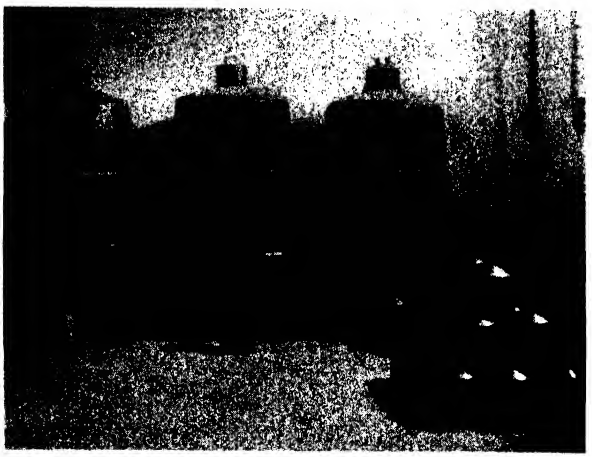


Fig. 51. Copper containers for storing essential oils.

Some authors have suggested storing citrus oils in an atmosphere of an inert gas, such as CO_2 or nitrogen. However, this seems to be superfluous if the containers are filled as described above. The introduction of CO_2 may even have a detrimental effect, as shown by the keeping qualities of French Guinea orange oil; when stored under CO_2 the oil deteriorated significantly. Kennerth[10] found that lemon and orange oils kept well when stored in bottles under nitrogen.

Careful examination of the effect of temperature, water, air, and light on limonene and the California citrus oils has been made by Poore (1932). The physical constants of the original oils did not change at all after 20 months storage in sealed bottles, whether kept in cold storage or at ordinary room temperature. However, orange oils, for instance, exposed to air and light, after 20 months had an

[10] Kennerth, R. A., "How Varied Conditions Affect Some Essential Oils," *Am. Perfumer Essent. Oil Rev.*, **20**, 695 (1926).

increased specific gravity (original 0.8511 increased to 1.025) and refractive index (original 1.474 increased to 1.498) and a much lower optical rotation (original +60°20′ decreased to +22°21′); the acid value increased from 0.9 (original) to 29.2 (at the end of the experiment).

Ogston and Moore[11] reported that samples of Italian lemon oil kept in sealed containers for 10 years showed practically no difference in analysis and no material change in quality.

Donovan (1937) reported analytical data of machine-pressed lemon oil kept under seal for nearly 4 years:

Date	Nonvolatile residue %	Citral %	$\alpha_D^{15.5°}$	$d_{15.5°}$
June 21, 1933..	4.12	3.45	60.05	0.8570
Feb. 16, 1937..	4.60	3.40	60.10	0.8568

There is ample evidence that machine-pressed oils, carefully prepared and stored under proper conditions, have definitely better keeping qualities than hand-pressed oils.

Recent examination of Brazilian orange oil,[12] left for three months in the laboratory exposed to light and in some cases to air, showed the following changes: $d_{25°}$ from 0.845 to 0.874; $n_D^{20°}$ from 1.4740° to 1.4780°; $\alpha^{20°}$ from 98.6 to 86.0; nonvolatile residue from 4 to 12.5%.

This last constant of nonvolatile residue is of the greatest interest. While oils extracted by machines (especially the rasping methods) contain higher nonvolatile residues due to the fact that they come in close contact with the chromophores and therefore dissolve more of the waxy materials, all citrus oils naturally tend to acquire higher residues as the season progresses. The following figures show differences in machine-pressed orange oils during a season in regard to their nonvolatile residues (%):[13]

	1945	1946	1947
January	4.80	3.83	3.86
February	5.26	3.76	...
March	6.30	5.14	...
April	8.34	...	7.96

It may be suggested that these changes could be caused by prolonged exposure to the action of ultraviolet rays of the sun. Maffei[12]

[11] Ogston, G. H., and M. Moore, "Alteration of Lemon Oil on Keeping," *Perfumery Essent. Oil Record*, **14**, 7 (1923).

[12] Maffei, F. J., "Anomalous Characteristics of Orange Oils," *Anais assoc. quim. Brasil*, **5**, 61 (1946).

[13] By courtesy of Dr. F. Stern, Jaf-Ora, Ltd., Rehovoth, Israel.

showed that an orange oil exposed in a closed glass flask to ultraviolet light (from a Hanau lamp with a focus of 30 cm) underwent the following changes:

	Original	After 40 hours	After 48 hours
d_{25}°	0.841	0.843	0.852
$n_D^{20^\circ}$	1.4729	1.4734	1.4751
α^{20°	97.4	96.5	92.2
Residue, %	1.6	2.27	6.5

According to Schimmel and Co. of Leipzig (Germany), lemon and orange oils kept well for 10 years with the addition of 10% absolute alcohol or 2–5% olive oil. Several other procedures have been suggested for keeping essential oils unaltered. According to a French patent (No. 736,984), small quantities (1.4–1.5%) of dioxynaphthalene and various condensation products of pyrogallol and acetone may be used. Similarly, oxidation of unsaturated compounds present in essential oils is prevented, according to a U.S. patent [No. 1,898,363 (Sept., 1932)], by small additions of multibasic aliphatic acids and their esters and salts, also of hydroxylized diaryl compounds in quantities of 0.001–0.1%. A mixture of wheat-germ oil and hydroquinone added to essential oils of lemon and orange in concentrations of 0.02–0.1% is claimed[14] to be excellent for improving their keeping qualities. It is questionable whether all these additions are practicable.

One important point must be carefully kept in mind: all citrus oils (unless distilled) deposit, for a considerable time after their extraction, large amounts of stearoptenes. It is, therefore, expedient to keep the bulk of the expressed oils for a few weeks in large copper containers until most of the stearoptenes have settled. The oils may then be carefully filtered, filled in containers, and sealed for shipment.

To accelerate the settling of stearoptenes, the oils may be chilled for varying periods before filtration.

10. Examination of Citrus Oils

The examination of citrus oils is usually required to evaluate the quality of various oils according to the presence of their most odoriferous components, or to ascertain adulteration, if any. For this purpose the most commonly accepted methods of examination have been compiled in this section. For each test only one method has been selected although, of course, for several of the determinations a

[14] Lakritz, W., "Lemon and Orange Oil Preservation," *Mfg. Confection.*, **23**, No. 9, 18 (1943).

variety of methods may exist. The methods chosen are, for the most part, those officially accepted by the Association of Official Agricultural Chemists (U.S.A.).

It is frequently desired to investigate a particular oil for its chemical components, which may be separated either by fractional distillation or by methods of organic chemistry; they are often identified, however, by comparison with the physical indices of the corresponding pure substances.

(a) Estimation of Oil Content in Fruit

No practical method has been found to extract the oils from the peel by chemical solvents without admixing a considerable quantity of water; the results are then always erroneous. The quantity of oil in the peel or in the blossoms is determined, therefore, by distillation. The method is known as that of Wilson and Young (1917), but some modifications in preparing the peel and in measuring the quantity of oil have been introduced by Donovan (1937) and others.

To obtain correct results the peel must be thoroughly ground. To prevent spurting and volatilization of the oil the sample of the weighed citrus fruit is peeled in a thin layer with a sharp knife, and the peel is thrown directly into a beaker containing a salt brine (20% NaCl solution), the peel remaining in the brine overnight. This gives the peel a certain softness and eliminates the turgor. The following morning, when the peel is comminuted in a meat mincer, very little oil is lost. The ground peel with the ruptured cells is washed into a two-liter, round-bottomed, short-necked flask, thereby creating a thin paste of minced peel and salt brine. The flask is fitted with a steam tube projecting to the bottom and is attached to a straight condenser by means of a Kjeldahl bulb (Fig. 52). The oil is then distilled by steam from a small generator, placing an additional small flame under the flask with the peel. Alternatively, the flask can be wrapped in a towel to be kept warm during the distillation, which must proceed rapidly but not violently. The oil is distilled until it ceases to come over: about 200 cc of distillate are usually sufficient. The distillate is conveniently received in a burette filled with water, the lower end of which is connected with a syphon, as shown in the figure. Such an arrangement makes it possible for the oil to separate in the upper part without being smeared over the whole burette.

The volume of oil collected is measured in cc and its weight is calculated by multiplying it by the previously established specific gravity. Duplicate determinations should check within 0.1 cc.

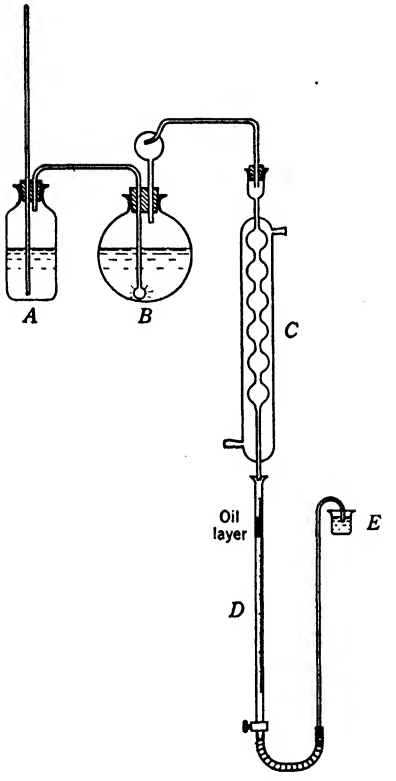


Fig. 52. Improved Wilson apparatus for determination of oil content in the peel of citrus fruits: *A*, steam generator; *B*, distillation flask with Kjeldahl bulb attachment; *C*, condenser; *D*, burette with constant-level attachment; *E*, receiver of excess distillate.

(b) Physical Tests

(1) SPECIFIC GRAVITY

When large quantities of oil are available and when the degree of accuracy is required only to the third decimal point, it is sufficient to determine the specific gravity with a hydrostatic balance, such as Mohr's or Westphal's. For more accurate determinations, or when only small quantities of oil are at the disposal of the investigator, the use of a pycnometer is required (Fig. 53).

The specific gravity at 20/20° (in air) is determined as follows: The pycnometer (previously cleaned with chromic mixture and

thoroughly rinsed with water), full of recently boiled H_2O and adjusted to level in a water bath at 20° C, is wiped dry and, after being allowed to stand for 15–20 min, weighed. The same pycnometer

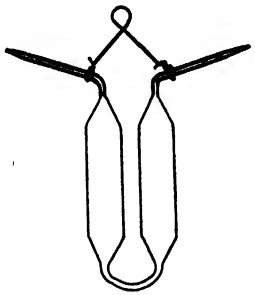


Fig. 53. Ostwald pycnometer.

is weighed empty after rinsing with alcohol and then with ether. The difference in both weights is the weight of H_2O at 20° C. The dry pycnometer is then filled with the essential oil and weighed, after adjusting the level in a bath at 20° C. The weight of the contained oil divided by the weight of the contained water is the specific gravity of the oil at 20/20° C.

According to numerous experiments made by Schimmel and Co., using the temperature of water at 15° C as a basis, the specific gravity of the volatile oils varies, on the average, 0.00075 for each degree centrigrade. If, therefore, the temperature at which the specific gravity is determined is higher than 15° C, the correction should be added for each degree difference; if lower, the correction should be deducted.

According to Schreiner and Downer, the correction factor for observations between 15° and 25° C is 0.00064 for each degree.

(2) OPTICAL ROTATION

The determination of the angle of optical rotation, a specific property of all citrus oils, is very important. This determination is made in any standard half-shadow polarimeter (Fig. 54); the source of light should be the sodium D-line. For citrus oils a 50 mm tube is used. The results are stated at 20° C in angular degrees on a 100 mm basis. If instruments having the sugar scale are used, the reading for orange oils is above the range of the scale, but readings may be obtained by the use of standard levorotatory quartz plates or by the use of smaller tubes, 25 or even 20 mm long. The true rotation cannot be obtained by diluting the oil with alcohol and correcting the rotation in proportion to the dilution.

The angle of rotation depends largely upon the temperature during examination: with increasing temperature the angle of rotation is reduced. Although the natural variations in the rotation of an oil, in general, are usually greater than the differences due to a variation of several degrees in temperature, orange and lemon oils are exceptions; their rotation is strongly influenced by even small changes in temperature. Hence, in order to obtain comparable data, the temperature during observation should be exactly recorded and then corrected to +20° C.

According to Gildemeister, for every degree centrigrade variation in temperature between 10° and 20° the difference in the angle of rotation is 14.5 minutes; between 20° and 30° the difference is 13.2′. When correcting for 20° C it is, therefore, necessary to deduct 14.5′ for every degree if the observation is made below 20° C and to add 13.2′ for every degree if the original reading is made above 20° C.

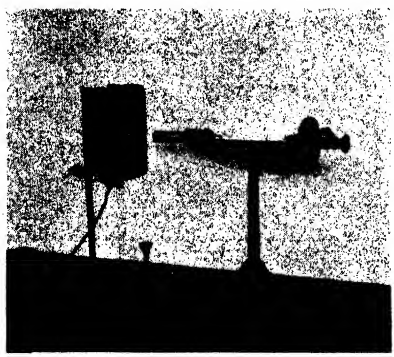


Fig. 54. Polarimeter.

The observed angle of rotation in a 100 mm tube with sodium light at 20° C is designated as $\alpha_D^{20°}$, while the specific rotation is designated as $[\alpha]_D^{20°}$. The specific rotation of an essential oil is calculated according to the formula:

$$[\alpha]_D = \frac{a}{ld}$$

where a is the observed angle of rotation, l the length of the tube in decimeters, and d the specific gravity of the oil.

(3) REFRACTIVE INDEX

Determination of the index of refraction, $n^{20°}$, is made with any standard refractometer (Pulfrich, Abbe, or Zeiss) at 20° C, using diffused daylight or some form of artificial light (Fig. 55).

To charge the instrument (Abbe or Zeiss), place 2 or 3 drops of the filtered oil on the surface of the lower prism and close the prisms firmly by tightening the screw head. Circulate through the prism a stream of water of constant temperature. Allow the instrument to stand for a few minutes before a reading is made, so that the temperature of the sample and the instrument will be the same. Move the alidade backward and forward until the field of vision is divided into a light and a dark portion. The line dividing these portions is the borderline of total refraction, and, as a rule, is not a sharp line

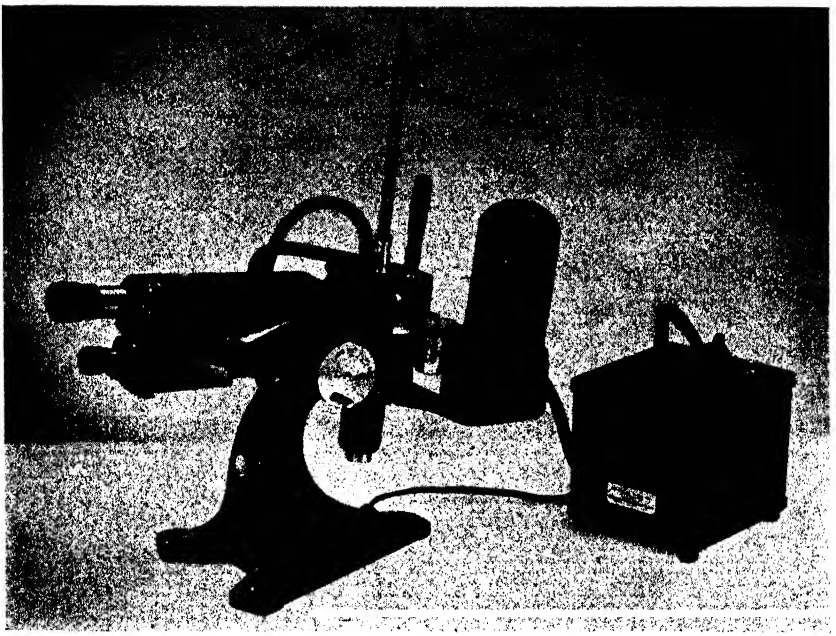


Fig. 55. Precision Abbe refractometer (courtesy Bausch and Lomb Optical Co.).

but a band of color. Eliminate the colors by rotating the screw head of the compensator until a sharp, colorless line is obtained. Now, adjust the borderline so that it falls on the point of intersection of the cross hairs. Read the refractive index of the oil directly on the scale of the sector.

As the temperature rises the refractive index falls. The index also varies directly with the specific gravity. The instrument used may be standardized with H_2O at 20° C, the theoretical refractive index of H_2O at 20° C being 1.3330; it may also be standardized by means of a quartz plate that accompanies the instrument, using monobromonaphthalene. Any corrections found should be applied to all readings.

The readings of the Zeiss butyrorefractometer on oils may be brought to standard temperature by the following formula:

$$n_D^{20°} = R' + 0.58\,(T' - T)$$

in which T is 20° C, T' the temperature of the reading, R' the reading at T', 0.58 the correction in scale divisions for 1°.

The readings of instruments that give the index of refraction directly can be reduced to standard temperature by substituting the factor 0.00038 for 0.58 in the formula.

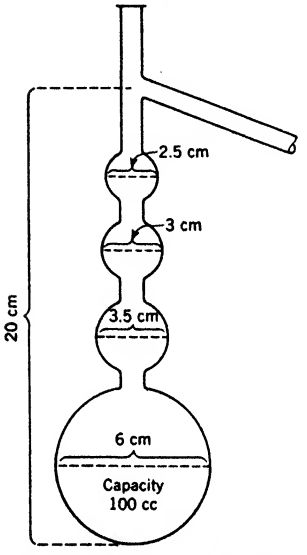


Fig. 56. Ladenburg flask.

The specific refractive power is calculated by the Lorenz and Lorentz formula:

$$\frac{n^2 - 1}{n^2 + 2} \cdot \frac{1}{d}$$

when n, the refractive index, and d, the specific gravity, are determined at the same temperature.

Further applications of the refractometer in citrus chemistry are discussed in the section on juices.

(4) SOLUBILITY TEST

One cc of the oil to be tested is placed in a small graduated cylinder fitted with a glass stopcock. Alcohol of a desired strength is added in small portions and the cylinder is vigorously shaken. If normally soluble oils show any abnormalities, it is sometimes possible to draw conclusions regarding adulteration from the character of turbidity and the separation of an insoluble part. Petroleum, for instance, floats on 70% alcohol, while a fatty oil settles in drops at the bottom.

(5) DISTILLATION TEST

This test may be performed to investigate roughly the different fractions of the essential oil and to determine their boiling points; principally, however, it is used in order to ascertain adulterants. When lemon oil, for instance, is adulterated with turpentine oil it reveals an abnormal optical rotation, which, however, is masked if orange oil or terpenes are added at the same time. For this purpose it is customary to test the first 10% fraction of the distillate.

Place 50 cc of the oil in a three-bulb Ladenburg flask (Fig. 56), having the main bulb 6 cm in diameter and of 100 cc capacity and the condensing bulbs of the following dimensions: 3.5, 3, and 2.5 cm. The distance from the bottom of the flask to the opening of the side arm should be 20 cm. Distill the oil at the rate of one drop per sec until exactly 5 cc have been distilled. Determine the refractive index and the angle of optical rotation of this distillate.

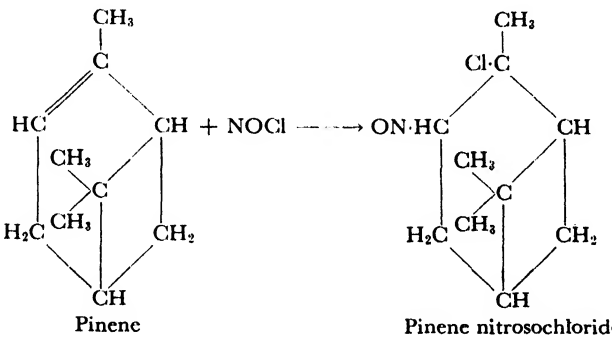
(c) Chemical Tests

(1) DETECTION OF PINENE (OFFICIAL METHOD)

This method, developed by Chace, is based on the preparation of pinene nitrosochloride crystals according to the following formula, and their examination under the microscope.

$$C_2H_5ONO + HCl \longrightarrow C_2H_5OH + NOCl$$

Ethyl nitrite



Pinene — Pinene nitrosochloride

Mix the 10% distillate obtained in the preceding test with 5 cc glacial acetic acid, cool the mixture thoroughly in a freezing bath, and add 10 cc of ethyl nitrite. Then add slowly 2 cc of HCl (2 : 1) with constant stirring. Keep the mixture in the freezing bath for 15 min. Collect the crystals formed on a filter, using suction, and wash with 95% alcohol. Return the combined filtrate and washings to the freezing bath for 15 min. Collect the additional crystals formed on the original filter. Wash the combined crops of crystals thoroughly with alcohol. Dry at room temperature and dissolve in a minimum quantity of $CHCl_3$. Add methyl alcohol to the $CHCl_3$ solution, a little at a time, until the nitrosochlorides crystallize out. Mount the separated and dried crystals in olive oil and examine under microscope. Pinene nitrosochloride crystals have irregular pyramidal ends, while limonene nitrosochloride crystallizes in needles.

(2) NONVOLATILE RESIDUE

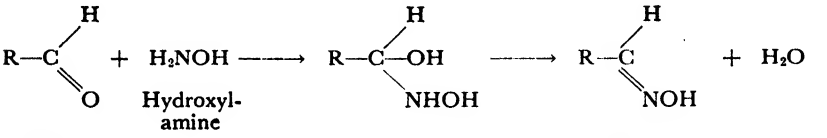
About 5 g of the oil are weighed, as accurately as possible, to the centigram in a tared glass dish and evaporated on a water bath until of constant weight, which requires a few hours.

Machine-pressed oils naturally have larger residues than hand-pressed oils because, during manufacture, machine-pressed oils are in longer contact with the peels and, as a result, incorporate more of the waxy constituents.

(3) DETERMINATION OF ALDEHYDES (CITRAL)

Numerous methods exist for the determination of aldehydes in citrus oils, but only a few give accurate results.

Walter-Bennett Method. This method is based on the reaction between an aldehyde and hydroxylamine:

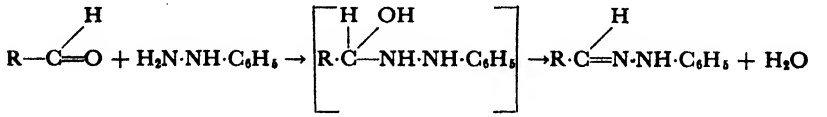


Aldehyde Aldoxime

Boil a mixture of 20 cc oil, 20 cc 0.5 *N* alcoholic (80%) hydroxylamine-HCl solution, 8 cc alcoholic *N* KOH, and 20 cc aldehyde-free strong alcohol for half an hour in a long-necked flask fitted with a reflex condenser. Cool and add 250 cc water, rinsing the condenser. Neutralize the HCl still combined with hydroxylamine, using phenolphthalein. Then titrate the hydroxylamine not combined with the citral with 0.5 *N* H_2SO_4, establishing the end point by removing drops to test with methyl orange as indicator.

A blank experiment without oil must be run to find the factor of hydroxylamine solution. From the difference in the 0.5 *N* H_2SO_4 consumed in the two experiments the amount of citral is computed by multiplication with 0.076.

C. Kleber's Method. This method is claimed to be the most reliable of all and is also officially accepted in the United States. It is based on the following reaction:



Phenylhydrazine Intermediary addition product Phenylhydrazone

Prepare a 10% solution of phenylhydrazine (previously distilled

in vacuo, rejecting the first portions containing NH_3) in absolute alcohol. To an accurately weighed sample of oil (15 g) in a glass-stoppered flask add 10 cc of the phenylhydrazine solution. Allow to stand 30 min and titrate with 0.5 *N* HCl, using either methyl or ethyl orange indicator. Titrate, similarly, 10 cc of the phenylhydrazine solution. The difference in the number of cc of 0.5 *N* acid used in these two titrations, multiplied by the factor 0.076, is the weight of citral in the sample.

If difficulty is experienced in detecting the end point, titrate until the solution is distinctly acid, transfer to a separatory funnel, and draw off the alcoholic portion. Wash the oil with H_2O, adding the washings to the alcoholic solution, titrate back with 0.5 *N* alkali, and make the necessary corrections.

Note: In the case of orange oil allow to stand with phenylhydrazine for 2 hours; decylic aldehyde reacts more slowly than citral.

British Pharmacopoeia (1932) Method. A simplified method of determining citral in lemon oil is given by British Pharmacopoeia (1932) in its latest edition (Jan. 1943):

Weigh accurately about 10 g of the oil into a stoppered tube, approximately 25 mm in diameter and 150 mm in length. Add 7 cc of hydroxylamine-HCl reagent in 60% alcohol and 1 drop of methyl orange solution, shake, and neutralize the liberated acid with 0.5 *N* KOH in 60% alcohol until the red color of the indicator changes to permanently full yellow in the lower layer, after shaking vigorously for two min and allowing separation to take place. The volume of the hydroxylamine-HCl must exceed by 1 to 2 cc the volume of 0.5 *N* KOH.

(4) ESTIMATION OF TOTAL ESTERS

Weigh accurately about 2 g of the oil to the nearest centrigram into a wide-necked flask fitted with a glass tube about one meter long serving as a reflex. Add a few cc of acid-free alcohol. Neutralize exactly the free acid with 0.1 *N* alkali and add a measured excess of 25 cc of 0.1 *N* alkali. Boil for an hour with the tube as a reflex condenser, cool, and titrate with 0.1 *N* acid.

Calculate the number of cc of 0.1 *N* alkali used in saponification of the esters either as ethyl acetate 1 cc. 0.1 *N* alkali = 0.0088 g) or as linalyl and geranyl acetates (1 cc 0.1 *N* alkali = 0.0196 g).

(5) IDENTIFICATION OF CONSTITUENTS

The isolation and identification of the individual constituents of a citrus oil, as of any other essential oil, is a tedious and difficult task,

requiring much skill and a thorough knowledge of the subject. The compounds isolated by fractional distillation or by various chemical means are tested for their physical indices or otherwise identified. The procedure, however, cannot be dealt with in detail here; one should consult special treatises on the subject or standard books on

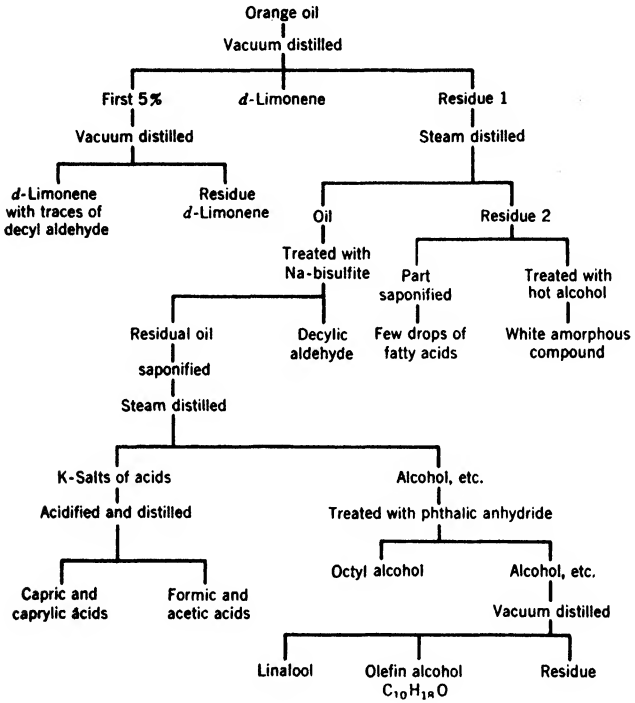


Fig. 57. Flow sheet showing the determination of the constituents in orange oil (after Poore).

organic chemistry. However, a general outline is given here diagrammatically (Fig. 57), taken from the work of Poore (1932) showing an example of the method used for the identification of the constituents of California orange oils.

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CHAPTER VI

PROCESSING CITRUS JUICES

1. Halving the Fruit

If the essential oils are recovered from whole fruit, the fruit again has to undergo a thorough rinsing with pure water. Halving the fruit is necessary if the juice is to be extracted by the burring principle, whether by hand or by machine. Halving is usually performed by a circular knife or saw of stainless steel, rotating at a considerable speed (about 1500 rpm). The fruit from the washer is run to the knife through a V-shaped chute inclined towards the blade at 40 or 45°. If the chute is sufficiently long the fruits arrange themselves in perfect order, one after the other, and usually orientated by the short axis. However, only a part of the fruits are cut across the growth axis since the fruit has a tendency to roll around the longest axis. To obtain a steady flow of fruit towards the knife, it has been proposed to place two V-shaped chutes one above the other, with a difference in height of only a few centimeters: the fruit, running down the first chute, will fall a short distance down into the second chute, thus acquiring a steadier and more regular flow.

In some plants the fruit is fed to the circular blades by an endless belt carrying spikes or prongs upon which the fruit is laid one by one; the prongs carry the fruit past the knife, which rotates in the opposite direction (Fig. 59). Such individual handling of the fruit is better in many respects, but the speed of halving is naturally greatly reduced and the feeding requires much labor.

2. Juice Extraction by Hand

The extraction of juices from citrus fruits is far more complicated than from any other fruit, as has been previously explained. Simple disintegration and squeezing will not yield the pure juice secreted in the segments but will incorporate much of the tissue fluids from the albedo, as well as peel oil and some of the undesirable glucosides and other foreign matter. The only acceptable method, therefore, of obtaining a high-grade juice for drinking purposes is to ream the

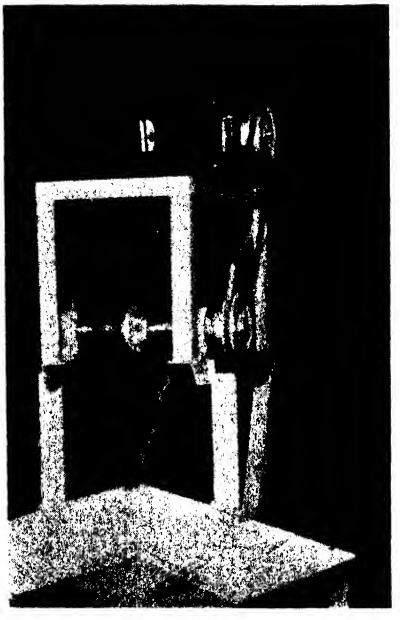


Fig. 58. Arrangement of V-shaped chute to circular knife.

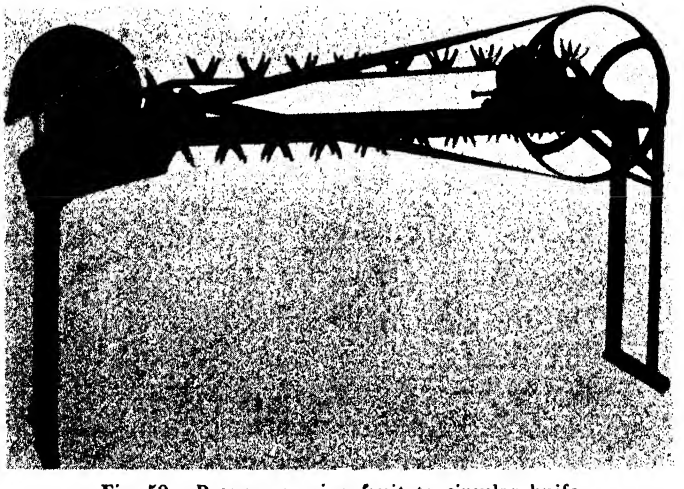


Fig. 59. Prongs carrying fruit to circular knife.

halves of a crosswise-cut citrus fruit on a suitable rosette. Such reamers are well known in the household as "lemon squeezers." The difficulty in translating this operation into a manufacturing procedure obviously lies in the fact that each half must be handled separately. Until recently, hand-reaming on revolving rosettes was the only known and generally accepted method. Practically all producers outside the United States and Palestine still ream fruit by hand.

Long reaming tables are set up in such a way that fruit cut in halves passes on a rubber conveyor between two rows of burring heads, made of stainless steel, aluminum, or possibly glass, rotating at a speed of 600 to 1500 rpm. These rosettes are usually conical and have ribbed or grooved sides; a variety of forms in use are shown in Figure 60.

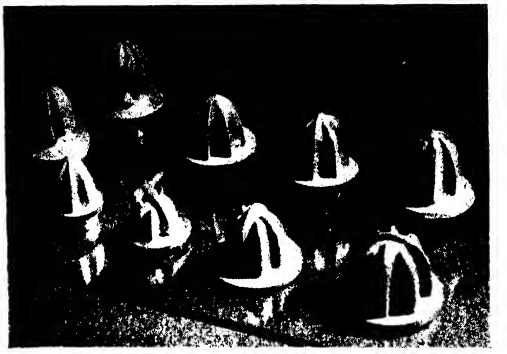


Fig. 60. Collection of reaming rosettes.

The higher the speed at which the reamer revolves the greater the tearing effect upon the tissues of the endocarp, but the high speed results in greater aeration of the extracted juice. The fruit from the belt can be diverted to the various workers by swinging gates opening to different rosettes. In some cases the reamers are set up horizontally (Fig. 61), in others they are set at an angle of 45 degrees; each is surrounded by a suitable pail to collect the expressed juice. The burring heads are often made interchangeable for use in handling either small fruit, such as lemons, or large ones, such as grapefruit. The operator picks up the halved fruit, presses it against the revolving rosette, and then tosses the peel through the refuse opening. The peel is carried away by another conveyor or by the returning part of the same belt which delivers the fruit. All rosettes have a common shaft with right-angle gears, or each rosette has its own individual small motor (about 1/3 HP). The extracted juice runs

through an opening in the pail and is carried away by a sanitary pipe or channel. All parts coming in contact with the fruit or the juices must be made of noncorrosive metal—stainless steel or aluminum—with as few seams as possible. The entire assembly must be accessible and easy to clean.

This method of juice extraction is the best for it does not break the oil cells of the peel nor crush the seeds; it is, however, very expensive, requiring individual handling, and there is a great difference in the output of many operators. The amount of juice an operator can ream per hour depends on the quality of the fruit, on the particular variety

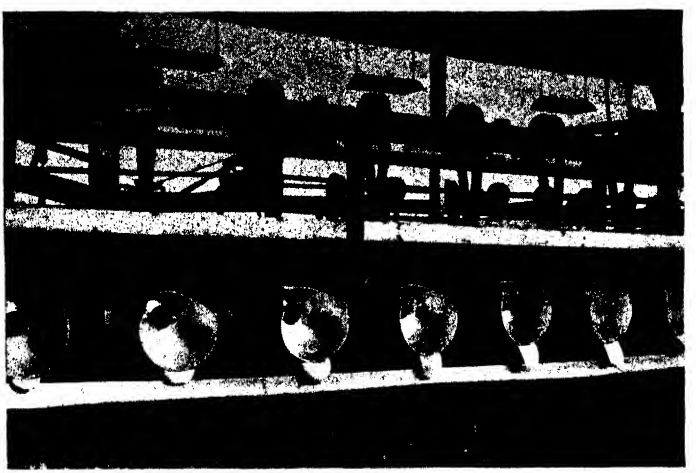


Fig. 61. Reaming table.

handled, and on his individual skill. It runs from as low as 25 liters for lemons to as high as 90 liters for grapefruit per hour, oranges being somewhere in the middle of these two extremes.

It is important to note that the acidity of citrus fruits, especially that of lemons and limes, may cause sensitive sores on the hands and wrists of the operator. To prevent these every worker employed in reaming, after thoroughly washing his hands, should rub on a very thin layer of paraffin oil or vaseline. Dipping the hands into a solution of sodium bicarbonate is not of much help; it may, in fact, aggravate the condition, rendering the skin more tender.

3. Mechanical Extraction

Many attempts have been made during the past 10 years to duplicate the hand method by mechanical means. Perhaps the first effort

in this direction was made early in 1932 by the writer and Criss,[1] who designed and patented a machine for the automatic extraction of citrus juices without injuring or pressing the peel. This machine consists of two belts moving in opposite directions and carrying specially designed cups or "holders" which close tightly when they pass

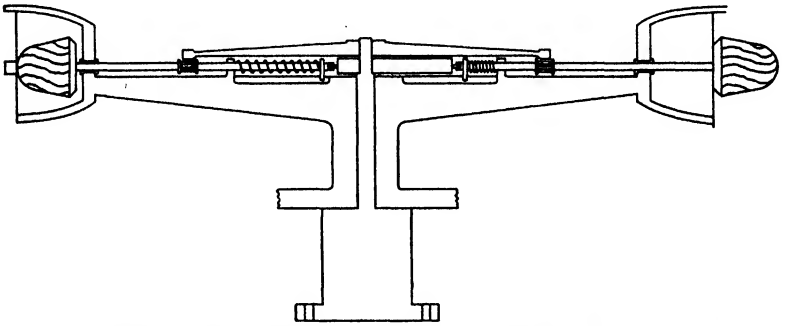


Fig. 62. Automatic juice extractor (vertical cross section).

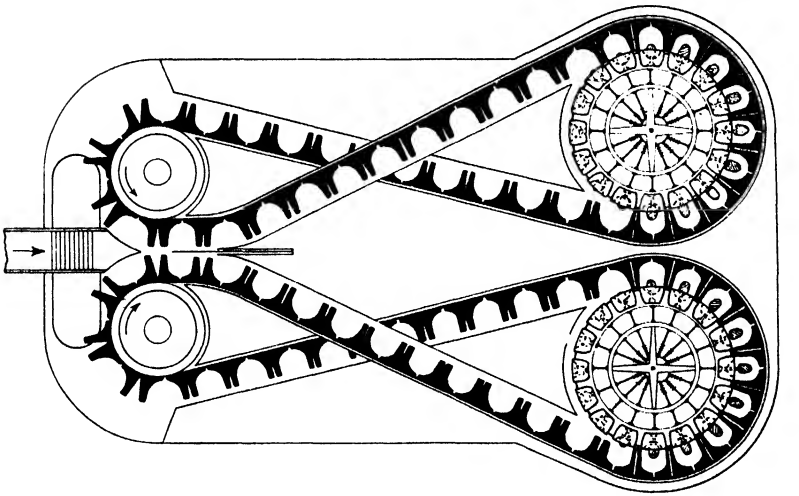


Fig. 63. Automatic juice extractor (horizontal cross section).

the meeting point, thus enabling the cups to grasp the fruit, which at this point is fed quickly in succession, one by one. The cups then grip the fruit firmly and hold it during halving by the circular knife and throughout the extraction process, releasing it later when extraction has been completed (Figs. 62, 63). The extraction of the juice

[1] Braverman, J. S., and B. Criss, Palestine Patent No. 224 (Mar. 16, 1932), and "An Improved Machine for the Continuous Automatic Extraction of Fruit Juices," Palestine Patent No. 245 (June 16, 1932).

takes place at two driving-extracting wheels with hollow "nests" carved in them, suitable for enclosing the rosettes and situated to coincide with the cups carrying the halved fruit. By means of a cam the rosettes, which are fixed to revolving rods, gradually penetrate the fruit and extract the juice; they then slide back while the extracting wheel continues to turn.

Due to technical and constructional difficulties existing in Palestine at that time, this patent was not commercially utilized.

The same principle served as a basis for the citrus-juice extractor invented three years later by Brown[2] in the United States. In this

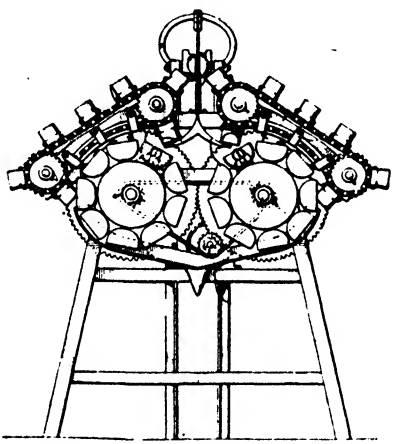


Fig. 64. Automatic juice extractor (Brown patent).

machine a carrier adapted to travel through a predetermined path, with a number of mounted cups made of resilient material (such as rubber), serves to carry the halved fruits to the point where they meet the reamers. The reamers are adapted to enter and leave the cups while the juice is extracted from the fruit sections contained therein (Fig. 64). This machine has been constructed and successfully used in California.

Later, Brown[3] improved his machine. A rotating table carrying the reamers is tilted and over it a rotary carrier, on which a number of inverted cups are mounted, is set in a path angularly disposed with relation to the path of travel of the reamers. The reamers on the

[2] Brown, William O., "Citrus Fruit Juice Extractor," U. S. Patent No. 2,130,610 (applied for Dec. 2, 1935).

[3] Brown, William O., "Juice Extracting Machine," U. S. Patent No. 2,199,876 (Mar. 22, 1937).

rotating table project upwards and are positioned to enter the cups during a portion of their path (Figs. 65 and 66). This machine, now manufactured in the United States, is very complicated but its capacity is enormous (300 fruits a minute). Neither this machine nor any other automatic extraction device is sold outright; they may only be leased against a certain royalty per gallon of juice.

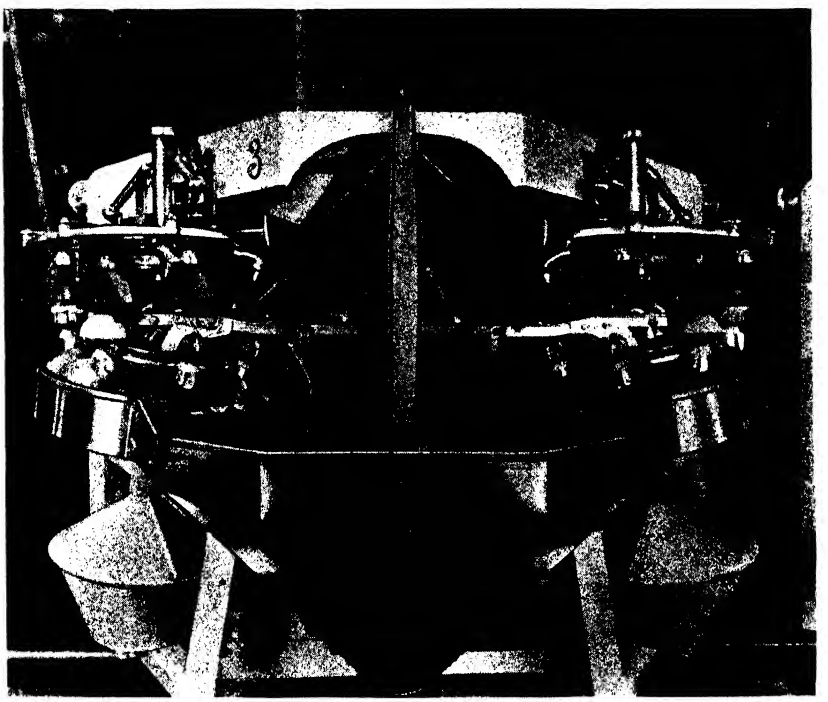


Fig. 65. Improved automatic juice extractor with upper hood removed (Brown patent).

Another patent, based on a system in which the individual citrus fruits are gripped, cut in half, and forced against a stationary squeezing head, was granted to Segovia[4] in 1935 (Fig. 67).

All other automatic extracting machines are not based on the method of reaming the juice with rosettes but on quite different principles. Although some of these are quite ingenious, all of them are inferior to reaming because they exert more or less pressure on the peel, thus squeezing out a considerable portion of the essential oil as

[4] Segovia, Crispin B., "Automatic Fruit Juice Extractor," U.S. Patent No. 2,078,737 (Nov. 9, 1935).

well as some tissue fluids from the albedo. Nevertheless, the entire citrus-products industry of North America and Palestine has recently changed from hand reaming to the use of mechanical extractors, due to the scarcity and high cost of labor.

In the previous chapter, when the centrifugal separation of oil was discussed, a method of crushing whole fruit between rollers and separating the juice in a centrifuge was mentioned. This method, at one time widely employed in the United States, has been abandoned. A similar fate has befallen another process in which the fruit was com-

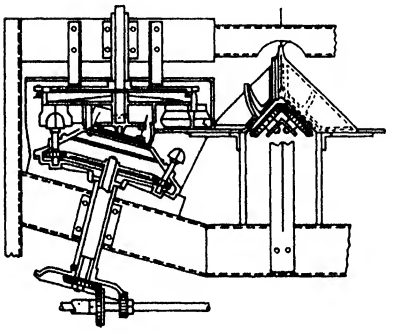


Fig. 66. Diagrammatic presentation of the same extractor illustrated in Figure 65.

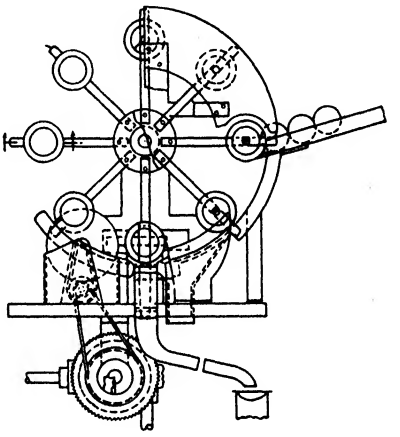


Fig. 67. Segovia automatic extractor.

pletely peeled and the juice extracted in a continuous screw-expeller press. Obviously, such procedures cannot avoid the introduction of peel-tissue fluids, the so-called "rag juice," even when the fruit is peeled. Machines of this type are good only for juices used for technical purposes, such as citric acid, vinegar, etc.

The Food Machinery Corporation of the United States has developed an automatic juice extractor of a plunger type (Skinner). The fruit passing through this machine must first be sized into four sizes, 5½, 7, 8¼, and 9½ cm in diameter. On the way to the extractor heads, the fruit is split in halves and is moved by a special arrangement under the plunger head which presses the fruit down on top of an inverted cup. The clearance between the plunger head and the cup is adjusted to a little more than the thickness of the peel to avoid pressing the peel.

The same firm has introduced another extractor (Polk mechanical juice extractor) which is now used with more success than the

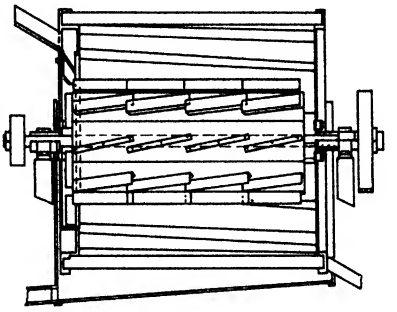


Fig. 68. Polk mechanical juice extractor.

plunger type. Whole fruits fed into this machine are quartered by stainless-steel knives; the quarters pass through specially designed

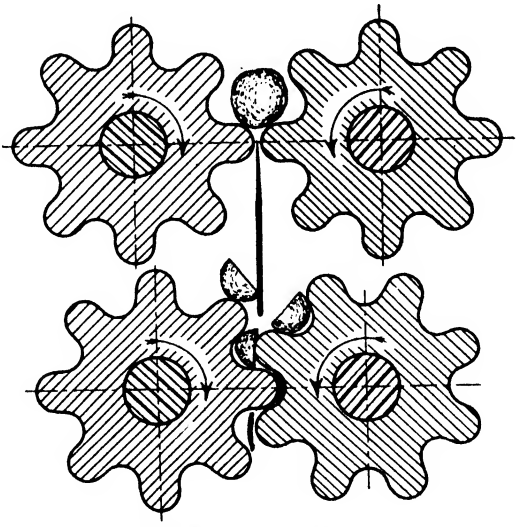


Fig. 69. Diagram showing the principle of Faulds mechanical juice extractor.

rollers which remove the juice. This method, patented[5] in 1937, consists in "repeated batting of freely-falling juice-cell masses, propelling these masses out of the zone of the batting action and straining the

[5] Polk, Ralph, Sr., and Ralph Polk, Jr., "Method of Extracting Fruit Juices," U.S. Patent No. 2,137,414 (June 3, 1937).

juices from them" (Fig. 68). It is claimed that quartering the fruit increases the yield of juice, but it is very doubtful whether it reduces contamination with oil, seeds, and other undesirable tissues.

A very ingenious method of extraction, invented by Fauld and manufactured by Rotary Juice Press of Dunedin, Florida, consists of four grooved cylinders rotating in opposite directions (Figs. 69 and 70). The two upper cylinders are placed so that each groove meets the groove in the opposite cylinder to create an enclosure which

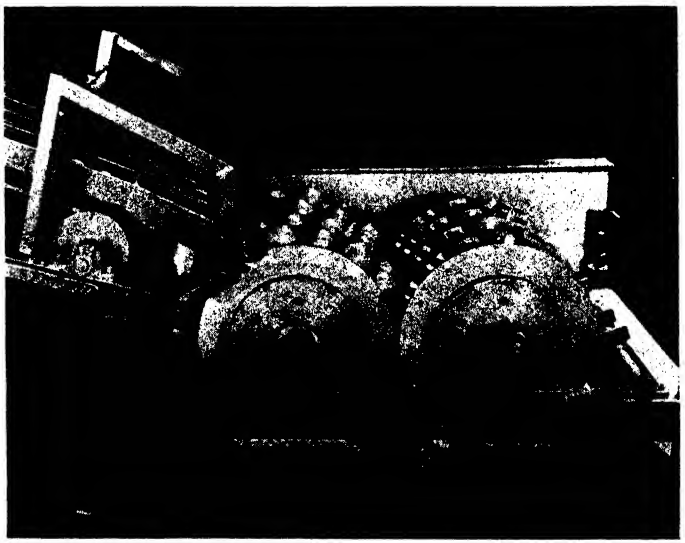


Fig. 70. Fauld's mechanical juice extractor (courtesy Rotary Juice Press).

is capable of holding the fruit and pushing it through the cutting blade. Thereafter the halves are caught by a second pair of cylinders in which, however, the grooves of one coincide with the protuberances of the other. Evidently this causes the juice to be squeezed out of the fruit, the peel being released as the cylinders rotate. One Rotary Juice Press at 10 rpm takes in some 360 fruits per minute, i.e., about 3 to 4 tons per hour. This automatic juice extractor is widely used in Florida. Although the machine is provided with self-regulating springs the juice from it is not free from peel oil. On similar lines, an automatic juice extractor has been constructed in Israel by Dr. M. Koffler, the outstanding feature of which is the separation of the main body of the extracted juice from a smaller quantity of

juice mixed with tissue fluids by placing two separate channels under the extraction head.

Another very popular automatic juice extractor is named "Citro-Mat," patented in the United States by Bireley's, Inc., of Los An-

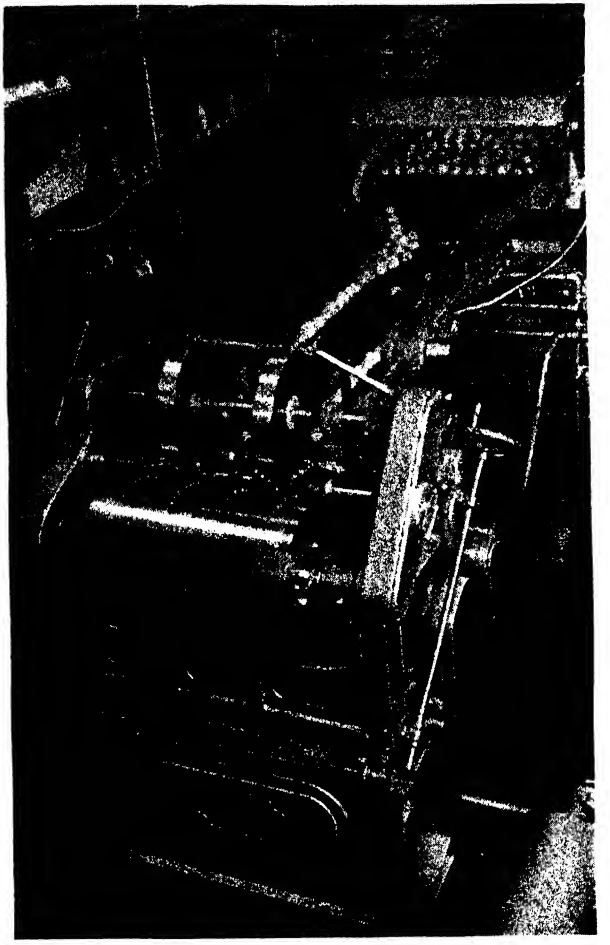


Fig. 71. "Citro-Mat" automatic juice extractor (courtesy Bireley's, Inc.).

geles, California. It consists of a revolving stainless-steel drum carrying small spikes. Halved fruits are fed into the drum, which drags them towards a fixed screen. The distance between the drum and the screen becomes gradually narrower; the juice is pressed out and

passes through the screen (Figs. 71, 72). The latest development of the "Citro-Mat" provides for the possibility of shaving off from the exhausted peel a thin layer of the flavedo, which is then used for the extraction of citrus oils.

A number of other inventions for mechanical juice extraction have been patented but only a few of them have been produced commercially.[6]

The writer is definitely of the opinion that unless mechanical extraction of citrus juice is based on reaming of the fruit without pressing the peel, it will hardly gain much of a foothold in the industry. It is true that during World War II many machines were brought into use and became popular even for the preparation of juice for concentrates. When normal conditions are restored, however, the consuming public would prefer juice with its full content of natural unbroken fruit cells which give it a better appearance and flavor. Juice of this kind can be obtained only by reaming the fruit on revolving rosettes. It has frequently been claimed that during reaming a good deal of air is incorporated, especially when the heads revolve at high speed. There is no evidence, however, that other methods of extraction incorporate less air (compare Table XVII on gaseous constituents), and, as already stated, regardless of the method of extraction the juice must be thoroughly deaerated before further treatment.

A new, very efficient automatic juice extractor, based on a quite different principle, has been put on the market, after thorough experimentation during several seasons, by the Food Machinery Corporation of San Jose, Cal.—the so-called Super Juice Extractor. This machine consists of a series of caps mounted on a circular rotating

[6] Clark, W. E., "Fruit Juice Extractor," U.S. Patent No. 2,241,081 (Feb. 23, 1937).
Deleray, B. R., "Juice Extractor," U.S. Patent No. 1,762,855 (June 10, 1930).
Edenfield, E. E., "Fruit-Juicing Machine," U.S. Patent No. 2,247,190 (May 26, 1938).
Edwards, G. W., "Machine for Extracting Juice from Citrus Fruits," U.S. Patent No. 1,764,158 (June 17, 1930).
Gum, G. C., "Automatic Fruit Juice, Pulp, and Seed Extractor," U.S. Patent No. 1,834,097 (Dec. 1, 1931).
Heinrich, E., "Juice Extractor," U.S. Patent No. 2,228,822 (Oct. 14, 1938).
Lorenzen, B. L., "Continuous Operation Centrifugal Juice Extractor," U.S. Patent No. 2,180,877.
Mocharnuk, J., and McCamey, "Citrus Fruit Juice Extractor," U.S. Patent No. 2,142,002 (Sept. 24, 1937).
Thompson, A. R., "Juice Extraction Method," U.S. Patent No. 2,181,218 (Dec. 21, 1936).
Walker, R. W., "Fruit Juice Extractor," U.S. Patent No. 2,067,555 (May 8, (1924).

table and arranged in fingerlike fashion, into which an entire fruit fits. Another similar cap closing in from above fits exactly in such a manner as to squeeze the whole fruit. The lower caps are provided with small tubes protruding into the inside of the fruit and through which the juice flows as the fruit is squeezed. There is, therefore, no contact whatsoever between the juice and the oil or any other tissue

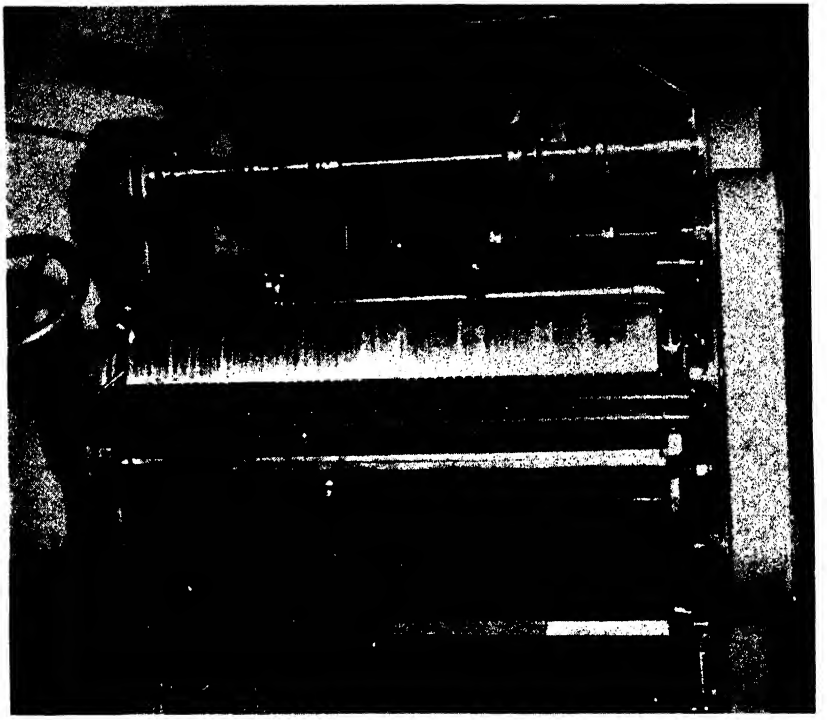


Fig. 72. Enlarged view of "Citro-Mat" drum and screen (courtesy Bireley's, Inc.).

fluids, which are expressed simultaneously but flow on the outside of the fruit and are collected separately. A fair yield of oil can be obtained in the same process. The machine takes in 480 fruits per minute and is, therefore, the most efficient and most promising of all existing automatic extractors. Although the resulting juice contains a lot of fruit cells it is practically free from peel oil or any other tissue fluids since the inner membrane of the uncut fruit remains intact and protects the juice from possible contamination from the albedo (Fig. 73).

4. Screening of Juices

Whatever method of extraction is used the juice has to be screened from incidental pieces of peel, from the carpellary membranes, and from coarse pulp, leaving only the natural fruit cells. The presence of such fruit cells in the juice not only attracts the eye and conveys an impression of authenticity but also has a marked effect on the flavor of the juice, although it has relatively little bearing upon its chemical constitution.

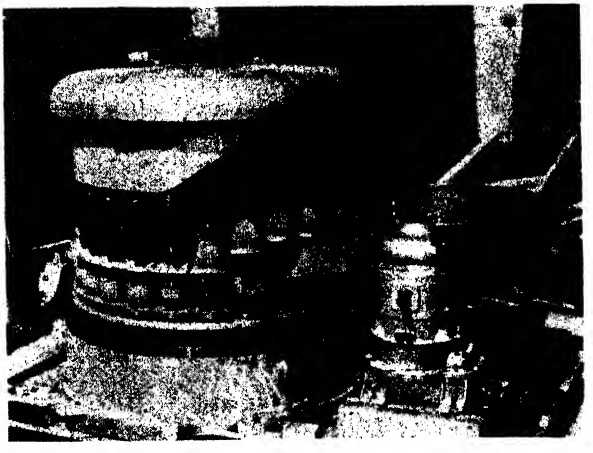


Fig. 73. The new Super Juice Extractor with top rotor removed (courtesy Food Machinery Corporation, San Jose, California).

In determining the amount of fruit cells in a given juice, sedimentation alone will not give definite results; it is advisable,[7] therefore, to use some sort of centrifugation, which affords a much better criterion.

Screening is preferably done in two stages: first, removing the coarse particles by letting the juice pass through a screen perforated with 3 mm holes; second, removing as many of the fruit cells as desired by further screening the juice through correspondingly smaller perforations. The first stage is sufficient for the preparation of so-called "raw juices" for squash manufacture and, in some cases, for pasteurized juices. Further screening, sometimes through perforations as small as 0.4 mm, is necessary if the juice is to be deaerated and especially if it is to be used for concentrates.

[7] Donovan, F. K., "Citrus Fruit Facts with Particular Reference to Determination of Cell Content," *Bottler and Packer*, 8, 50 (Sept., 1934).

Several technical methods exist for screening citrus juice. The main precaution to be observed should be to prevent undesirable ingredients of the peel or rag from being pressed or ground into the juice. Screening appliances which let the juice pass freely through the screens by gravity, without exerting additional mechanical pressure, are, therefore, preferred. The second important precaution

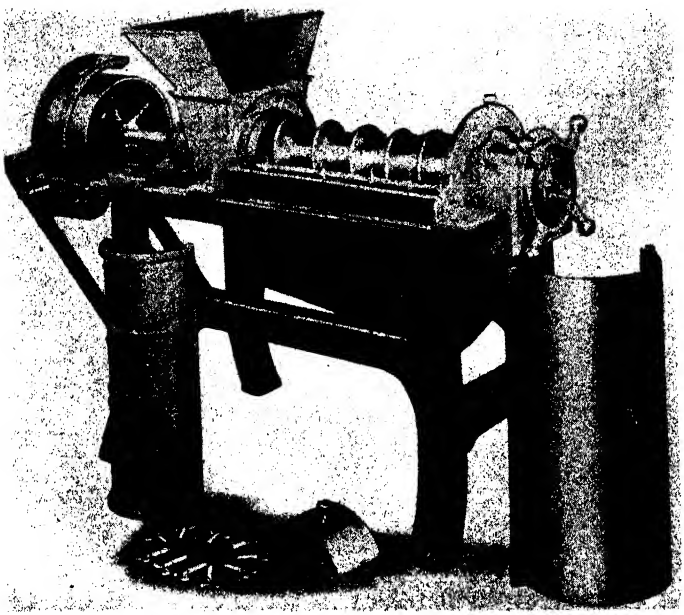


Fig. 74. Screw-type juice finisher (partially disassembled), for screening citrus juice (courtesy Food Machinery Corporation, Hoopeston, Illinois).

should be to prevent an excess of air from being beaten into the juice during the screening process.

The best method of screening consists in letting the juice run through a long, slightly inclined, slowly revolving screen made of stainless steel in the form of a drum. The juice which passes the perforations is carefully collected underneath the screen and carried away by gravity or pump, while the coarse particles left inside the drum move along to be discharged at the far end. Frequently the screens are stationary and the juice moves inside by means of slowly revolving paddles or helical screws made of stainless steel or aluminum. Another method used for screening consists of conical helical screw expellers very similar to those used in the wine industry (Fig.

74). Vibrating flat screens may also be used for this purpose (Fig. 75). Such arrangements are sold under the name of "juice finishers."

As previously mentioned, when whole fruits are crushed and the oil and juice are separated in centrifugals, the pulp remains in the separator as a solid deposit.

In some factories citrus juices have been passed through so-called homogenizers—a kind of colloid mill. The intention in such cases was to cut the pulp into very fine shreds to keep it more easily in suspension. However, as already pointed out, the only way to keep some of the pulp in suspension is to flash-pasteurize the juice after deaeration; by this process the pectic enzymes which are largely responsible for the settling of the suspended pulp are destroyed.

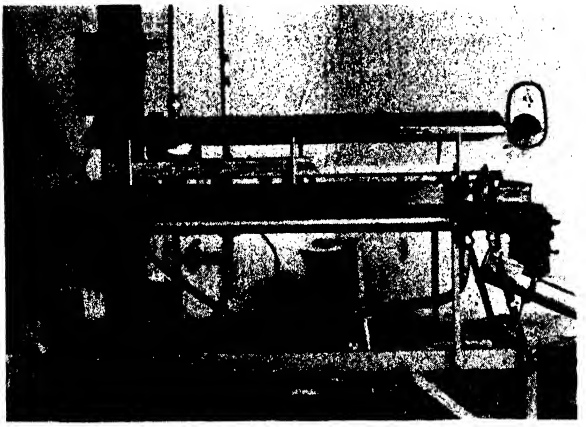


Fig. 75. Vibrating screen for screening citrus juice.

The complete removal of the pulp for the preparation of clear juices—so-called cordials—is discussed on pages 304-306.

5. Microbiology of Citrus Juices

Although practically sterile while still inside the endocarp, the juice after extraction invariably shows quite a rich microflora, consisting of yeasts, bacteria, and spores. All these microorganisms find their way into the expressed juice by various means: by contact with the peel or with various parts of the machinery, and probably also from the environment.

The microflora of the peel of citrus fruits is ordinarily very rich, and, unless the fruit is actually sterilized by means of strong antiseptics for a sufficiently prolonged time, these microorganisms are

carried along into the juice. This can hardly be prevented by ordinary washing of the fruit. It has been demonstrated that citrus fruits may carry on their surface as much as 5×10^5 microorganisms to each square centimeter of peel, e.g., an average fruit having a surface of 200 cm^2 carries about 100 million microorganisms.

No matter how clean the fruits are when they arrive at the juice extractors, it is obvious that at least a fraction of this microflora finds its way into the juice. In fact numerous tests showed that the extraced juice before being processed may contain as much as 10^4 to 10^6 microorganisms to each cubic centimeter. Nolte and von Loesecke[8] have isolated and identified the following yeasts and molds from raw grapefruit juice:

Saccharomyces cerevisiae	*Dematium sp.*
S. ellipsoideus	*Oidium sp.*
S. apiculatus	*Monila sp.*
S. pastorianus	*Penicillium glaucum*
Torula sp.	*Aspergillus glaucus*
Mycoderma cerevisiae	*Aspergillus sp.*

The authors have also isolated over 100 cultures of bacteria but no attempt was made to identify these organisms in the raw juice.

On the other hand, the authors isolated and identified the following microorganisms from the same juice after pasteurization and found them to consist of the following:

Bacillus mesentericus	*Bacillus cytaseus*
B. subtilis	*Lactobacillus thermophilus*
B. vulgatus	*B. nondiastaticus*
Mucor sp.	*B. cereus*
Penicillium glaucum	*Sarcina sp.*

The total bacterial count of pasteurized juice showed a minimum of zero and a maximum of 30 per cc. None of the organisms isolated would, however, grow on orange or grapefruit juice agars under either aerobic or anaerobic conditions at 25°, 37°, and 55° C. Their inability to grow in citrus juices, therefore, renders them unable to cause spoilage from a biological point of view. If pathogenic organisms should happen to be present in the citrus juice they would not survive pasteurization temperatures (especially of flash-pasteurization) because of the low *p*H of the medium.

Apart from the contamination of the juice by the outside peel it can also take place at various stages throughout the processing operations. Drops of stagnant juice, bits of pulp, etc., may cause serious infestations in the juice so that pasteurization (or any other method

[8] Nolte, A. J., and H. W. von Loesecke, "Types of Organisms Surviving in Commercially Pasteurized Citrus Juices in Florida," *Food Research*, 5, No. 11 (1940).

of conservation) may be inadequate to prevent further spoilage. Strict observance of industrial hygiene must be adhered to at every point of possible infection. Frequent, thorough cleaning and sterilization of machinery coming in contact with the products and of all ducts through which the juice flows must be carried out with due care.

The behavior of bacteria, yeasts, and molds at freezing temperatures or below will be discussed further later.

6. Preservation of Citrus Juices

Every weak solution of reducing sugars, if left unattended at ordinary temperature, will soon start to ferment. Citrus juices are no exception; in fact, they present a perfect nutritive medium for the development and growth of yeasts and molds. The primary object of the producer is, therefore, to prevent fermentation in the expressed citrus juices.

However, the prevention of fermentation alone is not sufficient, for the natural flavor and appearance of the juices must also be preserved as far as possible; these usually tend to change very rapidly owing to various enzymatic and oxidative reactions which take place freely in such complex solutions as citrus juices. The adverse effect of these chemical changes on the quality of the juices has frequently been mentioned in the discussions of juice constituents. Before describing the various methods of preservation it is important, therefore, to summarize the changes that take place during processing and storage.

(a) Changes Occurring in Juices

(1) FERMENTATION

Fermentation is due to yeasts which may have found their way into the juice during the interval between the halving of the fruit and the moment the juice is packed. It has been repeatedly demonstrated that sound fruit before being halved contains no yeast whatsoever and that any yeasts which may be present in the juice have come from the outside. Yeasts are microorganisms possessing peculiar powers of adaptability; it may often happen that yeasts present in juices will start fermentation after a long time, for they are seldom destroyed but usually are only inactivated by preservation.

Although the most favorable condition for fermentation is a slightly acid medium, the more acid the medium the less is the danger that fermentation will begin. Consequently juices which are more acid require less preservative (if chemical preservatives are

used) or a lower temperature of pasteurization. Studying the effect of hydrogen-ion concentration on the toxicity of several preservatives to microorganisms, Cruess, Richert, and Irish[9] found that it depends largely on the *p*H value of the juices, which should not exceed 4.5; the optimum is in the vicinity of *p*H 3.0. In actual practice it has been found advantageous to blend citrus juices possessing a higher *p*H value with others more acid such as lemon) in order to augment their acidity.

The foregoing discussion refers not only to alcoholic fermentation in citrus juices but also to other types of fermentation, such as acetic or lactic, which may take place owing to contamination of the juices by certain microorganisms.

(2) MOLD FORMATION

Citrus fruits coming from the grove carry on their surface varying amounts of spores of various molds, such as green (*Penicillium glaucum*) and blue (*P. digitatum*) molds, etc. Even if the juice is preserved molds will occasionally develop on the surface, especially when the headspace of the container retains sufficient oxygen to promote their growth.

(3) CHANGES IN AROMA

The nature of the substances responsible for the specific aroma of the citrus juices has been discussed previously at some length; it has been shown that they differ entirely in composition from the essential oils of the peel. These aromatic substances are present in very minute quantities and in most cases they deteriorate or evaporate very rapidly even if the juices are not subjected to high temperatures or to vacuum.

If the juices are not carefully reamed but are extracted by mechanical means, much of the peel oil is incorporated into the juice. In due course this oil deteriorates and develops off-flavors, due most probably to the decomposition or hydrolysis of the essential oil in the acid medium of the juice and the polymerization of its terpenes. It has been found[10] that objectionable off-flavors due to the incorporated essential oil of the peel may develop on prolonged storage with an oil content as low as 0.01%. In order to lower the oil content of the juice extracted by mechanical means the practice in Florida lately has been to steam the whole fruit before going into the juice extractor. It is

[9] Cruess, W. V., P. H. Richert, and J. H. Irish, "The Effect of *p*H on the Toxicity of Several Preservatives to Microorganisms," *Hilgardia*, **6**, No. 10, 295 (1931).

[10] Boyd, J. M., and G. T. Peterson, "Quality of Canned Orange Juice," *Ind. Eng. Chem.*, **37**, 370 (1945).

claimed that the chief beneficial action of this treatment is the softening or wilting of the peel, making it more pliable, so that few of the oil cells are broken during the extraction treatment.

(4) CHANGES IN FLAVOR

Changes in aroma are not the only causes of the development of off-flavors. There are, in addition, some very profound changes, mostly, as yet, of an unknown nature, which are largely responsible. Some of these processes, which have been discussed in the section on proteins and their decomposition in citrus juices (page 124), are undoubtedly caused by the action of enzymes. The change of sucrose into invert sugar is attributed to similar factors. In some cases of canned orange juice an increase in invert sugar from 5.4 to 9.14% has been observed, while sucrose decreased from 6.36 to 1.21% and the *p*H value dropped from 4.02 to 3.62 without any change in titratable acidity.[11]

When citrus juices are pasteurized or sterilized by various methods they very often develop a decidedly "cooked" taste in storage. Apart from the technique itself by which the pasteurization is performed, the main cause of the cooked taste lies, no doubt, in the presence of oxygen in the juice. The oxygen is then rapidly absorbed by oxidative reactions in the citrus juices, causing also a marked loss in the reducing substances (such as vitamin C) naturally present in the juice.

(5) CHANGES IN COLOR

Unless processed citrus juices are kept in cold storage or preserved with SO_2, their color gradually changes and in the course of time becomes brown. The browning of citrus juices represents not a change in the constitution of their coloring matter but apparently a much deeper change in some of their vital constituents; it goes hand in hand with the gradual loss of the reducing power of the juice. Conversely, when citrus juices are preserved with SO_2 they soon become somewhat bleached. (See under "Browning," page 271.)

(6) LOSS OF VITAMIN C

This important change in stored citrus juices has already been discussed at length (page 144). Numerous investigations have clearly demonstrated that the loss of vitamin C is entirely dependent on the presence of oxygen; unless the juices contain other antioxidants possessing a preferential capacity for binding the oxygen, or unless the

[11] von Loesecke, H. W., H. H. Mottern, and G. N. Pulley, *Ind. Eng. Chem.*, **20**, 1302 (1928).

juices are stored at low temperature, they soon lose their vitamin C potency.

(b) Chemical Preservatives

Having enumerated the main changes which may occur in citrus juices during processing and storage, the various methods of preservation will be described, with particular reference to their effectiveness in obtaining the ideal conditions.

Manufacturers of fruit juices have been using a variety of chemical compounds as preservatives for a long time. Chemicals such as formic

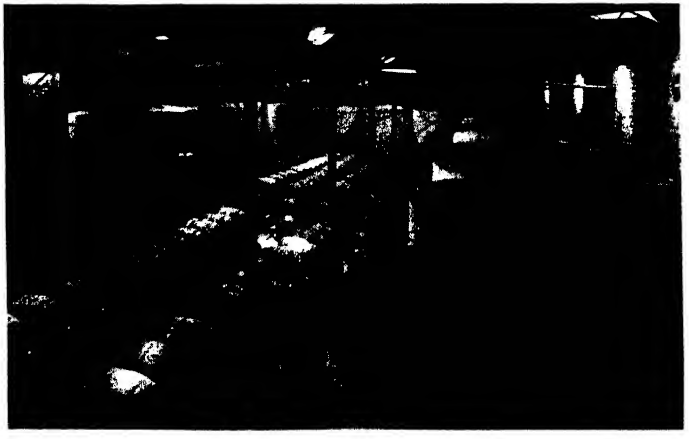


Fig. 76. A roomy, cool cellar storing large quantities of citrus juices in bulk (concrete glass-lined vats) and in barrels.

and salicylic acids and their salts were used for many years but have now been completely abandoned. In most European countries, the United Kingdom, and the United States, public health laws for pure foods permit, under certain restrictions, the use of only benzoic acid (or sodium benzoate) and sulfurous acid (SO_2) or sulfites. It is interesting to note that all the permissible chemical preservatives are acids, as, also, are the new preservatives, monochloracetic acid and propionic acid, which have been introduced only in recent years. In each case, however, the acidity itself does not exert the preserving action.

Chemical preservatives are used in citrus juices only when it is desired to preserve them in bulk, in barrels, or in large vats (Fig. 76), when pasteurization methods cannot be used effectively. It is important, however, that the existing food laws in each country for which the juices are intended be carefully studied to avoid infringe-

ment by shipping juices with the wrong preservative or with an excess of the permissible ones.

(1) BENZOIC ACID AND BENZOATES

Benzoic acid is the active principle when sodium benzoate is used as a preserving agent. It is found in nature in greengage plums, prunes, and cranberries. Cranberries, which contain some 0.2% of benzoic acid, consequently resist rapid deterioration in storage. Because of its superior solubility in water, sodium benzoate is more widely used than benzoic acid. The latter, relatively speaking, is only slightly soluble in water (0.34 g per 100 cc at 25° C) whereas sodium benzoate is more than 180 times as soluble (62.5 g per 100 cc). The preserving action of the benzoic acid radical is explained by the formation of a complex compound with the proteins of the yeast. This bacteriostatic action can be increased by the introduction of a side chain into the benzoic acid ring. Thus, the esters of *p*-hydroxybenzoic acid (such as methyl, ethyl, and propyl esters) have an increased effectiveness and have been extensively used, especially in Germany.

It has long been recognized that only juices of high acidity can be readily preserved with as much as 0.1% of sodium benzoate (1000 ppm) but that even 0.2% would not preserve low-acid products. As previously stated, the concentration of sodium benzoate required to preserve citrus juices has been found to depend upon their *p*H value. The retarding effect of this preservative on yeast multiplication is best at *p*H 2.0; this value should not, however, exceed 4.5. It is very interesting to note that only the undissociated acid is antiseptic and that the benzoate ions have practically no effect on yeast. It is further important to remember that, according to observations made by many workers in this field, small concentrations of benzoate (0.02–0.04%) stimulate rather than check the activity of microorganisms.

Apparently only a small part of the benzoic acid is utilized as a preservative, since the greater part is bound by the proteins of the juice. If a stronger acid is used to satisfy this acid-binding power of the proteins the disinfecting effect of the benzoic acid is increased.[12]

Although clear liquids, relatively free from fruit pulp, are more easily preserved with benzoates than turbid juices, raw citrus juices because of their high acidity can be preserved with 0.1% of sodium benzoate, or 1000 ppm. In Great Britain, under the Preservatives Act of 1927, only 600 ppm are permissible for fruit juices (except for

[12] Perry, M. C., and G. D. Beal, "The Quantities of Preservatives Necessary to Inhibit and Prevent Alcoholic Fermentation and the Growth of Molds," *J. Ind. Eng. Chem.*, **12**, 253 (1920).

grape juice, for which 2000 ppm are allowed). According to the American Food and Drug Act benzoates are the only permissible chemical preservatives, and although no restrictions are made as to the quantity which may be used, their presence and amount must be plainly shown on the label. Food Inspection Decision No. 104 (March, 1909) of the United States found that benzoates are not deleterious or poisonous or injurious to health.

Sodium benzoate in its dry form should never be added to the juice directly as it dissolves very slowly. A 25% solution in water (or 2 lbs of sodium benzoate in enough water to make exactly one gallon) is the best way of using this preservative. Furthermore, the addition should be made slowly, with continuous stirring, for otherwise the sodium salt upon contact with the acid juice will immediately be converted into benzoic acid which, being much less soluble, will be precipitated and will settle at the bottom of the container before it has time to dissolve properly.

Although benzoic acid is an effective agent for the prevention of fermentation in citrus juices packed in bulk, it has many disadvantages. First, it does not prevent the growth of molds on the surface of the liquid in the containers, especially when there is sufficient oxygen in the headspace. Secondly, in contrast to SO_2 (see the next paragraph), benzoic acid is a preservative which cannot be reextracted, as once it has been added to the juice it remains in solution. It imparts to the juice a disagreeable "burning" flavor which is readily perceptible to the taste. Citrus juices preserved in this way cannot, therefore, be used for direct consumption; their only possible use is in the manufacture of syrups to be subsequently used in mineral waters (so-called "soft drinks"), where the amount of preservative is diluted manifold. But the greatest objection to the use of benzoate in citrus juices is its incapability to prevent the loss of vitamin C. As benzoic acid is not a reducing substance, it cannot prevent the oxygen dissolved in the juice from attacking the ascorbic acid, which rapidly oxidizes and subsequently deteriorates. Concurrently with the destruction of vitamin C excessive browning of the juice takes place even on short storage. Obviously the quality of citrus juices is greatly impaired by benzoate.

(2) SULFUR DIOXIDE AND SULFITES

Citrus juices are very often preserved by sulfurous acid either in the form of SO_2 gas or as sulfites. If gas is used, liquefied SO_2 from inverted cylinders is measured into a special apparatus and then slowly introduced into the juice in the gaseous form, which is readily

absorbed by the juice. Where no such arrangements exist the preservative is added as a solution (about 15%) of potassium metabisulfite (or pyrosulfite), $K_2S_2O_5$, or of sodium bisulfite, which contains, on the average, 55 to 65% of available SO_2. The bisulfite is usually cheaper than the metabisulfite and is just as efficient. Any sulfites used must, of course, be carefully checked by analysis for their SO_2 content.

Differing in this respect from benzoic acid, SO_2 is very effective against molds because the oxygen present oxidizes some of the preservative and is thereby eliminated from the juice and the headspace. Against the fermenting action of yeast, however, the use of SO_2 as a preservative encounters some difficulties. As already mentioned, yeasts adapt themselves easily to various environments: some strains of yeasts could easily be trained to develop in an atmosphere of SO_2, especially if the amounts of this preservative are slowly increased. This important fact is successfully employed in the wine industry when it is desired to ferment grape juice by a specially selected strain of yeasts and to avoid undesirable fermentation by "wild" yeast. The selected strain of yeasts is "trained" to ferment in a juice containing a very small amount of SO_2; when the fermentation is in full progress, the whole is mixed with further lots of juice with progressively increasing amounts of SO_2. The yeasts then become SO_2-resistant and can be employed for the fermentation of the bulk of the juice, to which a large amount of SO_2 has been added and in which no other microorganisms or "wild" yeast, except the selected strain which has become SO_2-resistant, will develop. While this mechanism is of advantage to producers who desire to create fermentation it is obviously a drawback to those who desire to prevent it. The appearance of SO_2-resistant yeasts can, however, be avoided if the amount of preservative required be added in one lot and not in small portions. Furthermore, it is important that the containers and the working premises be sterilized from time to time by antiseptic materials other than SO_2 to prevent the yeast from becoming accustomed to an SO_2 environment.

Like all other preservatives, SO_2 is much more effective in an acid medium. In this connection, however, the writer cannot agree with the accepted view that a minimum *p*H value of 4.5 is required for the proper effectiveness of the preservative. The *p*H of the citrus juices, as mentioned earlier, changes only very slightly during ripening notwithstanding the fact that the total titratable acidity diminishes very appreciably. It is, however, well known from practice that juices extracted at the end of the season when the fruit is overripe lend

themselves with great difficulty to preservation, as compared with juices extracted earlier in the season. It appears, therefore, that total acidity and not the amount of dissociated acid plays the important role in the effectiveness of the preservative. The practice of producers preserving orange juices in bulk for squash manufacturers is to add citric acid to the juice or to blend it with lemon juice to assure perfect preservation.

Another difficulty encountered with SO_2 as a preservative lies in its chemical nature. When a fixed amount of SO_2 is added to fruit juices and afterwards is measured by direct titration with iodine, the value obtained is much smaller than the amount added. Only by letting KOH in solution act upon the juice for a while, and then acidifying it again, will titration with iodine show the true value of added SO_2. Evidently a part of the SO_2 has somehow combined with some other constituent in the juice and can be detected only after this combination has been destroyed. Sulfur dioxide is known to combine with aldehydes but pure unfermented juices seldom contain enough aldehydes to bind corresponding amounts of SO_2.

Experiments with pure solutions of various sugars have demonstrated that SO_2 partly combines with glucose but remains entirely free when added to a pure solution of sucrose. The combination with glucose seems to be very labile in many senses. When the proportion of glucose in solution increases, the proportion of combined SO_2 also increases, leaving less free preservative. As previously shown, citrus juices when freshly expressed contain a large proportion of sucrose which, however, slowly undergoes inversion owing to the action of acids and the enzyme *invertase*. While this takes place, the newly created invert sugar binds more of the SO_2 present. Preservation practice has shown that only free SO_2 has any toxic effect upon yeasts; the combined SO_2 is entirely or practically inactive. Here lies the main difficulty in using SO_2 as a preservative for citrus juices. In addition, a very small amount of SO_2 is oxidized by the oxygen present in the juice into SO_3 which, again, has no antiseptic value. Furthermore, some SO_2 constantly evaporates from the juice, thereby reducing the total amount of the preservative.

Samples selected from numerous analyses performed under the direction of Dr. F. Stern of Rehovoth (Israel), compiled from data collected through many years of periodical testing of various citrus juices preserved with SO_2, indicate the rate of disappearance of total SO_2 as well as the changes between the proportions of free and combined SO_2 (Table XXIII).

TABLE XXIII

Changes in SO_2 Content of Citrus Juices during Storage

	Total SO_2	Free SO_2	Combined SO_2	Percentage of combined SO_2
	In mg per liter or ppm			
Orange Juice				
Sample A				
at the time of preservation..	768	627	141	18.3
after 2 months.....	705	513	192	27.2
" 4 "	704	448	256	36.4
" 6 "	593	352	241	41.3
Sample B				
at the time of preservation..	777	616	161	20.7
after 2 months.....	793	563	230	29.0
" 4 "	768	486	282	36.7
" 6 "	623	356	267	42.8
Grapefruit Juice				
at the time of preservation..	819	627	192	23.5
after 2 months.....	755	538	217	28.7
" 4 "	640	373	267	41.7
Lemon Juice				
at the time of preservation..	756	616	140	18.5
after 2 months.....	614	460	154	25.1
" 4 "	512	333	179	35.0
" 7 "	873*	678	195	22.3
" 9 "	793	614	179	22.6
" 12 "	794	589	205	26.8

* More SO_2 added after 7 months of storage.

The juices from which these samples have been selected were preserved with gaseous SO_2 and stored in large glass-lined vats of 30,000 liters each. It can be noted that while the total amount of SO_2 decreases slightly during storage, the percentage of the combined in relation to the total SO_2 continuously and steadily increases. During the first few hours the proportion of bound SO_2 rises very quickly, then the rise becomes slower until it reaches some 40 to 50%, when it remains more or less constant with a slight upward tendency. Experience has shown that after many months of storage the proportion of bound SO_2 in raw citrus juices may suddenly jump to 75% of the total. At that point the risk of fermentation is very great and additional supplements of SO_2 will not always help to hinder spoilage. The graph of Figure 77 shows the general trend of behavior of SO_2 in citrus juices.

Kerp[13] in 1904 suggested that sulfurous acid or sulfites combine

[13] Kerp, W., *Arb. Reichsgesundh.*, **21**, 144 (1904); **26**, 231 (1909); **32**, 98 (1915).

with glucose to give an addition-product—namely, glucose-sulfurous acid or an ester:

$$CH_2OH\cdot(CHOH)_4\cdot CHOH\cdot SO_3H$$

or:

$$CH_2OH(CHOH)_4\cdot C(=O)H + HSO_3K \rightleftharpoons CH_2OH(CHOH)_4CH(OH)SO_3K$$

While glucose has been previously regarded as an aldehyde it has never been demonstrated that it could react with sulfites as all other

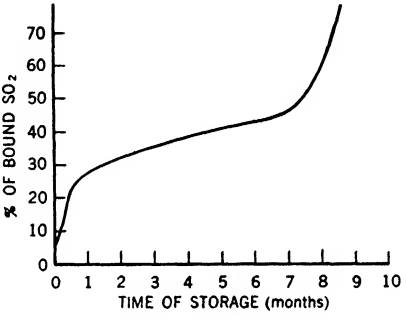
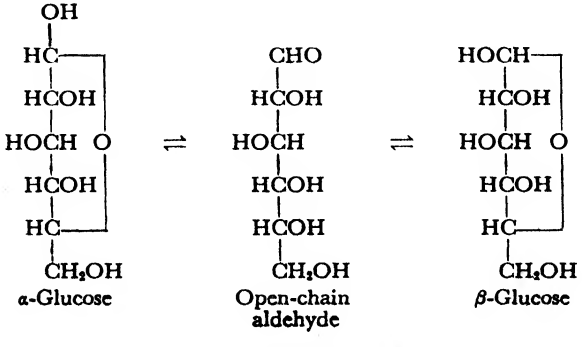


Fig. 77. Graph showing behavior of SO_2 in juices.

true aldehydes do, by forming stable compounds. We now know that glucose exists in two isomeric lactone forms, α- and β-glucose (see page 80), and it is rather improbable that these lactone configurations react with sulfites. Recently Conant[14] expressed the view that both lactone forms of glucose are in equilibrium with a very small amount of an open-chain aldehydic form:



[14] Conant, J. B., "The Chemistry of Organic Compounds," Macmillan, New York (1939).

It may not be inopportune to express a far-fetched theory suggesting that if the foregoing is true, it would explain the behavior of SO_2 in glucose solutions. The equilibrium shown above, with a very small amount of an aldehydic form seems to be responsible for binding the sulfite as an addition product, while the lactone forms do not. When this equilibrium is disturbed more of the cyclic forms open their chains, forming more aldehyde, which proceeds to bind additional amounts of SO_2. It would probably explain also why sucrose (which is a ring formation) does not bind SO_2 at all.

Evidence of the existence of such sugar-bisulfite compounds has recently been demonstrated by Browne,[15] who measured the optical rotations in 10% solutions of lactose, D-glucose, maltose, D-galactose, and D-ramnose containing 0.5–30 g of $NaHSO_3$ (per 100 cc). The optical rotation decreased with increasing amounts of $NaHSO_3$ and a correspondingly lower reducing value per unit concentration was found.

The ability of SO_2 to create a stable compound with a true aldehyde explains why, once fermentation has started, small additions of further quantities of the preservative will not help to check it. During alcoholic fermentation acetaldehyde is formed and this combines immediately with SO_2. When free SO_2 is added gradually to a juice in active fermentation it is converted almost instantly into an organic SO_2 compound and fermentation is not hindered.[16] Sulfur dioxide should, therefore, be added as a preservative in one lot, according to permitted standards, and only the free SO_2 should be reckoned with as having bactericidal action. Here again, each country has its specific regulations: in the United Kingdom no more than 350 ppm of total SO_2 are permitted. However, this applies only to juices used for direct consumption; when the juices are intended for the manufacture of squashes and for subsequent dilution, more SO_2 is permitted.

Apart from the foregoing considerations, sulfur dioxide has many advantages as a preservative. Owing to its ability to combine easily with oxygen, SO_2 is a powerful antioxidant, i.e., its presence prevents other valuable constituents (such as vitamin C) of the juice to oxidize and thereby to lose their nutritive value. In fact, citrus juices carefully treated with SO_2 preserve their vitamin C potency for a long time, especially if they are stored in a cool place.

[15] Browne, H. H., "Evidence of the Existence of Bisulphite Compounds of Sugar," *J. Org. Chem.*, **9**, 477 (1944).

[16] Bertin, Charles, "Auto-desulphiting of Musts," *Bull. Soc. Ind. Rouen*, **52**, 427 (1924).

While some earlier investigators[17] were of the opinion that sulfur dioxide, when used as a preservative for citrus juices, has a "definite destructive action on the antiscorbutic vitamin at laboratory temperature (15–18° C)," later observations[18] confirmed that SO_2 appears to exert only a slight destructive effect on the ascorbic acid, but the loss is largely outweighed by the reducing action of SO_2 as against benzoic acid, for instance, when the latter is used as a preservative. However, Hamburger and Joslyn[19] found no evidence for an induced oxidation of ascorbic acid by SO_2. On the contrary they state that SO_2 prevents particularly the rapid oxidation of vitamin C to dehydroascorbic acid and that this action is more pronounced in juices with 500 ppm than with 250.

Browning of citrus juices, which goes hand in hand[20] with the destruction of vitamin C, is also prevented by the use of SO_2 and the color of the juices is very well preserved; in fact, SO_2 has a bleaching effect on orange juice which can be strengthened by the addition of some permissible food color.

Finally, SO_2 is the only chemical preservative which can be removed from the juice by applying heat or vacuum. This fact alone is of great advantage, for, if properly treated, citrus juices can be preserved in bulk during the working season and then bottled as straight juice after the extraction of SO_2. For the convenience of those who may use vacuum for the extraction of SO_2, Table XXIV gives the partial pressures of this gas at various concentrations and temperatures.

TABLE XXIV

Partial Pressures of SO_2 at Various Temperatures

SO_2 (ppm)	Partial pressure in mm Hg			
	15° C	20° C	30° C	50° C
200	0.3	0.5	0.6	1.3
500	0.8	1.2	1.7	4.7
1000	2.2	3.2	4.7	12.0

[17] Williams, J., and J. W. Corran, "The Preservation of the Antiscorbutic Vitamin in Lemon Juice," *Biochem. J.*, **24**, 37 (1930). Also, A. H. Bennett, and D. S. Tarbert, "Vitamin C in Citrus Juices," *ibid.*, **27**, 1294 (1933).

[18] Downer, A. W. E., "The Stability of Ascorbic Acid in Citrus Juice Products," *J. Soc. Chem. Ind.*, **61**, 82 (1942).

[19] Hamburger, J. J., and M. A. Joslyn, "Auto-oxidation of Filtered Citrus Juices," *Food Research*, **6**, 599 (1941).

[20] Joslyn, M. A., G. L. Marsh, and A. F. Morgan, "Relation of Reducing Value and Extent of Browning to the Vitamin C Content of Orange Juice Exposed to Air," *J. Biolog. Chem.*, **105**, 17 (1934).

Sulfur dioxide must not be used in juices that are to be packed in tins or stored in any metal containers because SO_2 in contact with metal is reduced to H_2S, with the development of a most disagreeable flavor.

To determine the amount of free SO_2 in a solution it is ordinarily sufficient to titrate 10 or 25 cc against a 0.1 *N* solution of iodine using starch as an indicator, the end point being reached when the titratable solution remains slightly blue for a few moments. This procedure, however, is inadequate for citrus juices—first, because some of the SO_2 is combined and secondly, because citrus juices contain an appreciable amount of reducing substances (principally vitamin C) which give the same reaction with the iodine solution. On the other hand, total SO_2 is easily determined by distilling the juice after acidification with HPO_3 in a current of CO_2 through a condenser fitted with a bubbler which dips into bromine water. The excess of bromine is evaporated off, the distillate is concentrated to a suitable volume, and SO_3 is precipitated in the usual way with $BaCl_2$, exercising recognized precautions.

For routine work in the factory a very convenient method for the determination of total sulfurous acid consists of distilling the juice in a current of CO_2 and absorbing the SO_2 in H_2O_2 by using a special absorption tube designed by Jewsbury and Brown, Ltd., Manchester, England. The absorption tube assembled together with the distilling apparatus is filled with 25 cc of 3% H_2O_2 and a few drops of bromophenol blue as indicator. The guard tube (a Peligot tube) contains 10 cc H_2O_2 and a drop of the indicator and is made absolutely neutral with 0.1 *N* NaOH.

Into the flask run 25 cc of concentrated HCl and 450 cc of distilled water. Replace the reflux condenser and drive out all the air with a current of CO_2, then add through the funnel the sample of citrus juice: 25 cc in the case of the juice containing 700 ppm of SO_2 or 50 cc for that containing 300 ppm. Boil, keeping a steady current of CO_2 flowing through the apparatus, and continue boiling for 30 min. The HCl hydrolyzes any combined sulfite compound and liberates all the SO_2, which is driven forward by the current of CO_2 into the absorption tubes, where it is oxidized to SO_3. Titrate the contents of the absorption tube with 0.1 *N* NaOH. Using 25 cc of juice, each cc of 0.1 *N* NaOH is equivalent to 128 ppm SO_2 in the sample. It is important to ascertain the blank acidity of H_2O_2, to compensate for the acid contained therein as preservative, and to apply the correction.

Where no absorption tube such as that previously described is available, the same procedure can be carried out instead with an ordinary 200 cc Erlenmeyer flask provided with a Peligot tube and connected with a straight tube to the upper end of the reflex condenser.

When the total SO_2 is determined, the free and combined SO_2 together with the reducing substances (vitamin C) of the juice can easily be determined, as follows:

(*a*) Titrate 25 cc of juice with 0.1 *N* iodine solution, which gives the figure for free SO_2 + reducing substances.

(*b*) Add to the same sample a few cc of NaOH solution (10%) and wait for a few minutes, then reacidify with some HCl and titrate again with 0.1 *N* I solution; this gives the figure for combined SO_2.[21]

[21] Jensen, H. R., "Rapid Determination of Sulphites by Alkaline Liberation or Extraction and Titration," *Analyst*, 53, 133 (1928).

(*c*) Distill, as above, another sample of 25 cc of the same juice, which gives the figure for total SO_2.

Hence:

(c) = total SO_2
(b) = bound SO_2
$(c)-(b)$ = free SO_2
$(a)-[(c)-(b)]$ = reducing subtsances

To eliminate low results by direct titration with iodine, an excess of NaOH is suggested,[22] the mixture to be left for 10 min. and thereupon acidified with H_2SO_4, then titrated again with I_2.

In the presence of SO_2 the reducing substances of citrus juices (mainly vitamin C) can be conveniently determined also by adding to 25 cc of juice some 10 cc of acetone (which reacts with SO_2 to form a compound stable with I_2) and then titrating the juice with iodine.[23]

An electrometric indicator to replace starch in iodine titrations of sulfurous acid in fruit juices has been recently suggested by Ingram.[24] Using this electrometric indicator, consisting of a bright platinum electrode in conjunction with a very simple reference electrode and electrometer, particularly sharp end points are observed in various citrus juices as well as in concentrates and in general in highly colored juices.

For the use of SO_2 in concentrates, see page 284.

(3) OTHER CHEMICAL PRESERVATIVES

The use of various esters of benzoic acid as preserving agents for citrus juices has already been mentioned. Some of them, marketed under specified patent names (Nipasol, Nipakombin, Esterol, etc.), are only mixtures of ethyl or propyl esters of *p*-hydroxybenzoic acid with various amounts of sulfites which are generally added only for the purpose of circumventing the food regulations, which in most countries do not permit the simultaneous use of both SO_2 and benzoic radicals. In practice none of these preparations is particularly better than sodium benzoate when used with citrus juices.

Recently two very important new chemical preservatives have been extensively studied in connection with various foods. These are propionic acid or propionates and the monohalogen acetic acids. The first of the two is essentially an inhibitor of respiration and is, therefore, suitable for preventing the growth of molds. Of the mono-

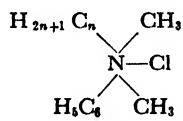
[22] Bennett, A. H., and F. K. Donovan, "Determination of SO_2 in Citrus Juices," *Analyst*, **68**, 140 (1943).

[23] Mapson, L., "Vitamin Methods; Vitamin C," *Biochem. J.*, **36**, 155 and 196 (1942).

[24] Ingram, M., "An Electrometric Indicator to Replace Starch in Iodine Titrations of Sulphurous Acid in Fruit Juices," *J. Soc. Chem. Ind.*, **66**, 50 (1947).

halogen acetic acids the monochloracetic acid ($CH_2Cl{\cdot}COOH$) has been found to be the least toxic. It is essentially an inhibitor of fermentation. The optimum quantity of monochloracetic acid required for commercial stabilization of fruit juices is 300 mg per liter. Although it presents no hazard to health, its use has recently been banned by the Food and Drug Administration of the United States. This preservative has been used under such trade names as "505," "Esterex," and "Clarex" in various citrus beverages. The advantages of monochloracetic acid over benzoates are that it blends well with the fruit products and has only a temporary preservative effect, being transformed on storage into the innocuous glycolic acid and finally probably into sodium acetate and sodium chloride. However, Wilson[25] has indicated that little if any loss of monochloracetic acid occurs when this preservative is used with canned orange and grapefruit juice in 30 months. Much more research will be required before these preservatives establish themselves in the food industry.

In recent years various quaternary ammonium compounds have also been suggested as chemical preservatives for fruit juices and other food products. As such, alkyldimethylbenzylammonium chlorides are used, where the alkyl groups range from C_8H_{17} to $C_{18}H_{37}$:



These are marketed under a number of trade names (Zephiran, Roccal, Onyx BTC, and Zephirol). A method for determination of quarternary ammonium compounds in orange juice has been elaborated recently by Harris.[26]

(c) Deaeration of Citrus Juices

Before approaching the subject of preservation by heat it is necessary at this stage to dwell upon one all-important step in the processing of citrus juices—namely, deaeration. Both ordinary and flash pasteurization of citrus juices must always be preceded by deaeration, i.e., more or less complete elimination of dissolved and occluded oxygen, to prevent oxidation and consequent diminution of the nutritive value or flavor of the juice.

Oxygen incorporated into the juice with the air during reaming is

[25] Wilson, J. B., *J. Assoc. Official Agr. Chem.*, **27**, 195 (1944).

[26] Harris, T. H., "Determination of Quaternary Ammonium Compounds in Fruit Juices," *J. Assoc. Official Agr. Chem.*, **29**, 310 (1946).

rapidly absorbed by some components present in the juice and unless this oxygen is rapidly removed the juice will deteriorate and its flavor will be greatly impaired. While this danger is lessened when the juice is preserved by sulfur dioxide, which quickly reacts with free oxygen, if the juice is subjected to heat preservation the adverse effect of oxygen is very much apparent. Undeaerated juices heated to a high temperature soon develop a pronounced "cooked" taste and become unmarketable after a short period of storage.

There is the possibility that oxidase activity may be responsible for some detrimental changes in the flavor of the juices, especially on

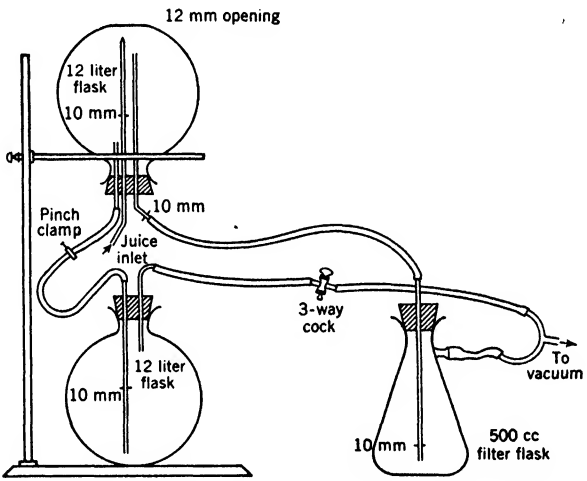


Fig. 78. Laboratory apparatus for deaerating juices (after Mottern and von Loesecke).

prolonged storage. If this is so the effect of deaeration is to inhibit the activity of oxidases which pass into the juice.

It was, therefore, customary to eliminate the oxygen by heating the juices in batches and agitating them slowly, thereby causing the air bubbles to rise. This process is still used in some places especially with grapefruit juice, but it is very inefficient. A continuous and quick deaeration was first suggested by Mottern and von Loesecke,[27] who forced the screened juice at a high velocity through a small nozzle against the dome-shaped base of an inverted flask, as shown in Figure 78. The flask was placed under a good vacuum; the juice, running in a thin film, contained numerous bubbles of air which, under these

[27] Mottern, H. H., and H. W. von Loesecke, "Deaeration and Flash Pasteurization of Orange and Grapefruit Juices," *Fruit Prod. J.*, **12**, No. 11, 325 (1933).

conditions, expanded and burst. This bursting or "flashing" of air bubbles is caused by their tremendous increase in volume. Berkness[28] calculated that when citrus juices enter the deaerator at a temperature of 24° C (75° F), with a pressure of about 18 mm Hg, any air bubbles contained in them will increase *forty-three times* in volume.

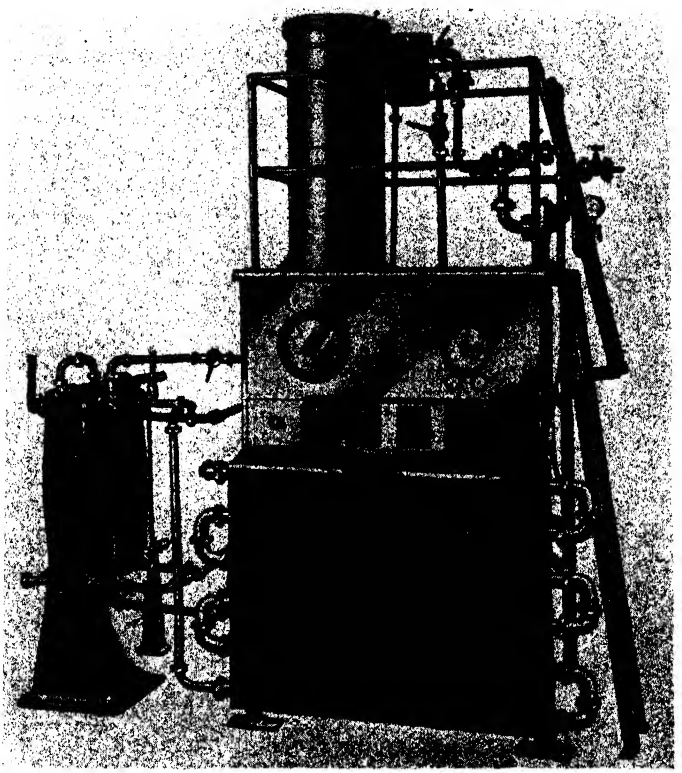


Fig. 79. Commercial deaerator and plate-and-frame flash-pasteurizer (courtesy Aluminum Plant and Vessel Co. Ltd.).

The arrangement above is conveniently used in laboratory experiments.

In actual practice three different types of deaerators are used, all of them based on the same principle of admitting the juice in the form of a spray or a thin film into a chamber with a high vacuum (about 29 in or 736 mm Hg). The temperature of the juice and the vacuum should, however, be so adjusted as to prevent the juice from

[28] Berkness, I. R., "Deaeration," *Food Prod. J.*, **19**, 138 (1940).

instantaneous boiling. One type of deaerating apparatus consists of a column built of stainless steel inside which is placed an array of trays made of porcelain or stainless steel. The juice, introduced at the top, runs from one plate into the second (a cascade effect) in a thin film, thus acquiring a very large surface subjected to the vacuum and becoming deaerated during its passage. In another type the plates are replaced by a stainless-steel screen or perforated mantel upon which the juice flows steadily downwards (Fig. 79). A third type of deaerating apparatus consists of a comparatively small vacuum chamber with a rapidly rotating plate inside. The juice, sprayed upon the rotating plate, is ejected by centrifugal force to the sides of the vacuum chamber and slowly runs downwards.

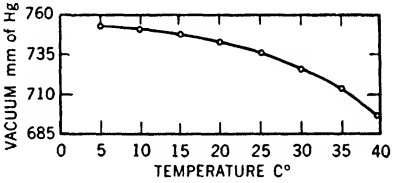


Fig. 80. Relation of vacuum to temperature for complete removal of dissolved gases during deaeration.

The question of selecting the suitable type of deaerating apparatus depends entirely on the ease with which the particular type can be cleaned. The juices are usually pumped into the deaerator under high pressure and are removed by a positive pump suitable to work against the vacuum. Vacuum in the deaerator can be created by special pumps or, still better, by a double-stage steam ejector. It is evident that all these deaerators, as well as all connections, taps, etc., should be made of acid-resisting materials such as pure aluminum or stainless steel.

The elimination of dissolved and occluded gases in any liquid depends on the temperature of the liquid. The removal of such gases from a liquid is based on the fundamental law that the solubility of a gas in a liquid is directly proportional to the partial pressure of the gas above the liquid; as this partial pressure depends on the temperature, the required vacuum for adequate deaeration should, therefore, be determined by the temperature of the juice, as shown in the accompanying graph (Fig. 80).

Assuming that the temperature of the juice is 75° F and that its physical properties are approximately the same as water, its vapor pressure will be 0.87 inch Hg; thus the partial pressure of air by

difference will be 29.13 inches Hg. If this juice can now be put under a vacuum of 29.13 inches or slightly higher, the partial pressure of air above the juice will become substantially nil and the dissolved air will immediately start flushing out of the juice. If ample time is given and sufficient surface contact is available so that the equilibrium can be reached, the dissolved air will be completely removed. In actual practice it is sufficient to remove 90 to 95% of the dissolved air.

It is considered[29] that 0.8 cc of air per 100 cc of juice left after deaeration is a fair result (the original juice contains some 3.65 cc of total gas per 100 cc of juice). The same deaerating apparatus can be used for the removal of SO_2 (desulfitation), as has previously been mentioned.

Subsequent to deaeration, the juice is immediately subjected to preservation by heat.

(d) Preservation by Heat

(1) PASTEURIZATION

In 1765 Spallanzani, experimenting on the origin of life, found that perishable materials could be preserved by heating them in hermetically sealed containers. However, Nicolas Appert, a French baker, should justly be credited as the father of the canning industry. In 1810, after several years of experimentation, he won the prize of 12,000 francs awarded by Napoleon for a method of preserving food for army use. Almost simultaneously Thomas Saddington, an Englishman, reported a method of preserving fruit by means of heating in a water bath. Since then sterilization by heat at boiling-water or higher temperatures and so-called pasteurization at lower temperatures have been extensively used for a great number of food products and the technique has been highly developed. Pasteurization means the destruction, through the application of heat, of all microorganisms causing spoilage. In citrus juices yeasts are killed by heating for a few minutes at 60–65° C, while some resistant molds which can grow in acid juices require a temperature of about 80° C for 20 min. The time of heating the juice is inversely proportional to the temperature at which pasteurization takes place, i.e., the longer the time during which the juice is exposed to heat the lower the temperature, and vice versa.

[29] Loeffler, H. J., "Processing of Orange Juice," *Bur. Agr. Chem. and Eng., U.S. Dept. Agr. Bull.*

The following table (according to Cruess *et al.*[30]) gives the relation between the length of heating and the killing temperature for yeast:

Temperature		Time
° C	° F	(min)
57	134.6	10
55.9	132.6	20
54.8	130.6	40
54.2	129.5	60
53.3	127.9	120

Pederson and Beavens[31] have shown that fruit juices can be preserved successfully by heating to a temperature of 57° C (135° F) for several hours, to 65° C (150° F) for 30 min, or to 75° C (170° F) for 1 min. Hence, the thermal destruction of microorganisms is a function of both time and temperature. All these temperatures are sufficient only for the destruction of yeasts. Other microorganisms, such as most of the bacteria, are more heat-resistant but are not capable of multiplying in as acid a medium as citrus juices. Also, molds are more heat-resistant than yeast but their growth is prevented by the elimination of oxygen from the headspace of the container.

Evidently heating citrus juices at lower temperatures kills many microorganisms but not all of them; however, it greatly weakens and delays the development of the survivors, which is an important factor in pasteurization. In order that pasteurized juices shall not spoil on storage they must be kept sterile in sealed containers, either tins or bottles, in such a manner that all live microorganisms are excluded. The containers, which must be thoroughly cleaned and sterilized, should be filled and sealed, leaving as little headspace as practically possible. Heating is then usually performed in a water bath the temperature of which can be easily controlled and kept comparatively constant. In lieu of the water bath pasteurization can, of course, be achieved by applying direct steam; however, as this method does not permit very exact regulation of temperature, overheating may result. The simple form of such batch-operated pasteurizers is a shallow rectangular tank or wooden vat filled with water and fitted with a steam coil or perforated tubes through which live steam is admitted for heating the water to the desired temperature. Into the pasteurizer, which has a false bottom, wire baskets, containing the bottles or tins

[30] Cruess, W. V., H. Aref, and J. H. Irish, "Pasteurization Investigations," *Fruit Prod. J.*, **12**, 358 (Aug. 1933).

[31] Pederson, C. S., and E. A. Beavens, *Food Industries*, **12**, 61 (1940).

filled with the juice and arranged in a horizontal position, are immersed for a predetermined length of time. After pasteurization the baskets are taken out and cooled quickly to about 38° C (100° F).

An important factor in connection with pasteurization of juices filled into containers is heat transfer. It is obvious that every drop of juice must reach the desired temperature and that the transfer of heat from the hot water into the center and to all points of the container must proceed as quickly as possible. This depends, of course, on the size and shape of the container, on its material (whether tin plate or glass), and on the viscosity of the juice.[32] The temperature at the center of the container should be regarded as the basis for any study of processing. The time necessary for heat to penetrate to the center of cylindrical tins of different sizes filled with the same juice is approximately proportional to the squares of the radii when the length of the tins is greater than the diameter.[33]

The best way of increasing the rate of heat penetration is the rapid rotation or agitation of the container. To achieve this end, large factories use continuous pasteurizers in which the containers, particularly tins, are carried through the water bath by roller conveyors: upon them the tins rotate at a speed sufficiently great to cause the entire contents to move in a body inside the tin. The critical speed above which this movement occurs naturally depends on the viscosity of the juice as well as on the headspace. For orange juice it is approximately 70 rpm. Other continuous pasteurizers consist of a basket conveyor which progressively carries the bottles or tins first through baths of water of increasing temperature and then, to cool the juice, through similar baths of decreasing temperature. In some factories the containers, after pasteurization, are cooled in a current of air; although this method is slower it is regarded as preferable to water cooling. The tins must be taken out of the coolers while still warm to the touch so that excessive water may evaporate from the surface of the tin; failure to do so may cause the tin to rust on the outside.

Mention should be made here of the special quick-heating, the so-called Stero-Vac[33a] process, applied also to citrus fruit juices. By this process the juice is flash-heated *in the final container* in the absence of air. The juice leaving the deaerating unit and filled in special tins

[32] Irish, J. H., M. A. Joslyn, and J. W. Parcell, "Heat Penetration in the Pasteurizing of Syrups and Concentrates in Glass Containers," *Hilgardia*, **3**, 183 (1928).

[33] Bigelow, W. D., "Heat Penetration in Processing Canned Foods," *Bull. No. 162, Nat. Canner's Assoc.*, Washington, D. C.

[33a] Ayers, S. H., "Recent Developments in Canning Fruit Juices," *Food Research*, **3**, 5 (1938). Also, *Fruit Prod. J.*, **17**, 41 (1937).

with seamed valve ends is then slightly preheated to about 43° C (110° F) and enters a special machine consisting of turret carrying headers through which first vacuum and then steam is applied for 10 seconds. During this period the temperature of the juice in the can is raised to 99° C (210° F) and the contents of the can are violently agitated by the steam. The valve is then sealed and the can cooled.

In discussing enzymes (see page 127) mention was made of a method of measuring the phosphatase activity as a useful indication of the extent of pasteurization of citrus juices. The older, so-called Scharer phosphatase test, extensively used in milk production, is now superseded by the improved Sanders and Sager test.

This phosphatase test depends on the fact that, while all normal raw milk contains a phosphatase enzyme, this enzyme is inactivated by heating at a temperature a few degrees higher than that required to destroy the most resistant of pathogenic or disease-producing organisms that may occur in milk. It is now possible to detect a decrease of as little as 1° F in the pasteurizing temperature. The test is so sensitive that it will detect, for example, the presence of 1 part of raw milk in 2000 parts of properly pasteurized milk, whether the test is applied to the milk or to products made thereof.

The application of this test to measure the degree of pasteurization of citrus juices is not yet fully developed.

(2) FLASH-PASTEURIZATION

Improper heat treatment during the pasteurization of citrus juices causes the development of an undesirable "cooked" flavor. It is obvious that the lower the temperature or the shorter the period at which pasteurization is performed the better it is for the retention of the natural flavor of juices. To attain this objective, methods have been devised to heat the juice to a high temperature in a thin film for only a few seconds and then to cool the juice immediately, while filling the containers under sterile conditions. This method, known as flash-pasteurization, was first suggested by Chace[34] in 1920. In a flash-pasteurizer the juice is usually heated to 85° C (185° F) for only 30 sec. Recent investigations by Boyd and Peterson[35] indicate that the temperatures to which the juice is subjected during pasteurization are a minor factor in the cause of "cooked" and other off-

[34] Chace, E. M., "A Method for the Clarification and Preservation of Fruit Juices," *Calif. Citrograph*, **5**, 264 (1920).

[35] Boyd, J. M., and G. T. Peterson, "Quality of Canned Orange Juice," *Ind. Eng. Chem.*, **37**, 372 (1945).

flavors. According to these investigators the length of time the juice is kept at elevated temperatures is the major factor in determining the amount of "cooked" flavor in the final product and, to some extent, the rate of flavor change during storage under normal conditions. They suggest, for instance, treating orange juice at 110° C (240° F) for only 2 sec and then rapidly cooling it to 38° C (100° F). These elevated temperatures are not only sufficient for the preservation of the juice and the prevention of fermentation, but are also the proper temperatures for the inactivation of the various enzymes, espe-

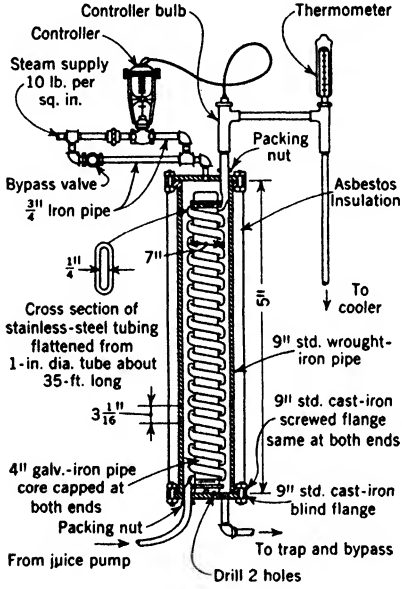


Fig. 81. Diagram of a flattened coil for juice flash-pasteurization (after Heid and Scott).

cially the pectic enzymes which otherwise cause the undesirable clearing of citrus juices and clotting of the fruit particles they contain. Incidentally, pectic enzymes are more heat-resistant, requiring for their inactivation at least 4 min at 85° C (185° F), 1 min at 88° C (190° F), or only a fraction of a minute at temperatures higher than 100° C (212° F). This method of flash-pasteurization in conjunction with deaeration, previously described, is finding more and more favor in citrus juice processing.

Flash-pasteurization is usually carried out in heat exchangers either of the plate type or of the coiled tube type. In plate-type heat ex-

changers (Fig. 79) the juice and the heating medium, usually hot water, run in countercurrent flow between stainless-steel plates which are separated by only about 1 mm. The same frame that holds the heating plates holds also an additional section for cooling the juice after pasteurization, thus preheating the entering juice at the same time.

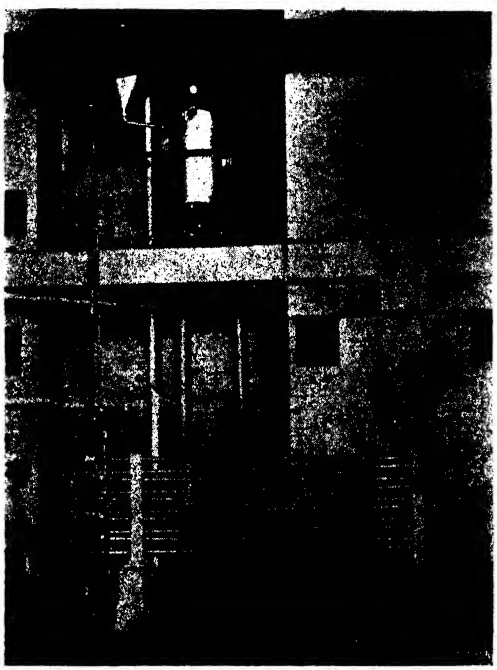


Fig. 82. Tubular flash-pasteurizer
(courtesy of Ontario Research Foundation, Toronto).

Tubular heat exchangers (Figs. 81, 82) consist of a series of stainless-steel flattened or coiled tubes immersed in a tank with the heating medium (steam or hot water). When coiled tubes are used they often contain continuous helical baffles inserted for the purpose of agitating the juice so as to obtain uniform heating. The velocities of the juice in the tubular heat exchanger should be at least 2 to 3 liters per sec and the heat transfer as high as 800 calories per hour per sq meter per degree difference in temperature (or 300 BTU per sq ft). When steam is used as the heating medium it should be directed downward and the juice upward to insure uniform counterflow heat exchange.

The velocity of juice flow in tubular heaters is an important factor

in determining the rate of heat transfer. The heat exchange rate is appreciably higher in the coiled tube, which is probably due to greater agitation of the juice.[36]

After leaving the flash-pasteurizer, juice can be cooled also by introducing it into a vacuum chamber, where it cools down instantly. Normally, however, cooling is attained by another exchanger. Figure 83 is a schematic diagram and Figure 83-A an external view of the arrangement of a coil pasteurizer using steam at a certain pressure, and the cooling system (Mallory No-Film Sterilizer), as described by Tressler, Pederson, and Beattie.[37] The juice pumped into a flash-pasteurizer of this type should enter at high speed and at a pressure of about 25 atm (375 lbs per sq in). Under such a high speed and high pressure there is no possibility for any stationary film of juice to adhere to the sides of the tubes and it is, therefore, claimed that citrus juices pasteurized by this method do not take on a "cooked" flavor.

Studies made[38] on the retention of ascorbic acid in pasteurized citrus juices indicate an average retention of 98–99% of vitamin C content in the fresh juices. At the end of a six months' period of storage, the same juices (orange and grapefruit) still retained 89–97% of their ascorbic acid when stored at 4° C (40° F)—and 75–83% when stored at room temperature (27° C or 80° F). A small quantity of dehydroascorbic acid (about 2%) apparently was present in these juices at the end of the storage period. (A more detailed discussion on the subject of vitamin C retention is presented in Chapter XI, under Vitamin C.)

Finally, high-temperature pasteurization is recommended to retain the "cloud" in marketed citrus juices, for during flash-pasteurization the pectolytic enzymes are inactivated for at least some months, thus preventing the settling of coarser particles and the clarification of the juice—a phenomenon greatly detracting from the appearance of the product.

(e) Other Methods of Preservation

Many attempts have been made during the last two decades to devise methods other than the application of heat or the use of

[36] Heid, J. L., and W. C. Scott, "The Processing of Citrus Juices. (Observations on Heating and Cooling Operations.)" *Fruit Prod. J.*, **17**, 100 (1937).

[37] Tressler, D. K., C. S. Pederson, and H. G. Beattie, "Fruit and Vegetable Juice Preparation and Preservation," *Ind. Eng. Chem.*, **35**, 96 (1943).

[38] Moore, E. L., E. Wiederhold, and C. D. Atkins, "Changes Occurring in Orange and Grapefruit Juices during Commercial Processing and Subsequent Storage of the Glass and Tin-Packed Products," *Fruit Prod. J.*, 270 (May, 1944).

chemicals for preservation of juices. However, all these methods take into account only the prevention of fermentation and, in most cases,

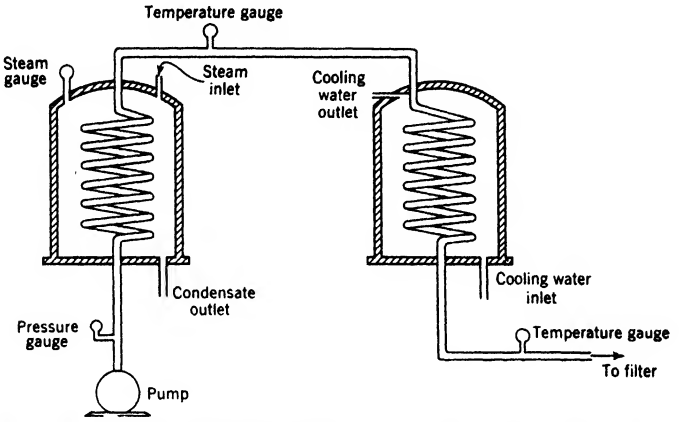


Fig. 83. Mallory No-Film Sterilizer (schematic)

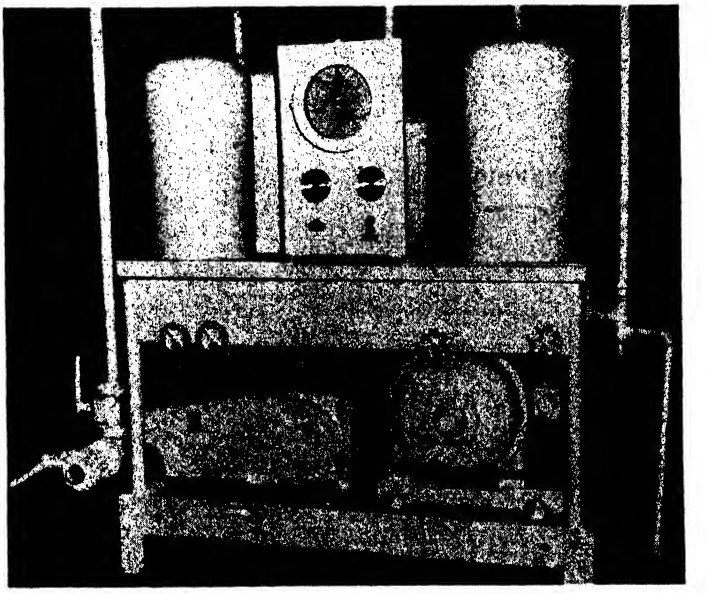


Fig. 83-A. 1800-gallon-per-hour Mallorizer
(courtesy B. C. Skinner Machinery Co., Dunedin, Florida).

entirely ignore oxidation, loss of vitamin C, and other radical changes. Hence, the new processes have failed and, as far as citrus juices are concerned, are not used in practice. Nevertheless, a short

description of the alternative methods may be useful from a negative point of view.

Some of the methods are based upon the so-called oligodynamic principle. Since ancient times it has been observed that wines kept in silver containers do not turn sour. In 1893 the botanist, C. von Nägli, discovered that inconceivably small quantities of copper (quantities which were too small to be determined analytically) hinder the growth in fresh water of the filamentous alga *Spirogyra,* known as pond scum. For the last 50 years a vast scientific literature has been accumulating on this oligodynamic principle, which shows, on the whole, that under specific conditions very small amounts of certain metals exert powerful antiseptic influences.

Commercially this method is used with sufficient practical success for water treatment under the patented process developed by G. A. Kraüse[39] and named Katadyn (abbreviated from Catalytic Oligodynamics). In this process either granulated silver is used or the water is introduced electrolytically between two silver electrodes. In the latter case a very low voltage (1.65 volts) is used, to prevent decomposition of the water; thus, silver ions pass into the water at the theoretical rate of 4 g. per ampere-hour. When precipitated granular silver is used, it is prepared so that it has a large absorptive surface of 1.62 sq meters per g. In other instances silver-coated sand is used in conjunction with other layers of colloidal material through which the water is filtered. The Katadyn process is used in Europe for vinegar and probably for other branches of the food industry, but it does not appear to have enjoyed any considerable development for fruit juices. The process is successful only with clear liquids; furthermore, the efficiency of Katadyn decreases when bacterial counts are high.

A further development of this method is the Matzka[40] process in which the use of ionized silver is combined with moderately elevated temperatures. The juices are passed between two tubes, one inside the other, the outer tube being made of silver and the inner of stainless steel, nickel, or aluminum. The juice is kept at 120–150° F (49–66° C). It is claimed that under these conditions the silver passes into the juice at the rate of about 1 part per 100,000,000 parts of juice. Although several installations have been using the Matzka

[39] Krause, G. A., Brit. Patent No. 293,385 (1929).

[40] Matzka, Wincenty, "Apparatus for the Sterilization of Liquids," U.S. Patent No. 1,812,105 (June 30, 1931); "Process for Sterilization of Liquids," U.S. Patent No. 1,850,594 (Mar. 22, 1932).

method in Canada, Holland, southern France, and other places, careful tests have shown that the entire process is nothing more than pasteurization and that the little silver which is added to the juices has no sterilizing effect whatsoever.

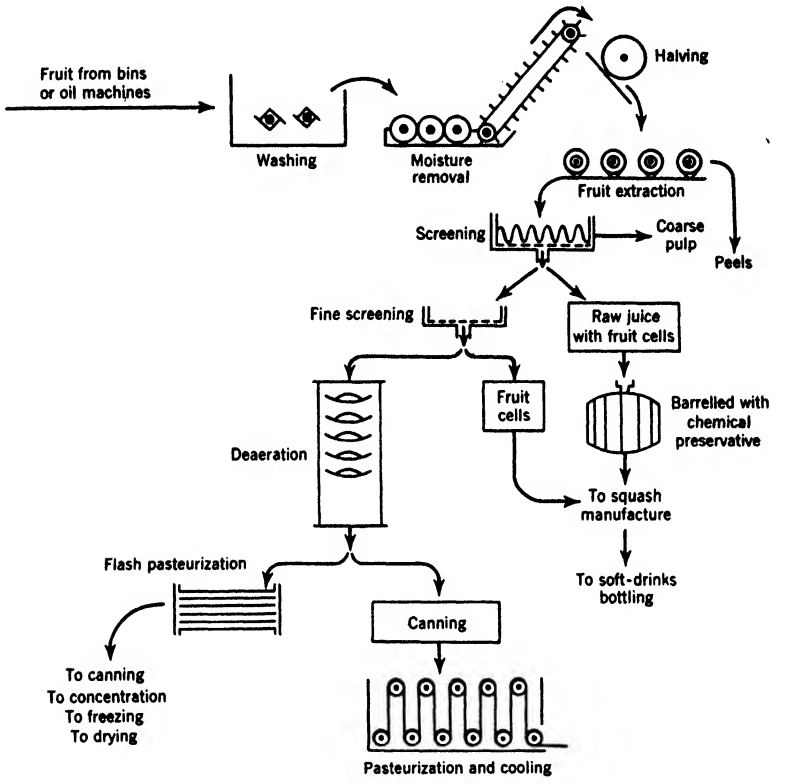


Fig. 84. Flow sheet showing the various steps in processing citrus juices.

Some other methods using enzyme action and bacterial filtration will be discussed in the section on the manufacture of cordials (pages 304–306).

To recapitulate, a flow sheet is presented to show the various steps in the processing of citrus juices (Fig. 84).

7. Darkening or "Browning" of Citrus Juices during Storage

Unless citrus juices (straight or concentrated) are preserved with SO_2, or unless some other antioxidants are added to them, they soon undergo a profound change in color: they darken and soon turn

quite brown if stored at room temperature. For the last twenty years, attempts have been made to elucidate the fundamental mechanism involved in this deterioration of color; however, there is still a lack of understanding of the real causes of this change.

As early as 1928, Wilson attributed the change of color to the possibility of Maillard reaction between sugars and amino acids. Since then other investigators reported data contradictory to Wilson's. Most of the workers in this field were of the opinion that the browning of citrus juices involves oxidation. It was found by Eddy[41] that a greater amount of oxygen is absorbed than indicated by potential reducing substances of the fresh juices. In fact Joslyn[42] indicated that darkening of citrus juices did not occur until all the free ascorbic acid had been oxidized into dehydroascorbic acid and when the latter was no longer oxidized to other stages. Most of the recent investigators have come to the conclusion that oxygen and ascorbic acid[43] or its decomposition products[44] are the principal factors involved in the darkening of packaged citrus juices.

It has been suspected that, during the decomposition of ascorbic acid, furfural is formed which upon polymerization is responsible for the browning of citrus juices as well as of other food products. It is known that uronic acids decompose in acid solutions, first into pentoses and carbon dioxide and subsequently into furfural.

It has been shown[45] that *d*-galacturonic acid forms, on heating, colored compounds at a rate exceeding that found with common sugars. On the other hand, pectinic acids, or heat-degraded pectinic acids, do not show an increased tendency to form color. It is probable, therefore, that when the pectins of the juice are ultimately degraded into the galacturonic units the latter begin to show color.

Similarly, ascorbic acid in the presence of acid also yields furfural with the evolution of CO_2. Furfural polymerizes giving rise to brown substances. It has been observed that concentrated orange juice when stored at room temperature gives rise to carbon dioxide without any signs of fermentation. This fact is ascribed to decomposition of ascorbic acid.

[41] Eddy, C. W., "Absorption Rate of Oxygen by Orange Juice. Effect of Catalysts," *Ind. Eng. Chem.*, **28**, 480 (1936).

[42] Joslyn, M. A., "Color Retention in Fruit Products," *Ind. Eng. Chem.*, **33**, 308 (1941).

[43] Moore, E. L., W. B. Esselen, and C. R. Fellers, "Factors Responsible for the Darkening of Packaged Orange Juice," *Fruit Prod. J.*, **22**, 100 (1942).

[44] Loeffler, H. J., *Report*, Glass Container Association, 1941.

[45] Seaver, J. L., and Z. I. Kertesz, " 'Browning (Maillard) Reaction' in Heated Solutions of Uronic Acids," *J. Am. Chem. Soc.*, **68**, 2178 (1946).

A recent study made by Rice, Kertesz, and Stotz[46] on color formation in furfural systems showed that dilute furfural solutions, treated with mineral acid, alkali or by heat, produced colored material and that the presence of such amino acids as glycine, aspartic acid, and arginine greatly accelerated the color reactions. Dialysis experiments undertaken by these investigators in order to characterize the brown precipitate indicated that the formation of this brown colored material involves a polymerization reaction. So far the only practical way to prevent browning in both straight and concentrated juices is to store them at low temperatures preferably at or below 4° C. Cold storage greatly retards the browning process and, as already mentioned, also the decomposition of ascorbic acid.

[46] Rice, R. G., Z. I. Kertesz, and E. H. Stotz, "Color Formation in Furfural Systems," *J. Am. Chem. Soc.*, **69**, 1798 (1947).

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Moore, E. L., W. B. Esselen, and C. R. Fellers, "Factors Responsible for the Darkening of Packaged Orange Juice," *Fruit Products J.*, **22**, 100 (1942).

CHAPTER VII

CONCENTRATED, FROZEN, SWEETENED, AND FERMENTED JUICES

A. CONCENTRATED JUICES

1. General Discussion—Difficulties of Concentration

Chemists and food technologists have long endeavored to reduce the volume of citrus juices by eliminating most of the water content without markedly impairing the quality. A reduction in volume is important for two main reasons: first, it may facilitate shipping by reducing the size of containers; secondly, it may be advantageous from the point of view of preservation since it is generally known that foods containing over 65% of sugar will not ferment if kept in a sanitary, closed container.

The term "concentrated juices" as discussed in this section is intended to signify only juices evaporated in vacuo at low temperatures. Concentration of citrus juices through the application of cold is described in the next section and several novel methods of condensing juices are mentioned in the last chapter.

While other foods, such as milk and some juices, lend themselves easily to condensation, numerous difficulties are encountered when concentration is attempted with citrus juices. The following reasons may be cited:

1. The total solids of juices such as orange or grapefruit juices, when the fruits are ripe, are in the vicinity of 12 to 13%, while their sugar content is only 8 to 9%. Such juices can be concentrated, at the most, only about sixfold (6:1), so that while their total solids will range at about 70%, their sugar concentration will reach only 50%, which is not sufficient to prevent fermentation if osmophylic microorganisms are present.

2. As has been pointed out, filtered citrus juices are deficient in flavor and, therefore, at least the fine pulp or fruit cells should be left in them. But even when citrus juices are very finely screened the re-

maining pulp with the contained pectin makes a sevenfold concentrated juice too thick to be easily handled. In other words, in order to obtain good concentrates a lower concentration is preferable.

3. The composition of the flavoring constituents of citrus juices has been described on pages 104–105. They are quite different from the peel oils and are of a very delicate nature. Concentration of citrus juices, even under the most advantageous conditions and at very low temperatures, destroys most of the flavoring constituents, leaving the final product rather flat in taste and lacking the freshness of the original juice.

4. Enzymatic action, which causes numerous changes in the appearance, flavor, and nutritive value of the juice and which has been discussed on pages 125–132, proceeds freely also in concentrated juices; unless full precautions are taken by the operator and the juices are properly prepared and stored the final products will be practically valueless.

Notwithstanding the foregoing difficulties the technique of preparing concentrated citrus juices has recently made good progress. It is hoped that in the near future concentrated products may be manufactured which upon reconstitution will be indistinguishable from fresh juice.

2. Preparing the Juice Prior to Concentration

At normal atmospheric pressure of 760 mm Hg, water boils at 100° C (212° F), however, when the pressure is only 22 mm (a vacuum of about 29 in) water will boil at 24° C (75° F). Solutions, or in the present instance juices, will boil at slightly higher temperatures.

When juice is concentrated in vacuo at a temperature of about 37–40° C it is actually subjected for a considerable length of time to optimum conditions at which microorganisms, if any are present, may freely develop and multiply. It is, therefore, of primary importance that the juice entering the concentration apparatus be processed under sterile conditions. Flash-pasteurization also inactivates the pectic enzymes and prevents the fruit cells in the final reconstituted product from settling down. However, previous to flash-pasteurization the juice must be thoroughly deaerated. Before entering the evaporator citrus juices should, therefore, be finely screened, deaerated, flash-pasteurized, and cooled—all in a single uninterrupted procedure.

Concentrated juices, when ready, contain 65 to 70% of total sol-

uble solids, some 6 to 7% of citric acid, and sufficient pectin to create a jelly (see page 322). To prevent the jellying effect it is suggested that an equivalent of 200 parts per million of sodium citrate be added to the juice before concentration is begun; this salt acts as a buffer and helps to inhibit the gelling of concentrates. Instead of sodium citrate, sodium bisulfite may be added. The SO_2 ion is largely removed in the vacuum during concentration, leaving the Na ion which combines with citric acid and forms sodium citrate, acting as described above. At the same time SO_2 may help to prevent the juice from becoming contaminated with microorganisms.

However, this last method may be used only when concentrated juices are packed in barrels or in glass. If packed in tins they obviously should not contain any SO_2—not even a trace of it—for fear of corrosion. In such cases excessive viscosity or jellification of the concentrated juice should be prevented by careful screening of the juice prior to deaeration.

In this connection it is interesting to note that, while juices extracted by hand-reaming or by such automatic machines as the Brown extractor using the same reaming principle, are very viscous when concentrated to 65° Brix, juices extracted by other machines using the pressure principle and containing, therefore, much less pulp, give rise to quite free-flowing concentrates. There is every belief that the abundance of solid particles, which contain more pectic substances probably in the form of protopectin than the juice itself, enrich the juice with soluble pectin while in close contact with the juice, and especially so during the pasteurization process when the protopectin is quickly transformed into soluble pectin by the action of heat and at acid *p*H of the juice.

To prevent excessive viscosity of the concentrates made from such juices, one must pay special attention to the screening operations, namely, to the size of screen and the method of screening. Perforated stainless steel sheets with holes not larger than 0.8 mm (or 24 mesh stainless steel screens) are recommended for this purpose. Furthermore, rotating or vibrating screens should be preferred to the drastic action of paddle or screw finishers, which tend to bring the pulp in closer contact with the juice.

Another method of reducing viscosity may be sought in applying some pectolytic enzymes to the pasteurized juice during the process of concentration and prior to its pasteurization for a second time before hot-filled in cans. Such methods may be used with advantage if the process of concentration is a batch process and not continuous,

and if the temperature in the vacuum evaporator is not too high to inactivate the action of enzymes.

For a 65° Brix concentrate to be sufficiently free-flowing for practical convenience in use, the relative viscosity of the concentrate at 20° C should not exceed 200.

3. Evaporating Apparatus

Concentration may be performed in one of the numerous existing types of vacuum evaporators. However, in order to obtain a good

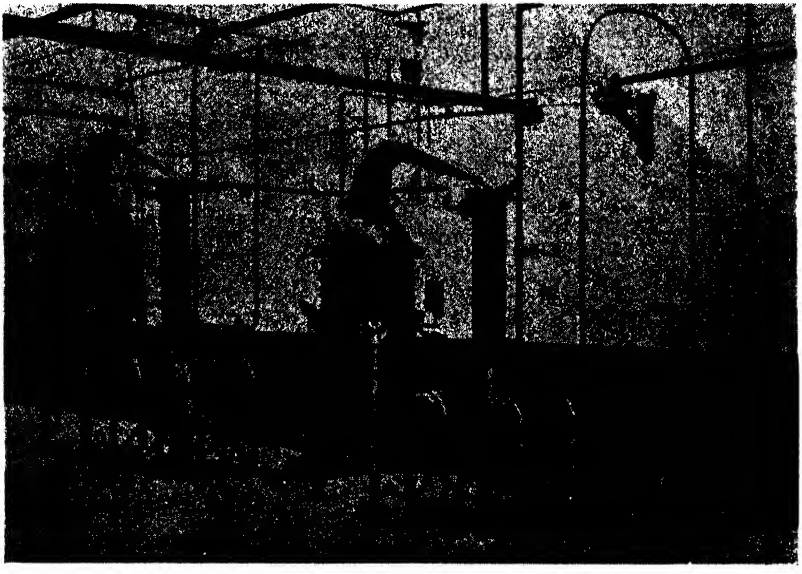


Fig. 85. A battery of jacketed vacuum evaporating pans.

product great care should be exercised in selecting the most appropriate type. The simplest type of evaporator, shown in Figure 85, consists of a cylindrical pan, the bottom portion of which is surrounded by a steam jacket and the head connected with a wet vacuum pump suitable for condensing the vapors. This simple design is capable only of batch operations and exposes the juice to the danger of overheating if its level falls below the top of the steam jacket. Furthermore, the heat-exchange surface of such a pan and consequently its evaporating capacity are very small.

To increase the evaporating capacity and to create a continuous natural circulation of the juice the so-called short-tube or Claassen-type evaporator is used (Fig. 86). This device consists of an upright

cylinder within which a nest of vertical 2-in tubes (varying from 2 to 6 ft in height) is inserted. In the center, between these tubes, a wider tubular "downtake"—its diameter generally equaling 50% of the total cross section of the tubes—is provided. The steam circulates outside the tubes while the juice fills the bottom of the tubes and cylinder. During evaporation the bubbles of the evaporated juice lift it inside the tubes, causing the juice to move continuously upward and to return through the central downtake. The evaporation is greatly

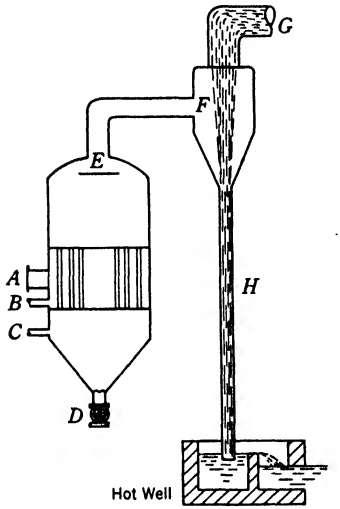


Fig. 86. Claassen-type evaporator (single effect): *A*, inside calandria; *B*, steam inlet; *C*, juice inlet; *D*, concentrate outlet; *E*, baffle to check foam; *F*, condenser; *G*, water inlet; *H*, barometric leg.

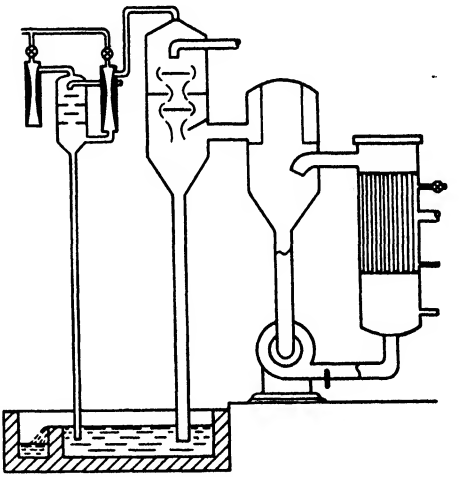


Fig. 87. Forced-circulation vacuum evaporator with outside calandria and two-stage steam ejector.

promoted by this natural circulation (or thermosyphon) which constantly disturbs the film clinging to the inner walls of the tubes, thus avoiding overheating.

Although this type of short-tube evaporator constitutes a great improvement over the simplest type of steam-jacketed vacuum pans, further improvements have been introduced comparatively recently. To the natural thermic movement of the juice a forced circulation is added by means of a centrifugal pump; consequently the clinging film is more intensely disturbed and a greater amount of heat is transferred per unit of heating surface. To achieve this objective longer

tubes—so-called "calandria"—are placed outside the main body of the evaporator (Fig. 87). These forced-circulation evaporators with outside calandria, containing vertical heating tubes of small diameter, are preferred for large citrus-juice concentrating operations (Fig. 88). A positive centrifugal pump is placed just below the calandria

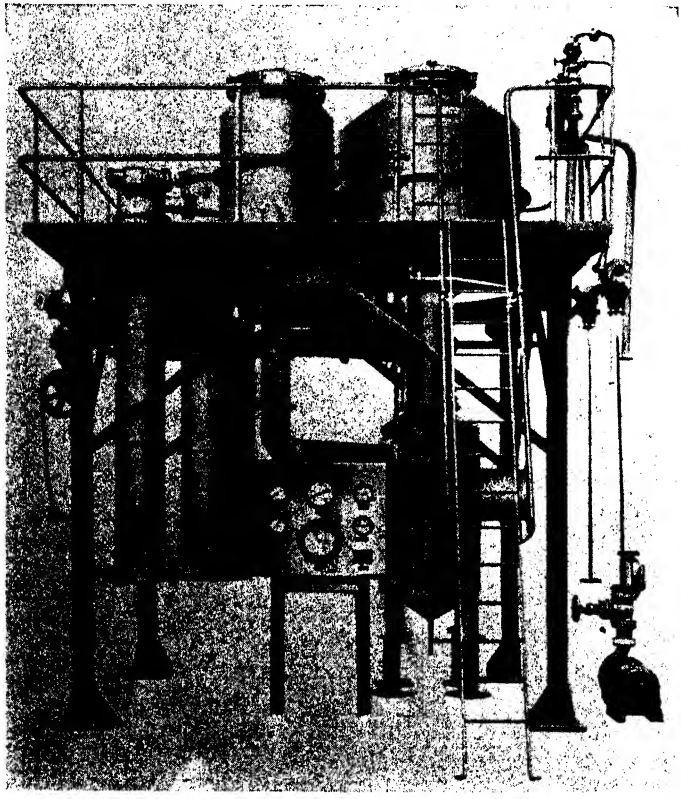


Fig. 88. Latest type of two-stage citrus juice evaporator with forced circulation, outside calandria, and recompression arrangements (courtesy Aluminium Plant and Vessel Co., Ltd.).

to force the juice upward at high speed through the heating element. With the aid of this pump the juice upon reaching the desired degree of concentration can be removed from the evaporator without breaking the vacuum, and fresh juice drawn in by the vacuum, so that the operation is continuous.

To avoid entrainment losses—that is, the carrying away of bubbles and droplets of the juice by the vapor—the juice entering the flash

chamber from the heater should be directed downward, tangentially to the wall; furthermore, to minimize entrainment, the top of the flash chamber should be equipped with baffles or a built-in spiral separator.

It is of the utmost importance that whatever type of evaporating apparatus is used, it should be built of suitable acid-resistant material; for this purpose the most suitable would be glass-lined equipment. However, inasmuch as glass lining, at the present time, is made only with plain steel, thin cracks frequently occur in the evaporator, thereby allowing contact between the juice and the iron. Stainless steel (more particularly 18-8) is preferred for concentration of citrus juices; for lemon and lime concentrates stainless steel with 1 to 4% of molybdenum is preferred. It is important that all connections, tubes, valves, fittings, and pumps be made of acid-resistant material and that no trace of iron, or especially of copper, be in contact with the juice during the process.

4. Steam Requirements

The heating agent in all evaporators is, of course, steam, but at a low pressure—perhaps 0.5 to 2 atm. It should not be superheated, for superheating in this case does not furnish a sufficient number of heat units to be of importance; the task of evaporation is delegated, instead, to the great store of latent heat, or heat of condensation, of steam. Steam can be used after it has previously been utilized for other equipment; however, steam taken directly from the main should first go through a reducing valve before entering the heating chamber.

To realize a considerable saving in fuel many industries using evaporators—for instance, the sugar industry—make use of so-called multiple-effect evaporators. These consist of a series of two, three, or more evaporators interconnected so that vapors leaving the first evaporator are piped to the steam chest of the second evaporator to concentrate another volume of juice or solution, and so on. In each case the incoming steam is cooler than the original steam from the main, and that, of course, requires that the temperature of the first evaporator be raised in order to maintain the required minimum temperature in the last apparatus of the series. This requirement of the multiple-effect evaporators, coupled with the large initial outlay for more complicated machinery, prevents its use in the concentration of citrus juices for, as already mentioned, the aim is to concentrate at the lowest temperature practically possible. However, in lieu of the

multiple effect, it is highly recommended, especially when large quantities of juice are to be concentrated, that two single-effect units, one achieving half concentration (say 3:1), and the other, a smaller unit, completing the operation. Such an arrangement to some extent shortens the time during which the juice is subjected to the process of concentration and results in an appreciable saving in steam.

Another very effective way of saving steam is to use recompression. In this system a part of the vapor given off during evaporation is re-

Fig. 89. General view and layout of deaerator, flash-pasteurizer, and concentrator (Jaf-Ora Ltd., Rehovoth, Israel).

compressed either mechanically or thermally and returned to the steam chest. The recompression evaporator must, however, have about four times the heating surface of the ordinary single effect in order to possess the same evaporation capacity. Recompression may be recommended in the case of citrus-juice concentration because evaporation must be conducted at a low temperature, and it is most economical only when high-pressure steam is used. Where steam is cheap and low-pressure "worked-up" steam is available, a single effect without recompression will be smaller, cheaper, and just as adequate.

5. Securing the Vacuum and Condensation of Vapor

To secure a vacuum and to condense the created vapors a number of methods exist. The simplest is a centrifugal vacuum pump, or so-

called wet pump, capable of entrapping the vapors, condensing them, and mixing them with cold water running through the pump. However, such pumps, on the whole, provide a poor vacuum and may not be suitable for large concentration plants. For large operations the vacuum is best secured by a two-stage steam-jet ejector working on the same principle as the well-known laboratory water-jet suction pump. These steam-jet ejectors create a good vacuum if the steam is run through them under a pressure of at least 7 atm. The two ejectors usually work in series, with a small intercondenser between the two stages; the first creates a vacum of about 610 mm (24 in) and the second increases the vacuum to about 735 mm (29 in).

When the vacuum is secured by means of steam ejectors it is necessary, of course, to eliminate the vapors created during concentration. This may be accomplished either by specially built surface condensers or by multiple-jet condensers provided with a barometric leg. Barometric condensers, which are widely used for large juice evaporators, are simple in design and operation (Fig. 86, above). They consist of a comparatively small condensing chamber provided with baffles, where the vapors entering from the bottom are intimately mixed with water running in multiple jets from the top. Condensing water and condensate drop through a 10-meter-long barometric leg to a hot well from which they are pumped, cooled, and continuously returned to the condenser. The temperature of the cooling water must be at least 15° C lower than that of the desired temperature inside the concentration vessel. As previously pointed out the temperature at which citrus juices are concentrated should not exceed 37 to 40° C.

To secure a good product it is important, therefore, to take the following precautions:

1. To prepare the juice in a suitable way, as described above, by proper screening, deaerating, flash-pasteurizing, and cooling.

2. To concentrate at as low a temperature as possible, preferably at 37° C.

3. To attain the highest vacum practically possible and to remove the vapors as quickly as possible.

4. To keep the juice inside the evaporator in constant motion, to break the adhering film, to avoid caramelization of the concentrate.

5. To remove the concentrate and to cool it as quickly as possible. Prolonged heating and local overheating cause more trouble than excessive temperatures.

Much effort has been spent by numerous scientific workers and private enterprises to recover some of the volatile citrus-juice constituents lost during the process of concentration, as is commonly done in the case of grape and apple juices; so far the results have been unsuccessful. When fractions of condensates have been collected and returned to the concentrate, the only result has been the development of off-flavors. Research fellows of the Florida Citrus Commission have recently worked on this problem of ester recovery in conjunction with the U.S. Citrus Products Station at Winterhaven, Florida; their report, however, is not yet available.

6. Preservation and Storage of Concentrates

The preservation of concentrated citrus juices is a difficult problem. As previously pointed out, concentrates, still containing some 20% of moisture and less than 15% of citric acid, are perishable and will spoil unless they are kept continuously in cold storage. The choice of the method of preservation is very closely connected with the method of packing.

If the concentrated juices are to be packed in wooden barrels they must be chemically preserved, for no matter how carefully the concentrates are packed or how scrupulously clean they are kept, absolutely sterile conditions are impossible. The juices should be packed in new paraffin-lined white-oak or chestnut barrels, preferably of 135 to 225 liters (30 to 50 Imp gal) capacity. The barrels should be thoroughly cleaned, steamed, dried, and warmed with dry air before they are waxed. Hot molten paraffin is poured into the barrel, which is rolled quickly about, rinsed again with cold water and "sulfured" (a piece of sulfur is placed in a special cup and burned) before being filled with the concentrate. Sodium benzoate can be used as a preservative but sodium bisulfite is preferred for reasons that were previously discussed. Gaseous SO_2 cannot be used with concentrates as it is practically impossible to incorporate the gas into such a viscous product. The preservative in solution should, therefore, be added to the concentrate at the end of the evaporation process; it should be poured directly into the apparatus while the vacuum is still on. By means of forced circulation the preservative is mixed into the concentrate for a few moments and filled directly into the barrels.

Usually about 1000 to 1500 ppm of SO_2 are added. It should be borne in mind that in a medium such as citrus concentrates the bulk of the added SO_2 readily combines with the glucose and, because of its high concentration, very little free SO_2 is left. Since only free SO_2 exerts a preserving effect the aim should be to obtain about 500 ppm

of the free gas. According to Downer,[1] to attain this amount at least 2000–2500 ppm of total SO_2 must be added to a fourfold concentrate; by careful processing, 1500 ppm should be sufficient. If the juices are then used for soft drinks more SO_2 can be added, for it is ultimately greatly diluted. Sodium benzoate can be used in the proportion of 0.1%, an amount which will effectively inhibit fermentation, especially if the concentrate is stored at 0° C. However, SO_2 will not only inhibit fermentation but at the same time will retard browning of the juice and loss of vitamin C. Again, cold storage, when used as an adjunct to chemical preservatives, further retards destructive changes.

If concentrated juices are packed in tin or glass containers they may be pasteurized without adding any chemical preservatives. Because of their high viscosity, however, pasteurization of concentrates presents special problems. Pasteurization in sealed tins requires considerable time, owing to slow heat penetration; by using flash-pasteurization in jacketed tubes of large diameter one runs the risk of scorching the juice, unless the tubes are equipped with helical baffles to insure uniform high velocity and agitation. The containers, either tins or bottles, should be completely filled to the brim from the bottom (to prevent aeration), sealed, inverted, and allowed to stand in this position for ½ min. They should then be cooled rapidly by rotation while immersed in cold water, or by means of strong water jets (Fig. 90).

Whatever method is used for packing or preserving concentrated juices, they should be stored in cold storage at 0 to +4° C and should preferably be transported in refrigerated cars and ships to preserve the qualities and vitamin C content of the products.

If the concentrate is not filled hot after pasteurization, there is always a danger of fermentation with accompanying rapid swelling of the cans. According to investigations made at the U. S. Citrus Products Station Winter Haven, Florida,[1a] gas formation at 80 and 95° F (27 and 35° C) may be due either to fermentation or to chemical decomposition. At 120° F (49° C), however, the microorganisms disappear and gas formation in the swelled cans of concentrated juice appears to be due to chemical action only. As already mentioned, this gas formation which is not caused by fermentation is assumed to be due to the decomposition of ascorbic acid, giving as end products, furfural and carbon dioxide.

Concentrated citrus juices being, of course, hypertonic solutions

[1] Downer, A. W. E., "Preservation of Citrus Juices with Sulphurous Acid," *J. Soc. Chem. Ind.*, **62**, 124 (1943).

[1a] Curl, A. L., E. L. Moore, E. Wiederhold, and M. K. Veldhuis, "Concentrated Orange Juice Storage Studies with Particular Reference to the Development of Swells," *Fruit Products J.*, **26**, 101 (1946).

should prevent the development and growth of any yeasts or bacteria causing their plasmolysis (whereby water will pass out of the bacterial cells into the hypertonic solution causing the death of the cells). However, there are instances of osmophilic microorganisms which may have adapted themselves to these environments of con-

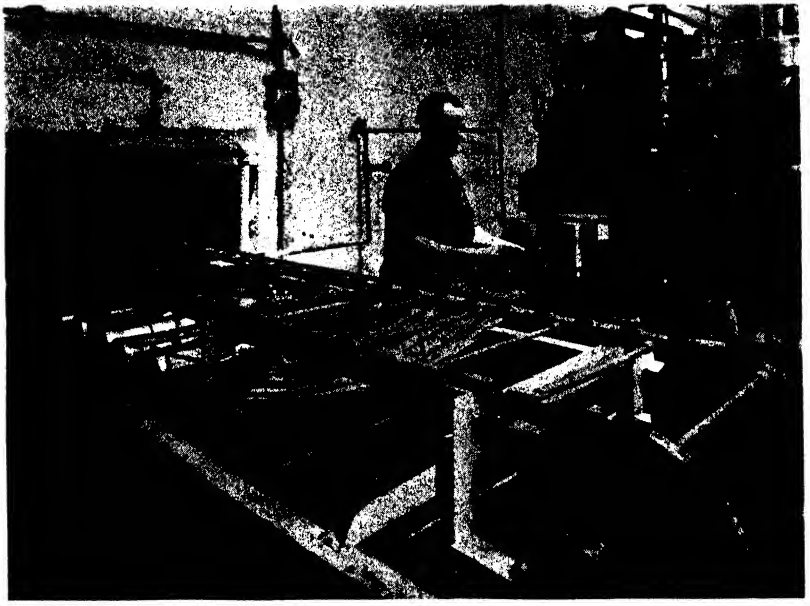


Fig. 90. Hot filling of concentrated juices: an automatic closing machine and cooling arrangement for concentrated orange juice. (Note the chain conveyor pushing the tins forward while the wire conveyor underneath, moving much faster, causes the tins to revolve continuously. Such an arrangement requires comparatively limited space.)

centrated solutions and may cause trouble. Ingram[1b] has isolated such osmophilic yeasts in concentrated American orange juice of 65° Brix.

7. Testing Concentrates

The determination of the degree of concentration of the product, indicating the proper moment at which concentration is to be terminated, is of primary importance. The most exact procedure would be to determine the specific gravity of the concentrate—a method, however, which is beset with difficulties because of the viscous nature

[1b] Ingram, M., *private communication,* Low Temperature Research Station, Cambridge, England.

of the end product. A simpler, although not absolutely exact, procedure is to determine the refractive index of the concentrate—a test which can be performed in less than a minute. The use of a pocket refractometer has previously been described. The same, or a similar refractometer, can be built in into the evaporator. The refractive index found is compared with the specific gravity (Table XXV). The table includes also the degree of concentration which, however, is specific for each country of origin and the season at which the original juice was squeezed; the figures should be adjusted accordingly. The table permits the estimation of the average degree of concentration to the nearest quarter when either the specific gravity or the refractive index is known.

When an exact determination is required the specific gravity is obtained with the aid of a pycnometer in the following way (the use of a hydrometer or a Westphal balance is usually impractical because of the high viscosity of the concentrates):

Weigh 20–25 g of the juice into a 150 ml conical flask fitted with a well-fitting rubber bung. Add a quantity of distilled water equal to three times the weight of the juice taken, and reweigh. Thoroughly mix the contents of the flask, agitating it by imparting a swirling motion, which minimizes the formation of air bubbles; then put it aside for one hour. Take a 50 ml specific gravity bottle, which has been standardized at 20° C, and fill it to the neck with the diluted juice, swirling the flask at intervals to insure that the proper proportion of cellular matter enters the specific gravity determination. Different quantities of juice and water may be taken according to the capacity of the apparatus in use in different laboratories, but it is always absolutely essential that the ratio of the quantities taken is, as nearly as possible, one part juice to three parts water by weight.

Calculation: Let

x = weight of concentrate originally weighed out (in g)
y = weight of water added (in g)
s = specific gravity of the diluted juice
b = Brix value of the diluted juice
B = Brix value of the original concentrate
S = specific gravity of the original concentrate, corresponding to Brix B

In Domke's table (Table XXVI) of apparent specific gravity of sucrose solutions at 20° C, find the degrees Brix (b) corresponding to s, interpolating so that the Brix value is expressed to two decimal places. The Brix value (B) of the original concentrate can be calculated from the equation:

$$B = \frac{b\ (x + y)}{x}$$

In Domke's table, look up the required specific gravity (S) of the original concentrate corresponding to Brix value B.

Besides specific gravity or degree of concentration, citrus concentrates may be tested for citric acid, vitamin C, amount of SO_2, etc.; these determinations have been described in previous sections.

TABLE XXV[2]

Degree of Concentration with Reference to Specific Gravity and Refractive Index

Degree of concentration*	LEMON		ORANGE			GRAPEFRUIT	
	Specific gravity	Refractive index	Specific gravity	Refractive index	Sucrose % (Brix)	Specific gravity	Refractive index
3	1.111–1.120	1.3681–1.3710	1.139–1.150	1.3816–1.3860	30.5–32.5	1.121–1.130	1.3756–1.3790
3.25	1.121–1.128	1.3711–1.3740	1.151–1.163	1.3861–1.3905	32.5–35.0	1.131–1.143	1.3791–1.3835
3.5	1.129–1.135	1.3741–1.3770	1.164–1.175	1.3906–1.3950	35.0–37.5	1.144–1.155	1.3836–1.3880
3.75	1.136–1.143	1.3771–1.3795	1.176–1.188	1.3951–1.3995	37.5–40.0	1.156–1.168	1.3881–1.3925
4	1.144–1.150	1.3796–1.3820	1.189–1.200	1.3996–1.4040	40.0–42.5	1.169–1.180	1.3926–1.3970
4.25	1.151–1.160	1.3821–1.3850	1.201–1.213	1.4041–1.4085	42.5–44.5	1.181–1.190	1.3971–1.4010
4.5	1.161–1.170	1.3851–1.3880	1.214–1.225	1.4086–1.4130	44.5–46.5	1.191–1.200	1.4011–1.4050
4.75	1.171–1.180	1.3881–1.3910	1.226–1.238	1.4131–1.4180	46.5–49.0	1.201–1.210	1.4051–1.4085
5	1.181–1.190	1.3911–1.3940	1.239–1.250	1.4181–1.4230	49.0–51.5	1.211–1.220	1.4086–1.4120
5.25	1.191–1.200	1.3941–1.3975	1.251–1.262	1.4231–1.4280	51.5–53.7	1.221–1.230	1.4121–1.4160
5.5	1.201–1.210	1.3976–1.4010	1.264–1.275	1.4281–1.4330	53.7–56.0	1.231–1.240	1.4161–1.4200
5.75	1.211–1.220	1.4011–1.4040	1.276–1.288	1.4331–1.4380	56.0–58.7	1.241–1.250	1.4201–1.4235
6	1.221–1.230	1.4041–1.4070	1.289–1.300	1.4381–1.4430	58.7–60.5	1.251–1.260	1.4236–1.4270
6.25	1.231–1.238	1.4071–1.4095	1.301–1.313	1.4431–1.4475	60.5–62.5	1.261–1.270	1.4271–1.4310
6.5	1.239–1.245	1.4096–1.4120	1.314–1.325	1.4476–1.4520	62.5–64.5	1.271–1.280	1.4311–1.4350
6.75	1.246–1.253	1.4121–1.4148	1.326–1.338	1.4521–1.4565	64.5–66.4	1.281–1.290	1.4351–1.4390
7	1.254–1.260	1.4149–1.4175	1.339–1.350	1.4566–1.4610	66.4–68.3	1.291–1.300	1.4391–1.4430
7.25	1.261–1.270	1.4176–1.4208	1.351–1.360	1.4611–1.4650	68.3–70.0	1.301–1.313	1.4431–1.4475
6.5	1.271–1.280	1.4209–1.4240	1.361–1.370	1.4651–1.4690	70.0–71.5	1.314–1.325	1.4476–1.4520
7.75	1.281–1.290	1.4241–1.4270	1.371–1.380	1.4691–1.4735	71.5–73.5	1.326–1.338	1.4521–1.4565
8	1.291–1.300	1.4271–1.4300	1.381–1.390	1.4736–1.4780	73.5–75.2	1.339–1.350	1.4566–1.4610

* The degree of concentration is indicated for ranges in specific gravity (at 20°/20°C) or refractive index (at 20°C).

[2] Report from the Panel of Chemists Concerning the Estimation of Concentration of Citrus Juices, S. G. Kendrick, Chairman. Issued under the authority of the Ministry of Food, London.

TABLE XXVI

Domke's Table of Apparent Specific Gravity* of Sucrose (Cane Sugar)

Bé	Specific gravity	Per cent $C_{12}H_{22}O_{11}$ (Brix)	G per liter	Bé	Specific gravity	Per cent $C_{12}H_{22}O_{11}$ (Brix)	G per liter
—	0.9982	0	—	24.4	1.2025	45	541.1
0.3	1.0021	1	10.02	25.0	1.2079	46	555.6
0.9	1.0060	2	20.12	25.5	1.2132	47	570.2
1.4	1.0099	3	30.30	26.0	1.2186	48	584.9
2.0	1.0139	4	40.56	26.5	1.2241	49	599.8
2.5	1.0179	5	50.89	27.1	1.2296	50	614.8
3.1	1.0219	6	61.31	27.6	1.2351	51	629.9
3.6	1.0259	7	71.81	28.1	1.2406	52	645.1
4.1	1.0299	8	82.40	28.7	1.2462	53	660.5
4.7	1.0340	9	93.06	29.2	1.2519	54	676.0
5.3	1.0381	10	103.8	29.7	1.2575	55	691.6
5.8	1.0423	11	114.7	30.3	1.2632	56	707.4
6.4	1.0465	12	125.6	30.8	1.2690	57	723.3
7.0	1.0507	13	136.6	31.3	1.2748	58	739.4
7.5	1.0549	14	147.7	31.8	1.2806	59	755.6
8.1	1.0592	15	158.9	32.3	1.2865	60	771.9
8.7	1.0635	16	170.2	32.8	1.2924	61	788.3
9.2	1.0678	17	181.5	33.4	1.2983	62	804.9
9.8	1.0721	18	193.0	33.9	1.3043	63	821.7
10.3	1.0765	19	204.5	34.4	1.3103	64	838.6
10.8	1.0810	20	216.2	34.8	1.3163	65	855.6
11.4	1.0854	21	227.9	35.4	1.3224	66	872.8
12.0	1.0899	22	239.8	35.9	1.3286	67	890.1
12.5	1.0944	23	251.7	36.4	1.3347	68	907.6
13.1	1.0990	24	263.8	36.9	1.3409	69	925.2
13.6	1.1036	25	275.9	37.4	1.3472	70	943.0
14.2	1.1082	26	288.1	37.9	1.3535	71	961.0
14.7	1.1128	27	300.5	38.4	1.3598	72	979.0
15.3	1.1175	28	312.9	38.9	1.3661	73	997.3
15.8	1.1222	29	325.4	39.4	1.3725	74	1016.
16.3	1.1270	30	338.1	39.9	1.3790	75	1034.
16.9	1.1318	31	350.8	40.4	1.3854	76	1053.
17.5	1.1366	32	363.7	40.9	1.3920	77	1072.
18.0	1.1415	33	376.7	41.4	1.3985	78	1091.
18.6	1.1463	34	389.8	41.8	1.4051	79	1110.
19.1	1.1513	35	402.9	42.2	1.4117	80	1129.
19.6	1.1562	36	416.2	42.7	1.4184	81	1149.
20.1	1.1612	37	429.7	43.2	1.4251	82	1169.
20.7	1.1663	38	443.2	43.7	1.4318	83	1188.
21.2	1.1713	39	456.8	44.2	1.4386	84	1208.
21.7	1.1764	40	470.6	44.7	1.4454	85	1229.
22.3	1.1816	41	484.5	45.2	1.4522	86	1249.
22.8	1.1868	42	498.4	45.6	1.4591	87	1269.
23.3	1.1920	43	512.6	46.1	1.4660	88	1290.
23.8	1.1972	44	526.8	46.6	1.4730	89	1311.

*Specific gravity of aqueous sugar solutions at 20°/4° C.

It must be remembered that unless the concentrates are kept in cold storage (at 0° C or slightly above) their vitamin C content rapidly decreases, as shown in Table XXVII, derived from tests performed by Dr. F. Stern of Jaf-Ora, Ltd., Rehovoth, Israel.

TABLE XXVII

Vitamin C Content in Stored Concentrated Orange Juice (Pasteurized)

After months	BOTTLED				CANNED			
	Ordinary temp.		Cold storage		Ordinary temp.		Cold storage	
	Vit. C	Acidity	Vit. C	Acidity	Vit. C	Total solids	Vit. C	Total solids
0	244	6.16	253	6.30	233		253	
1	223	6.18	211	6.30	230		250	
2	197	6.16	211	6.24	217		240	61.4
3	203	6.16	207	6.24	194	61.2	232	
4	140 } deterio-		203	6.18	161		208	61.4
5	148 } rated		210	6.06	154	61.5	203	62.0
6	148		231	6.18	148		211	61.4
8			211	6.18	127	62.1	194	61.4
10			151	6.00				
			very good flavor and bright color		deteriorated; metallic taste		color bright, taste good	

Table XXVII shows that the vitamin C content of concentrates kept in cold storage is still high after 10 months storage, while at ordinary temperatures a marked reduction is already noticeable after 3 months. It is noteworthy that a slight reduction in acidity is discernible on prolonged storage.

8. Powdered Citrus Juices

In addition to vacuum concentration numerous attempts have been made to remove the entire moisture content from citrus juices, leaving only the dry material in the form of a powder. It was thought that instantaneous desiccation would prevent material changes in the quality of the juice. Thus, citrus juices have been atomized in a current of heated air or inert gas and the resulting finely divided, suspended, solid particles have been collected by settling, filtration, or electrical precipitation. The whole process is very similar to that employed in the manufacture of milk powder. Figure 91 shows schematically a spray-drying procedure described by Merrell,[3] where the final product is collected by an endless belt. As the resulting product tended to coalesce into slimy hygroscopic masses, many attempts have been made to overcome this difficulty by adding various drying promoters, such as starch, malt extract, glucose, lactose,[4] small amounts of water-soluble gums,[5] etc.

[3] Merrell, J. S., "Process of Treating Juice and Product," U.S. Patent No. 1,398,080 (Nov. 22, 1921).

[4] Jameson, E., E. D. Stewart, and C. P. Wilson, "Food Product and Process of Making the Same," U.S. Patent No. 1,810,276 (June 16, 1931).

[5] Böhm Börnegg, C. von., "Method of Drying Fruit Juices," U.S. Patent No. 1,800,501 (Apr. 14, 1931).

Instead of atomizing the juice into a stream of hot gas, a spray-processing system could be applied in which the atomized juice is preheated and sprayed into the chamber where precooled gas at low temperature circulates. From 15 to 30 seconds is a fair estimate of the time juice particles stay in the spray-drying (or the spray-cooling) chamber when passing from liquid to solid form.[5a] This short period required for drying makes this process particularly adapted to the drying of heat-sensitive materials.

Recently Leo and Taylor[6] patented a process by which citrus juices are first freed of suspended solids by centrifuging, heated for 5 min at

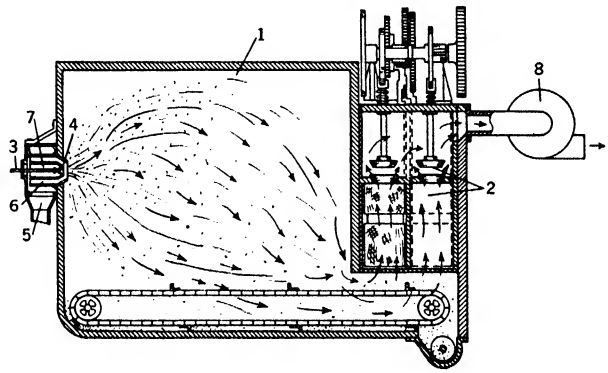


Fig. 91. Schematic drawing of spray drying:
1, drying chamber; 2, vacuum pump; 3 to 7, spray nozzle; 8, exhauster.

200° F (93° C) to inactivate all enzymes, cooled, and concentrated in vacuo. To this concentrate dextrinized glucose in the form of corn syrup is added, the mixture being spray-dried. The resulting white powder is claimed to redissolve easily in water to form a cloudy beverage closely resembling the original product.

Although considerable experimentation has been undertaken in this direction and in some instances has even attained commercial production on a limited scale, there is hardly any future in the process, for the resulting dry powder, upon reconstitution, could hardly be acknowledged as citrus juice, having also lost most of its flavor and its vitamin potency.

Another disadvantage of spray-drying in comparison to other drying processes, such as drum-drying, is the fact that the resulting product is more bulky due to the hollow form of the spray-dried particles. The necessary addition of conditioners, such as mentioned

[5a] Fogler, B. B., and R. V. Kleinschmidt, "Spray Drying," *Ind. Eng. Chem.*, **30**, 1370 (1938).

[6] Leo, H. T., and C. C. Taylor, "Citrus Juice Powder," U.S. Patent No. 2,367,-131 (Jan. 9, 1945).

above, still further unnecessarily augments the volume of the finished product. A sample of such orange powder described recently[6a] contains: 25% citrus solids, 75% corn syrup solids, 0.27% citric acid, and only 15 mg ascorbic acid per 100 gm.

Additional novel methods of concentration based on drying citrus juices from the frozen state are described in the last chapter.

B. APPLICATION OF COLD

1. Alternative Methods of Freezing

An ideal way of preserving fruit juices would seem to be to freeze them below zero and to store the frozen product in cold storage. Some fruit juices, such as apple or berry juices, freeze particularly well, preserving their fresh aroma and flavor, but this method applied to citrus juices has encountered many technical as well as commercial difficulties. Large-scale experiments in freezing orange juice have been undertaken in the United States by strong industrial concerns which finally abandoned the process, apparently with considerable financial loss.

Although rapid strides have been made during the last decade in the technical development of the frozen-food industry, many difficulties must still be overcome from both the theoretical standpoint and from the equally important point of view of distribution and consumption.

The application of cold for preserving citrus juices is considered from two aspects—namely, freezing the juices in their original form, and concentrating the citrus juices by freezing out the water.

2. Frozen Citrus Juices

(a) Microbiology of Frozen Juices

It was formerly assumed that keeping juices at low temperatures would cause the absolute destruction of all microorganisms. However, a number of investigators have reported that certain psychrophilic bacteria, certain molds, and Torula yeasts might survive and grow even at very low temperatures (—10 to —20° C).

Pathogenic bacteria are markedly reduced in numbers when frozen in ice, but as James[7] has shown, not all the cells are killed and sub-

[6a] Holzcker, R., "Two Methods for Dehydrating Citrus Juices," *Food Ind.*, August, 1943, p. 62.

[7] James, L. H., "The Microbiology of Frozen Foods," *Fruit Prod. J.*, 12, 110 (1932).

cultures from defrosted spores are as toxigenic as they were before being frozen. Acid media such as citrus juices produce a greater killing effect during freezing than alkaline media.

In other words freezing kills many, but not all, microorganisms. In fact some reports show that molds, yeasts, and bacteria, if previously present in the juices, are preserved by the cold; when the frozen products are thawed and the temperature rises, the microorganisms begin renewed activity. Citrus juices prepared for freezing have, therefore, to be sterile *before* being frozen.

(b) Physical and Chemical Changes

Other very deep changes occur during freezing or after thawing which affect the quality of frozen citrus juices. After thawing, juices which have been frozen are usually quite different in texture and general appearance compared with fresh juices. This "breakdown" or flabbiness of the texture was originally believed to be caused by mechanical rupture of the plant cells due to the increased volume of the ice formed during the freezing process. However, it was later acknowledged that the water freezes not in the cell proper but in the intercellular spaces; thus, the cells are only pushed apart and this separation alone would not be sufficient to cause the breakdown of the cells.

Although Chandler[8] made the statement, in 1932, that he knew of no experience in freezing plants that could not be explained by the theory that death is due to pressure against the protoplasm by ice masses, the general opinion prevailing now is that due to the freezing temperature most of the colloid matter of the cell protoplasm irreversibly precipitates, liberating the so-called "bound water." This water is not reabsorbed by the colloids on thawing, thus causing the death of the cell. The irreversible precipitation of the cell colloids is differently explained by various investigators; some attribute it to the salting-out of the protein fraction of the protoplasm after losing its water, due to the high salt concentration; others believe the effect is due to an increased hydrogen-ion concentration, or to the increased viscosity.

(c) Quick Freezing

It has been found that the more rapidly the juices are frozen the smaller are the ice crystals formed, causing less damage to the frozen

[8] Chandler, W. H., "How Freezing Kills Plants or Plant Parts," *Fruit Prod. J.*, **12**, 50 (1932).

products. Increasing the rate of freezing can be attained by using, for instance, solid CO_2 rather than air at 0° F. Quick freezing leaves less time for the diffusion of salts in the cell and the separation of bound water; it also prevents the development of microorganisms (if they are present in the juice) and retards the action of enzymes. The temperature zone for crystal formation, according to Birdseye,[9] is between —0.6 and —3.9° C; the food should be passed through this zone as quickly as possible to the lower temperatures. Physical chemists define quick freezing as the "zone of maximum crystal formation." Hence, quick-freezing methods seem to be much more advantageous than slow freezing. Joslyn and Marsh[10] failed, however, to disclose any real advantages of the quick-freezing method over the slow one.

(d) Enzymatic Changes

Apart from the physical and chemical changes mentioned in preceding paragraphs, many changes are induced by enzymes which continue their action on the frozen products even at a low temperature. Although the action of enzymes is greatly reduced at temperatures below zero, there is ample evidence that oxidative enzymes such as catalase, pectic enzymes, and also invertase are still active under these conditions.

This phenomenon has already been touched upon earlier in the section on enzymes. It has been customary to assume that the disruption of the cells during freezing and subsequent thawing permits better access of enzyme to substrate. Although such mixing doubtless occurs, the fact is that juices are more apt to disintegrate after prolonged storage than after short storage, notwithstanding the fact that in both cases freezing and thawing were effected in the same manner. This leads to the belief that at low temperature enzymatic activity goes through its first phase of attacking the substrate; when the frozen juice is thawed the final stage of enzymatic action proceeds at a quick pace.

The addition of sugar syrup to sliced fruit, for some reason not fully understood, greatly retards oxidation during freezing. But darkening of frozen juices kept in cold storage over long periods of time may not always be prevented even when airtight containers have been used and the juices have been filled under vacuum.

To prevent the action of the oxidative enzymes it is necessary to

[9] Birdseye, C., "Some Scientific Aspects of Packaging and Quick Freezing Perishable Flesh Products," *Ind. Eng. Chem.*, **21**, 414 (1929).

[10] Joslyn, M. A., and G. L. Marsh, "Observations on the Effect of Rate of Freezing on the Texture of Certain Fruits and Vegetables," *Fruit. Prod. J.*, **11**, 327 (1931).

deaerate the juice as completely as possible, as described in the previous chapter. Subsequent flash-pasteurization inactivates the pectic enzymes and eliminates their influence during freezing.

It is evident, therefore, that citrus juices must be deaerated, flash-pasteurized, and, in general, be treated with the utmost care before freezing, just as in any other method of preservation. Nevertheless, some changes in color, flavor, texture, and odor will occur notwithstanding the frozen state. These considerations coupled with the difficulties of storage, transportation, and thawing do not afford frozen citrus juices much preference over juices preserved by other methods.

(e) Technical Methods of Freezing

Tressler and Evers[11] have described in detail the methods used by various firms in California and Florida to freeze orange juice. All three existing methods of quick freezing have been used with orange juice:

(1) FREEZING BY DIRECT IMMERSION IN BRINE

Paraffin-waxed cardboard containers (Sealcones) are cooled to about 40° F and subsequently frozen in $CaCl_2$ brine at 0° F by immersion, using a conveyor onto which the paper bottles are hooked. The total freezing time is 40 min ("Inver" freezer). One plant, using the same method, fills the strained juice in one-gallon cans, vacuum-seals them, and conveys them through a brine tank by means of a roller conveyor. Six hours are required for freezing.

(2) FREEZING IN A BLAST OF COLD AIR

Orange juice is frozen to a slush under vacuum in vertical direct-expansion ice-cream freezers, the slush being forced by compressed inert gas (nitrogen) into wax-lined cardboard containers or 30-lb enameled tin cans and the freezing completed in a blast of cold air at a temperature of about —10° F. This method takes only 6 min.

(3) FREEZING BY INDIRECT CONTACT WITH REFRIGERANT

This rather old method has been used in ice making and in fish freezing. Orange juice has been frozen by a similar process in a tubular machine patented by Finnegan in 1933.[12] The juice is placed in so-called cartridges which are inserted into guide tubes. The cool-

[11] See bibliography at the end of this chapter.

[12] Finnegan, W. J., "Method and Apparatus for Rapid Freezing and Handling of Comestibles," U.S. Patent No. 1,925,033 (Aug. 29, 1933).

ing is effected by a quick turbulent motion of the refrigerating liquid through the space between the adjacent walls of the cartridge and the guide tube.

Recently a firm in Detroit, Michigan (Pure Fruit Juices, Inc.), has marketed frozen orange juice in enameled cans, previously deaerated by a specially patented "Vaporlokt" process, and quickly congealed in the Finnegan tubular freezer. The whole operation is described in detail by the inventor.[13]

To allow for the expansion which occurs when the juices are frozen, it is necessary to leave in all containers a headspace of about 10% of the total volume. According to Shrader and Johnson,[14] orange juice expands 7.5% on freezing.

(f) Cool Storage

After being frozen by one of the foregoing methods the juice is stored at 0 to +10° F.

Most up-to-date citrus-products manufacturers store their products, particularly canned juices, concentrates, oils, and the like, in refrigerated rooms. Any standard refrigerating unit using compressible gases, such as anhydrous ammonia, methyl chloride, freon, or sulfur dioxide, may be used in the storage rooms.

When the juices are kept in large tanks they must be chilled before being run into the tanks. The juices may be precooled by Baudelot coolers or similar equipment, consisting of tubular heat interchangers, or the tanks may be refrigerated by interior coils. It is sufficient to bring the juices to a state of frozen slush; this preserves them quite satisfactorily for several months. Satisfactory preservation at ordinary cold-storage temperature of about +5° C, with no loss in vitamin C content, may be achieved by the addition of very small amounts of preservatives such as SO_2 (about 200 to 300 ppm).

Camp and Stahle,[15] investigating the methods of handling orange juice, suggest that milk-distributing companies in large cities should establish central juice-extraction plants, keep the juice in cold storage at 50° F, and distribute it in milk bottles. They claim that for temperatures below 50° F, 72 hours can be considered a safe period for storage and handling of a nonpreserved orange juice which will

[13] Finnegan, W. J., "Freezing Fruit Juices in Cans," *Fruit Prod. J.*, **20**, 141 (1941).
[14] Shrader, J. H., and A. H. Johnson, "Freezing Orange Juice," *Ind. Eng. Chem.*, **26**, 869 (1934).
[15] Camp, A. F., and A. L. Stahl, "Cool Storage Methods of Handling Orange Juice," *Fruit Prod. J.*, **13**, 361 (1934).

be as near to the freshly expressed juice in flavor and appearance as possible.

Recently considerable progress has been made in manufacturing eutectic ice—i.e., a frozen salt solution with 23.3% NaCl and 76.7% H_2O—which melts at a uniform temperature of —6° F. Eutectic ice is manufactured in strips; it can be economically used for refrigerating or storing cold-packaged citrus juices. In fact 3 kg of eutectic ice do the work of 1 kg of Dry Ice (solid CO_2) and can be manufactured at only one-tenth the price.

3. Concentration by Freezing

Since time immemorial it has been known that when a solution of salt is congealed, the water constituting the solvent is frozen first into ice, while the salt remains in solution, thus making the residual brine more concentrated. This fact was made use of in preparing kitchen salt from sea water in northern countries. With the introduction of artificial refrigeration, concentration by means of freezing out the water was applied to fruit juices. The method seemed to be ideal and in every respect preferable to evaporation since it left the concentrated juice in a state of unimpaired aroma and flavor. The first to advance this idea and to experiment with it on a commercial scale was Professor Eudo Monti in Italy at the beginning of the present century.

When water is evaporated it is converted into a vapor phase which requires an energy of 560 calories per liter, but when the same quantity of water is converted into ice only 80 calories are required. This saving of energy would in itself give concentration by freezing a substantial advantage over the evaporation method. Furthermore, the flavor of all fruit juices is largely due to very minute quantities of volatile substances which are destroyed or distilled over during evaporation; this loss can be easily avoided during freezing. Similar considerations apply also to the formation of foam, which takes place in most juices when heat is applied during concentration.

However, concentration by freezing has met with difficulties of its own. The foremost obstacle is the mechanical separation of the ice crystals from the concentrated liquid phase. The simple method of draining the liquid away from the ice crystals results in heavy losses of the valuable concentrated juice, which still adheres to the crystals. A further difficulty, which sometimes makes the separation quite impossible, is due to the solid pulp particles.

(a) Monti Method

Monti,[16] who first experimented with grape juice and later tried his process on lemon juice, froze the juices to a solid ice block and let the concentrated solution drain slowly from the block. He then "washed" away the concentrate from the ice by fresh juice and finally rinsed the remaining ice crystals with pure chilled water.

As one freezing did not bring the required degree of concentration the juice was frozen in two or three stages, each time successively using less-enriched juices for the rinsing. Extraction of the soluble substances interposed between the ice crystals by displacement and progressive extraction with solutions of increasing degree of dilution obtained by the fractional freezing of a previous operation is the distinguishing characteristic of the Monti process, patented in Italy in 1906.[17]

By pushing the congelation too far Monti experienced difficulties in displacing the concentrated liquids, due to the diminishing permeability of the frozen mass, the dissolved substances being precipitated or crystallized in the interpolated water. According to Monti the limit of cooling for juices is, therefore, about twice as low as their freezing temperature but not more than the point of congelation of the saturated solution.

Attempts have been made to make the concentration-by-freezing process continuous. La Cauza[18] patented a receptacle in which juices were subjected to refrigeration, forming an ice which deposited on the walls. The concentrated solution collected at the central portion of the receptacle, whence it was continuously drawn off. The receptacle consisted of star-shaped moulds.

(b) Gore Method

Instead of letting the concentrated liquids drain, Gore,[19] working originally on cider, suggested freezing the juices in plain ice cans at a temperature of 10 to 15° F, breaking up the ice blocks in a crusher, and centrifuging the resulting mush of crystals in a basket centrifuge.

[16] Monti, Eudo, "Sulla concentrazione a freddo del succo di limoni e d'altra frutta," *Citrus*, 14, 47, 134 (Feb., 1928).

[17] Monti, Eudo, "The Concentration of Lemon and Other Juices by Freezing," U.S. Patent No. 1,158,261 (Oct. 26, 1915).

[18] La Cauza, Giuseppe, "An Improved Process and Apparatus for the Concentration of Juices, etc.," British Patent No. 316,167 (July 23, 1929).

[19] Gore, H. G., "Apple Syrup and Concentrated Cider," *U.S. Dept. of Agriculture Yearbook No. 639*, 227 (1914).

The centrifuge is revolved at moderate speed; the concentrated liquid phase is thrown by centrifugal force through the perforations of the basket and collected separately, while the fairly pure ice remains inside the centrifuge. Two subsequent freezings are necessary at a still lower temperature (0 to 5° F) to obtain a concentrate of 50° Balling.

These rather crude methods have been further fully studied by T. N. Morris[20] of the Low Temperature Research Station of Cambridge who published the results of a series of experiments on the concentration of orange juice by freezing and the storage of the obtained product. Morris found that applying quick freezing resulted in the formation of smaller ice crystals, which tended to retain the concentrate to a greater degree than the large ice crystals formed during slow freezing. He therefore applied quick freezing in a brine at —28° C only up to the moment when ice crystals began to appear; then he "tempered" the juice at a higher temperature (—10° C) with intermittent stirring until sufficient crystals have formed. Centrifuging was performed in baskets with holes 2 mm in diameter. In this case, also, two stages of freezing were necessary, the first giving a concentrate of about 25–30% total solids, and the second freezing and centrifuging a concentrate of 45% total solids. Exactly the same results have recently been confirmed by Stahl.[21]

(c) Krause Method

The most successful method of concentration by freezing has been developed by Krause of Munich; the main feature of his system is to freeze the juice so as to facilitate the separation of the liquid phase from the crystals.

In all other methods the ice crystals are left to form in any haphazard way, but Krause has ingeniously made use of the fact that in circular freezing vessels the ice crystals form radially from the center to the periphery, thus determining the orientation of the crystals formed and facilitating the expulsion of the liquid phase during subsequent centrifuging.

Two plants, actually using the Krause system on a commercial scale, fill the fruit juices to be concentrated in large cells, each of a capacity of about 250 liters. Each cell, exactly equal in shape to a

[20] Morris, T. N., "Concentration of Orange Juice," *Dept. Scientific and Industrial Research, Dept. of Food Investigation Board,* **95** (1932), and **161** (1933).

[21] Stahl, A. L., "Concentration of Citrus Juice by Freezing," *Citrus Ind.*, **25** (No. 9), 5 (1944).

centrifugal basket, represents an annular ring which tapers slightly from top to bottom to facilitate the withdrawal of the ice block. A framework made of aluminum or stainless steel is immersed into the juice to provide a means of holding the ice block when it is transferred to and from the centrifuge.

The cells, after being filled with juice, are immersed into the freezing tanks where the brine circulates outside and inside the annular ring, thus insuring an evenly distributed temperature. Under these conditions the ice crystals practically all grow in a horizontal direction and along the radial gradient of the ring. After remaining in the freezing tanks for two hours the cells are transferred into other tanks, where they are left at slightly higher temperatures to temper for one hour, thus receiving an even temperature throughout the whole frozen mass. In order to remove the ice block, the cell is plunged into warm water for a few seconds. By the aid of the framework the block is then easily transferred to the centrifugal basket and is spun at moderate speed until the whole of the concentrate is released from the ice crystals. Provided the liquid phase is not too concentrated the separation under these conditions is very easy, because the radial interstices formed in the ice crystals have the same direction as the centrigual force. The ice block is then washed with juice and later with chilled water from previous operations, as already described. The procedure is continued in two or three stages according to the desired strength of the concentrate. It is claimed that by using this method the losses do not exceed 2% of the original juice.

In further developing this method Krause introduced special machinery by which the process became more or less continuous. The ice cake in this patented machine is formed as a wide continuous layer.[21a]

(d) Storage of Concentrates. Recent Developments

Since fermentation of juices can be inhibited only if total solids content is increased to about 65%, it is obvious that the concentrates obtained by the methods described above must be stored in cold or be treated by the addition of chemical preservatives. Keeping them at room temperatures will undoubtedly cause spoilage. Morris, however, found that even when concentrates are stored at low temperatures their keeping qualities are not entirely satisfactory, for some enzymatic changes still go on, if the products are kept for some

[21a] Krause, G. A., "Method of Concentration," U.S. Patent No. 2,248,634 (July 8, 1941).

months. It is obvious, therefore, that deaeration of the juice before concentration by freezing is an absolute necessity, although there is no doubt that the product obtained is far superior in quality to any concentrates obtained by evaporation.

To obtain a greater concentration, Grove[22] combined the freezing method with a final evaporation in vacuo to the desired density. Recent developments in this direction have been introduced in the United States where the citrus juices have been concentrated by freezing and finally have been dehydrated into a powder at a very low temperature and in vacuo. These methods are still in the experimental stage and have not reached commercial production.

A new method based on both concentration in vacuo and subsequent freezing is now in process of commercial development in Florida. It consists in concentrating orange juice under a vacuum, somewhat higher than commonly used, up to 65° Brix and then adding to it fresh single-strength juice until a consistency of about 40° Brix is reached. The addition of fresh juice is made in order to improve the flavor of the concentrate. The resulting mixture is then frozen to a slush in a continuous freezer, filled into tin containers, and stored at 0° F (—15° C). The product is reconstituted by adding three parts of water to one of frozen concentrate and is difficult to distinguish from fresh juice. This method eliminates the difficulties of reconstitution by thawing usually met in ordinary frozen juice.

Mention should be made of a recent new application of cold to grapefruit sections. The methods for canning grapefruit sections are described further in this book. However, since the 1944 season in Florida, grapefruit "hearts" instead of being canned are placed in lined, waxed-paper cartons, a 40% sugar syrup is added, the liner is sealed, and the whole is frozen rapidly. In some instances antioxidants are added to preserve the vitamin C potency and to prevent the development of off-flavors during storage.

It is claimed that a mixture of ascorbic acid (4%) and citric acid (96%) prevents browning of cut or frozen fruits owing to the combination of the enzyme-inactivating effect of the citric acid with the antioxidant effect of ascorbic acid. Caffeic acid ethyl ester is also used as a stabilizing agent for frozen grapefruit sections.[22a]

Freezer storage is of course essential for this product.

[22] Grove, O., *Ann Rep. Agr. and Hortic. Res. Sta., Long Ashton, Bristol,* **209** (1930).

[22a] Kremers, R. E., "How the Organic Chemist Can Best Serve the Food Field," *Food Ind.,* **19,** 91 (1947).

4. Summary

In summarizing the results obtained so far by the application of cold to the conservation of citrus juices, it is evident that the greatest difficulty associated with frozen juices lies in the method of thawing them, which must be quick enough to present the housewife with a product ready for use. Although the best method of preservation of the aroma and flavor seems to be concentration by freezing, here one encounters the insurmountable difficulty involved in separating the heavy concentrate from the ice in its last stages, a step which is attended by great loss. One may, therefore, safely suggest that concentration by freezing to a degree of probably 3:1 or 30% total solids, storing the concentrate, delivering it in a *frozen state,* and attaining quick and perfect thawing simply by adding the required amount of water to bring the juice to its natural strength, is probably the best and most adequate method known at present, provided that general safeguards as to selection of fruit, careful handling, and deaeration are strictly adhered to.

Very valuable research on every phase of refrigeration is still going on in Cambridge at the Low Temperature Research Station and in numerous laboratories of the United States Department of Agriculture, such as the Frozen Pack Laboratory at Seattle, Washington, and others.

C. SWEETENED JUICES

1. Syrups or Squashes

Pasteurized, frozen, or canned juices, which have been discussed in previous sections, are used in their original form for consumption as straight juice; most of the preserved or concentrated citrus juices, however, serve as a raw material for further treatment. They are used mainly for the manufacture of so-called syrups or "squashes," or "dairy-base drinks." All of them consist, in the main, of varied mixtures of citrus juices with pure cane sugar or cane-sugar syrups, and with additional flavoring ingredients such as citric acid, essential oils or essences, coloring matter, etc. These syrups are either bottled and marketed as such for home use or are sold in bulk as "Bottlers' Base" to manufacturers of soft drinks—orangeades, lemonades, etc.

There are probably as many formulas for the manufacture of syrups as there are manufacturers; each varies the proportion of the ingredients according to the specific requirements and generally accepted

taste of his consumers. On the whole, however, there are two methods of preparation—the cold and the hot.

According to the cold method, natural raw citrus juices are mixed with about equal weights of pure cane sugar to make a 50% syrup (by weight). It should be noted that sugar when dissolved occupies, on the average, a volume equal to only 0.6 of its original weight (using metric units), i.e., one kg of sugar when dissolved occupies a volume of only 600 cc, or in other words when one kg of sugar is dissolved in one liter of juice the whole will occupy, at ordinary temperature, only 1.6 liter (1600 cc). To this mixture of sugar and citrus juice some citric or tartaric acid is added, if necessary, to improve the flavor. Very good results are obtained by blending various citrus juices in suitable proportions, such as orange juice with a little lemon or 2/3 of orange with 1/3 of grapefruit juice. As additional flavoring, essential oils of the peel are used, having previously been emulsified with some juice or with a pectin solution (which serves as a powerful emulsifier). Although syrups freshly made in this way possess a pleasant flavor, they tend to acquire on storage a "terpenized" off-flavor. A much better way of fortifying the flavor of squashes is to add citrus essences, i.e., either partially or wholly deterpenized oils dissolved in 80% alcohol or in glycols. Lemon and grapefruit juices, on the whole, conserve their original specific flavor better than orange juice and require less fortification. Since all squashes or syrups used at home or by bottlers are diluted with water before they are consumed, they are usually slightly colored by permissible food colors (Orange I, Sunset Yellow, Ponceau 3F, or Naphthol Yellow S dyes).

According to the hot method, a strong sugar syrup is prepared by first dissolving sugar in hot water and then, after cooling, by adding the natural juices or the concentrates. In squashes prepared in this manner the proportion of sugar by weight can be as high as 65% or more, and the amount of juice is reduced to only 35%. It is evident that squashes made entirely in the cold will better retain their fresh aroma and flavor.

The juices used in both the cold and the hot processes may contain their full proportion of unbroken fruit cells or, as is sometimes desired, the juice is previously passed through a colloidal mill to reduce the large cells to only finely suspended matter. Unless the original juice is flash-pasteurized to inhibit the action of pectic enzymes, the suspended matter will ultimately settle in the container with the clear supernatant liquid above—a very undesirable feature of most squashes. Even if the juice is flash-pasteurized the inhibition of the enzymes is temporary and will last for only a few months.

Squashes bottled directly may be pasteurized again, or else chemical preservatives such as SO_2 or sodium benzoate should be added to prevent fermentation. The British public health laws permit as much as 350 ppm of SO_2 or 600 ppm of sodium benzoate in final squash, but the presence of both these preservatives at the same time is not allowed. The United States pure food laws permit one-tenth of 1% of sodium benzoate (i.e., 1000 ppm), but require an explicit declaration to this effect upon the label. When the squashes are intended as a base for soft drinks and are packed in bulk, either in large glass carboys or in barrels, additional chemical preservatives may be added to correspond to the requirements of preserving agents in the final drinks.

Although the addition of sugar greatly decreases the tendency of fruit juices to darken, this browning effect, fully described in previous sections, can be eliminated by preserving the squashes with SO_2 rather than with benzoates, or by pasteurization, for browning is largely a result of oxidation and SO_2 is a very strong antioxidant. Furthermore, sulfur dioxide prevents to a large extent the destruction of vitamin C, which is an important factor in citrus squashes, although the amount of vitamin C consumed in a greatly diluted state in one drink is rather small.

Citrus squashes when properly preserved are, therefore, well suited for fortification with additional ascorbic acid (vitamin C). As shown by Downer,[23] when 80 mg of ascorbic acid are added to each 100 cc of grapefruit squash or syrup only 20 to 30% is lost in 14 weeks, even if stored at room temperature.

2. Cordials

Citrus juices and squashes are now generally accepted if they contain fruit cells which give them a natural appearance. An exception is lime juice (and sometimes also lemon juice), which is marketed in a clear filtered form. Filtered fruit juices are known in the trade as "cordials."

To obtain a clear juice without any cells or detritus whatever, the raw juices may be preserved by chemicals (especially by SO_2), stored in bulk, and the whole allowed to settle for several weeks. The supernatant liquid may then be decanted and filtered through a neutral filtering medium (such as Filter-Cel or pure asbestos). However, if the juice to be clarified is not to be preserved by chemicals, clarification

[23] Downer, A. W. E., "The Stability of Ascorbic Acid in Citrus Fruit Juice Products," *J. Soc. Chem. Ind.*, **61**, 80 (1942).

should, of course, take place immediately after extraction and for this purpose special methods have to be employed. It is evident that in such cases flash-pasteurization, which inhibits the action of pectic enzymes, is out of the question. On the contrary, a mixture of pectic enzymes is added to the juice to accelerate the destruction of pectin, which holds the fruit cells in suspension and which in itself gives the juice a colloidal appearance. A commercial preparation manufactured by Rohm and Haas, Philadelphia, and marketed under the name Pectinol is very well designed for clarification of fruit juices. Pectinol has been found to contain over 90% sugars (mainly dextrose and levulose), the rest being a mixture of pectic enzymes. Fish and Dustman,[24] who prepared a sugar-free enzyme concentrate from Pectinol by extracting the sugars with cold 80% ethyl alcohol, claim that such a concentrate will completely hydrolyze ten times its weight of pectin to galacturonic acid in about 24 hours. Fish and Dustman worked with Pectinol A; other Pectinols have a much higher enzyme content, the sugar being added for standardization.

This enzyme can be prepared from the orange flavedo after extraction in a dilute salt solution at a slightly alkaline *p*H, separating the solid residue from the extract and precipitating the enzyme with $(NH_4)_2SO_4$. After filtration and centrifugation the enzyme is finally dried to a powder.

It has been recently demonstrated that the commercial fungal enzyme preparations used as filter-aids for clarification of juices, etc., act only on de-esterified pectin. The rates of action of such enzyme preparations are rather limited because of their low content of pectin esterase. Hence, optimum efficiency can be obtained only if approximately equivalent amounts of pectin esterase and commercial enzymes (glucosidic in nature) are present in such clarification preparations.

The enzymes appear to act in two ways: part of the pectin is precipitated as a white curd while another fraction is rendered soluble. An elevated temperature has, of course, an important effect on increasing the speed of enzyme action, but then citrus juices are also increasingly likely to ferment. To avoid this it may be necessary to protect the juice from fermentation by flash-pasteurization prior to the adding of the enzyme.

An older method of clarification, used especially in the wine industry, consists in adding about 10 g of tannin and 20 g of gelatin to every 100 liters of juice. A tannin solution is first thoroughly mixed with the juice and the gelatin and then slowly stirred in. The precipitated flocks resulting from the reaction between the tannin and the gelatin settle slowly and carry down the colloidal and suspended matter of the juice.

[24] Fish, V. B., and R. B. Dustman, "The Enzyme Activities of Pectinol A on Pectin and Other Substances," *J. Am. Chem. Soc.*, **67**, 1155 (1945).

The clarified juices are mixed with sugar and flavoring extract, if desired.

Whatever method is used in clarification the best way of separating the precipitate is by centrifuging. For this purpose a Sharples centrifuge is most suitable as it is built of stainless steel and its bowl can be very easily cleaned in a short time. While the centrifuge separates most of the sediment it is rather difficult to obtain a perfectly clear juice; consequently, some sort of additional filtration is always necessary.

The patented Schoop process of clarification consists of the use of a very small quantity of sodium chloride in association with a special plant catalytic substance, both to be added immediately after expres-

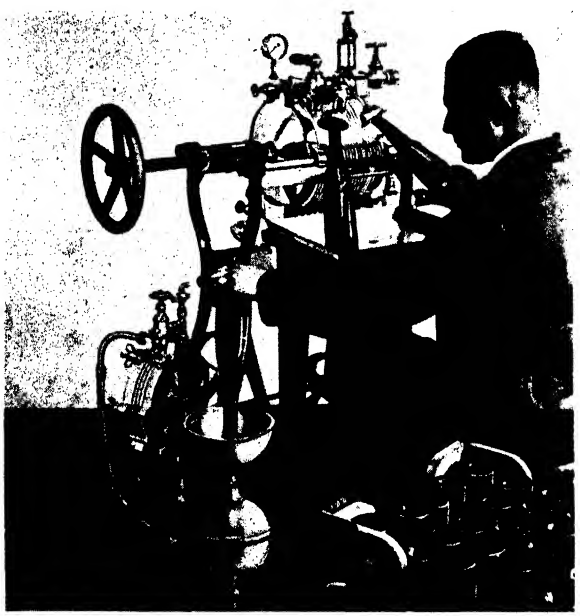


Fig. 92. Seitz E. K. filter.

sion of the juice. The precipitation or settling occurs while the juice is stored at 0° C. It is claimed that this process produces a remarkable clarification.

After sweetening, the clarified juice is bottled and pasteurized. However, when pasteurization is not desired the juice may be sterilized by passing it through special filters, such as the Seitz E. K. filter, the filtering discs of which are of a composition preventing the pas-

sage of yeasts and bacteria. Bottles previously sterilized are then filled and corked, taking every precaution that the bottling is done under sterile conditions (Figs. 92, 93).

3. Carbonated Citrus Beverages

Among the numerous flavored, bottled, and carbonated beverages, both natural and synthetic, citrus fruit juices maintain an eminent position. These so-called "soft drinks" contain syrups, squashes, or citrus concentrates diluted with water and carbonated with CO_2 gas.

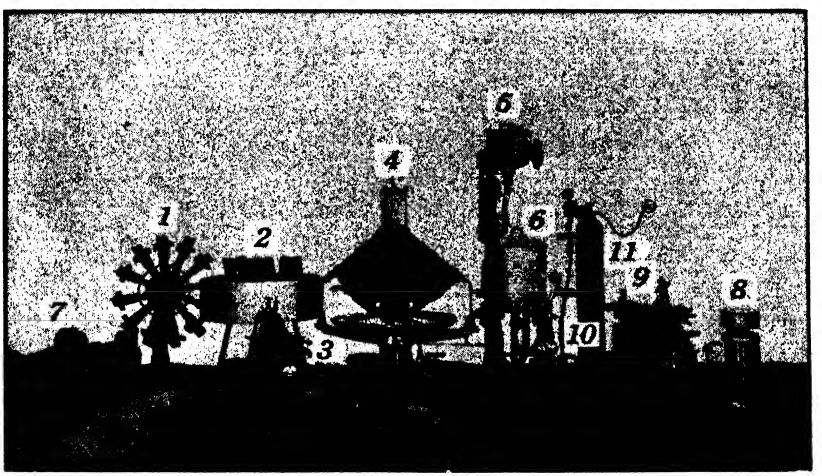


Fig. 93. Seitz sterile bottling line: *1*, sterilizer for bottles; *2*, circulating bottle holder; *3*, rinser; *4*, filler; *5*, crowner; *6*, sterilizer for crown corks; *7*, bottle rack; *8*, pump; *9*, Seitz E. K. filter; *10*, CO_2 impregnator; *11*, CO_2 cylinder with electrical heater.

They are intended as palatable thirst-quenchers, not as substitutes for pure fruit juices.

The usual procedure is to use, for an ordinary 12-oz bottle (about 1/3 liter, a "throw" of 40 cc. of a 60–65° Brix squash, adding carbonated water and capping the bottle. The squash should contain sufficient chemical preservative to prevent fermentation, or the closed bottle should be pasteurized in a water bath at about 70° C for 20 min. According to the British public health laws the prescribed preservative is a maximum of 70 ppm (mg per liter) of SO_2 or 120 ppm of sodium benzoate. In the United States carbonated citrus beverages are usually pasteurized; if sodium benzoate is added (0.05%), it must be prominently declared on the label.

Tastes, of course, differ greatly, so that when a squash is used in soft drinks, regional preferences should be taken into account. It is generally known that people living in southern (subtropical) countries prefer sweeter beverages than do those living in northern countries.

Table XXVIII shows the average composition of citrus-flavored carbonated beverages.

TABLE XXVIII

Composition of Citrus-Flavored Carbonated Beverages (after Toulouse)

Beverage	Sugar % (° Brix)	Gas volume	Citric acid g/l	pH
Lime (lithia)	9.17	4.0	1.40	3.02
Lemon and lime	11.04	3.2	1.75	3.01
Lime	11.10	3.7	2.28	2.90
Lemon	11.18	3.2	1.20	3.07
Orange	13.40	2.3	1.93	3.39

In some countries special regulations assign the proportion of natural juice required to be present in a citrus carbonated beverage (from 15 to 25%). As a basis for approximating the proportion of juice present the ash content can be used, although the P_2O_5 content of the ash has been suggested as a better index (see also p. 125).

When beverages containing natural fruit cells or pulp are bottled the squash must be constantly agitated to maintain the pulp in suspension, thereby attaining an equal distribution. During agitation the excessive incorporation of air should be avoided. The carbonation is performed in ordinary high-pressure carbonating systems or preferably in a low-pressure carbonating system, as described by Irish,[25] in which the syrup and water are mixed in a large tank and kept almost at freezing point; at this temperature the solubility of CO_2 is much greater than at room temperature. Low-pressure carbonation is preferable because it gives a finer flavor to the beverage and does not cause excessive foaming during bottling. Carbonation ranges from 1½ to 2½ volumes of CO_2 (Fig. 94).

Notwithstanding carbonation, some air is incorporated into the beverage during bottling or during the agitation of the squash, and the oxygen contained in this air may result in grave oxidative changes during storage. In bottled carbonated beverages containing citrus fruit, these oxidative changes may occur to a greater extent than in other types of soft drinks. Toulouse (1934), on testing a great num-

[25] Irish, J. H., "Fruit Juices and Fruit Juice Beverages," *Calif. Agr. Exp. Circular No. 313* (1928).

ber of soft drinks, observed that air present in the headspace of the bottle changed considerably in composition and that the greater portion of its oxygen was lost. Eliminating air as completely as possible by proper adjustment of bottling machinery and protection of the beverage from microorganisms are the only means of preventing oxygen-consuming phenomena which have an adverse effect on flavor.

With the purpose of establishing minimum standards for carbonated fruit beverages, Aref and Cruess (1933) made extensive comparative tests on pure juices, syrups, and soft drinks. Table XXIX, compiled from their paper, shows that the actual fruit con-

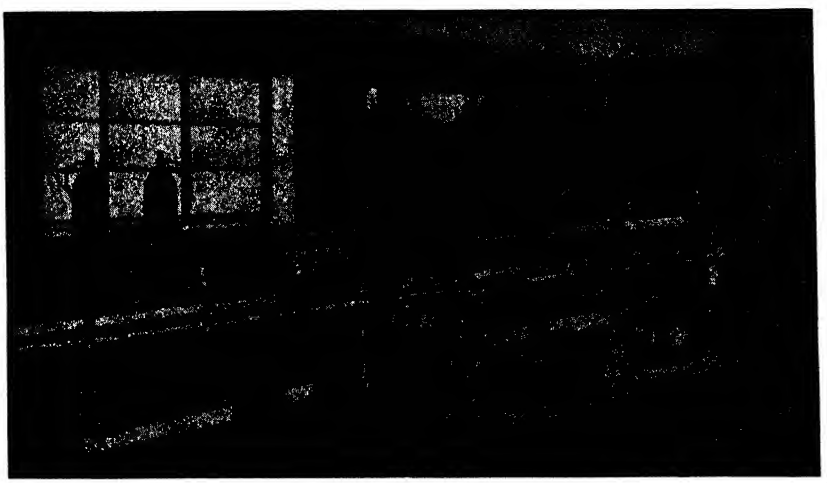


Fig. 94. Automatic continuous bottling line.

tent of carbonated fruit beverages produced in the United States varies greatly.

The general precautions necessary during bottling and the technique of bottle-washing are not described here; the reader is referred to any standard treatise on the subject.

D. FERMENTED JUICES

1. Citrus Wines

Like every other fruit juice containing sugars, citrus juices also can be fermented into alcoholic beverages. Only wines made from grapes should really be labeled unqualifiedly by the term "wine"; other fruit wines should always bear the name of the fruit used.

Owing to their high acidity lemons and limes can hardly be used

TABLE XXIX

Total Solids, Alkalinity of Ash, and Protein Content of Citrus Juices, Squashes, and Carbonated Beverages

	Brix	% Ash content in 100 g			Alkalinity of ash*			Acidity g/100 cc	Protein %
		Total ash	Soluble ash	Insoluble ash	Total alk.	Soluble alk.	Insoluble alk.		
PURE JUICES									
Grapefruit	12.85	.3204	.2404	.0800	42.58	23.82	18.76	1.98	.32
Lemon	12.1	.2860	.2231	.0629	40.96	26.32	14.64	6.12	.32
Navel orange (late)	17.1	.4986	.3994	.0992	87.1	60.9	26.2	0.83	.65
Valencia orange	14.75	.5149	.4341	.0808	78.5	55.4	22.1	1.4	.58
Navel orange	11.5	.328	.273	.055	83.7	56.8	26.9	1.19	....
SYRUPS AND SQUASHES									
Lime rickey	57.1	.161	.136	.025	9.32	5.57	3.75	0.293	....
Lemon syrup	56.5	.132	.068	.064	9.20	5.38	3.82	0.262	....
Orange syrup	55.6	.145	.116	.029	9.10	6.69	2.41	0.394	....
COMMERCIAL CARBONATED BEVERAGES									
Lemon	15.35	.1901	.1496	.0405	3.96	2.51	1.45	0.21	.03
Orange	18.0	.0196	.0162	.0034	1.0	0.6	0.4	0.27	.03
Grapefruit	15.05	.1082	.0690	.0392	19.7	16.8	2.9	0.455	.125
Lime rickey	15.05	.0300	.0286	.0014	1.3	0.7	0.6	0.403	.02

* Alkalinity of ash in cc 0.1 N HCl for ash from 100 g. of drink.

for the manufacture of alcoholic beverages. Even oranges, grapefruit, and tangerines, which are best suited for this purpose, are still too acid and contain, on the average, too little sugar to constitute an ideal source for wines of good quality: for, while grapes contain from 16 to more than 24% of sugars, citrus fruits ordinarily contain only from 6 to 8% and when very ripe no more than 10%. This deficiency in sugar not only makes citrus wines fermented from straight juice weak (they contain, on the average, some 3 to 4% of alcohol) but, owing to their insufficient alcoholic strength, also causes them to be unstable so far as their keeping qualities are concerned. A typical orange juice, for instance, will yield on fermentation a wine with less than 5% of alcohol and as much as 1.2% of acid, which is a tart product, very susceptible to souring, wine flowers, and other diseases, and which may develop objectionable flavors.

The alcohol content of citrus wines should, therefore, be considerably strengthened; this can be done in one of three ways: (*1*) by adding sugar to the juices before fermentation, (*2*) by adding citrus brandy or alcohol to the finished wines, and (*3*) by fermenting partially concentrated juices or admixing citrus concentrates to the juices before fermentation.

The last method should, of course, give the best results so far as quality is concerned inasmuch as it makes use of the maximum amount of juice without admixing sugars or alcohol from other sources. As the aroma of citrus juice is very elusive, any dilution with water will greatly diminish the bouquet and will tend to make the wine watery. Unless excessive amounts of sugar or concentrated juices are added the wines will lack body. The amount of sugar to be added to citrus juice depends upon the Brix reading of the original juice. In general, however, sufficient sugar or concentrated juice is added to bring the juice to a total solid content of about 25%, which on fermentation will yield a wine of approximately 13% alcohol (by volume). It is advisable, however, that sufficient sugar (or concentrate) be added and that the fermentation be stopped at the moment when approximately 4% of sugar, in addition to the alcohol content noted above, are left unfermented. It should be borne in mind that the amount of alcohol obtainable on fermentation equals about 55% of the sugar present in the juice.

To avoid excessive sourness in the resulting citrus wines only very ripe fruit of the less sour varieties should be selected at the end of the season. Partial neutralization of acidity by adding calculated amounts

of $CaCO_3$ is undesirable because in such cases the wines will acquire a modified and disagreeable flavor.

Citrus wines do not necessarily possess the characteristics of the fruits from which they are prepared, in the same way that wines proper do not resemble the flavor of the grapes from which they are fermented. During fermentation, and especially on aging, alcoholic beverages acquire a specific aroma and flavor. Nevertheless, special precautions should be taken when preparing juices for fermentation. The best juices will, of course, be those obtained by hand reaming, but as this method is very expensive, some mechanical method of extraction should be selected (page 230), provided that a minimum amount of peel oil is incorporated during extraction. Essential oil present in the juice in large proportions hinders fermentation and may even totally inhibit it. A suitable juice is obtained when the fruit is first peeled and then passed through a continuous screw-press expeller made of stainless steel. Prior to fermentation the extracted juice is best centrifuged in order to separate as much of the pulp as possible. This is especially important in the case of grapefruit, which otherwise yields a very bitter wine. Centrifugation is also important to eliminate any essential oil present in the juice. The juice may then be partially concentrated or sugar added in the desired amount.

To attain best results it is advisable to ferment the juice with a "starter" made of pure cultures of wine yeasts. Bakers' yeasts should never be used for it results in a "musty," "bready"-flavored wine. Yeast cultures best adaptable are *Saccharomyces ellipsoideus;* it has also been suggested that such yeasts as *Torulopsis dattila,* which ferment sugar twenty times as quickly as ordinary yeasts, may probably be the most suitable. Citrus juices present excellent media for alcoholic fermentation, but they may also contain wild yeast or acetic bacteria, so that it is advisable to add as much as 75–100 ppm of sulfur dioxide to the juice and to start fermentation with pure yeast (SO_2-resistant yeast) accustomed to this chemical, which exerts a selective action on various microorganisms. The presence of SO_2 also prevents any undesirable action of molds or vinegar bacteria; furthermore, it prevents the wine during aging from spoilage for which lactobacilli, which are very sensitive to SO_2, are largely responsible. Sulfur dioxide, being a strong antioxidant, also prevents vitamin C from oxidation during fermentation; thus, it is quite feasible to obtain palatable citrus wines containing their full amount of this vitamin.

If SO_2 is not added to the juice the temperature during fermenta-

tion has to be maintained quite low, at about 15–16° C (60° F), otherwise spoilage may occur from the action of acetic acid bacteria. The maintenance of such a low temperature during active fermentation, especially during the first three days, is possibly only by cooling the fermenting must. This is best done by pumping it through a tubular heat exchanger immersed in an ice bath.

Nitrogen is an essential element in fermentation processes as a nutrient for the propagation of yeasts. However, citrus juices contain comparatively little of such nitrogenous substances as are necessary for most favorable fermentation. An addition of about 1.0 g of ammonium carbonate per liter of juice has been found to stimulate the fermentation and to produce a wine of better aroma and flavor.

The culture is added to about half a liter of sweetened sterile juice and allowed to incubate for 48 hours at 24° C. When the juice is in active fermentation it is added to 5 liters of similar juice (previously pasteurized for 15 min and then cooled). This "starter" is sufficient to inoculate further 50 liters, to be subsequently transferred to larger quantities of juice. If the temperature is regularly controlled and if a vigorous starter of yeast is used, active fermentation is completed in about 10 days in open tanks. The supernatant wine, which is then cloudy, is siphoned off from the sludge and filtered through a plate-and-frame filter press, using 1 to 2% of a neutral filter-aid. The wine is finally "polished" through an asbestos filter-aid after aging and just before bottling. If it is desired to obtain a wine with a certain residual sugar content, the juice should be allowed to ferment until the desired alcohol content is reached, then racked and pasteurized to stop fermentation.

Citrus wines should be aged for at least six months in large storage tanks made either of redwood or of white oak.

2. Brandy and Liqueurs

By adding more sugar to the fermenting citrus juices the alcohol content of the resulting product will also proportionally increase. If the latter is then distilled, quite good brandy can be obtained. The product must then be aged in small oak kegs (3 to 5 gallons capacity); if aged in charred barrels it acquires a taste similar to whisky and appears to age more rapidly.

Products of great popularity are various liqueurs, prepared on the basis of citrus wines sweetened with pure sugar and with the addition of various flavoring extracts, derived mainly from citrus peel with its full oil content. One of the very popular and widely distributed

liqueurs is the so-called "curaçao," made with the peel of the sour or bitter orange. The finely cut peel is infused with alcohol of about 60° proof made of citrus juice. After several days the spirit is filtered and then mixed with sugar or sugar syrup.

3. Citrus Vinegar

(a) Mechanism

While it is very questionable whether citrus wines will ever occupy a place of their own in the general market of alcoholic beverages, vinegar made of citrus, especially orange vinegar, is a very excellent and much-favored product.

Vinegar is in reality "sour wine" (French: *vin-aigre*); it is made from grapes, apples, malt, or any other sugar-containing material by first fermenting them into alcohol and then, by subsequent acetous fermention with the assistance of bacteria of the genus *Acetobacter,* into the final product. Vinegar thus made should contain not less than 4 g of acetic acid in every 100 cc (at 20° C). Vinegar made of orange juice has a very fine flavor and, in addition to acetic acid, or the sour principle of other vinegars, contains also the natural fruit acid of the orange—namely, citric acid—which in itself is a valuable dietary constituent. Good orange vinegar contains about 5% of volatile acid, as acetic, and about 1% of fixed acid, as citric.

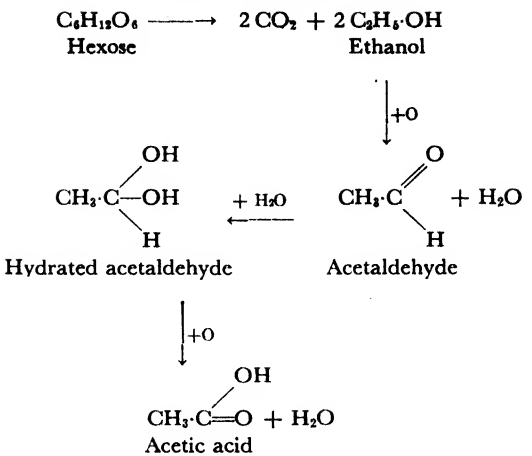
Although vinegar has been known since ancient times, its microbiological nature was realized only a little more than a century ago, in 1837, by Kützing and confirmed by Pasteur.

Before acetic fermentation, causing the conversion of juice into vinegar, can take place, the sugar in the orange juice must be totally converted into alcohol by yeast fermentation, as previously shown. The fermented juice is then acidified by a vinegar which has not been previously pasteurized. This initial acidification is carried out for seed purposes to supply the desirable acetic acid-producing bacteria and to inhibit the development of undesirable types of bacteria.

It has been established that acetaldehyde is an intermediate product of this fermentation.

In normal aerobic acetic-acid fermentation oxygen acts as the hydrogen acceptor in the conversion of ethyl alcohol to acetaldehyde. The latter is then hydrated and the two hydrogen atoms of the hydrated acetaldehyde are activated and donated to oxygen, the hydrogen acceptor, thus creating acetic acid.

The reaction runs, therefore, as follows:



The juice should never be acidified (seeded with vinegar) before alcoholic fermentation is completed because the acetic acid formed by *Acetobacter* retards yeast growth and activity, so that the two fermentations, alcoholic and acetic, cannot continue simultaneously. In fact, it has been proved that the presence of acetic acid in concentrations exceeding 0.5% stops the growth of *Saccharomyces*.

(b) Manufacture

Vinegar can be made by two methods: a slow or so-called "roller process," and a rapid or "generator process."

(1) ROLLER PROCESS

The "roller process" is a modification of a very old French method also called the Orleans process. It makes use of an ordinary whisky barrel into which a small rack is fitted in such a way that throughout its entire length, for about 15 cm below the bung, a compartment is created which is filled with beechwood shavings or corn cobs (Fig. 95). The purpose of the shavings is to create a greatly increased exposed surface area and, therefore, a closer contact with atmospheric oxygen, which acts as the hydrogen acceptor. It is evident that the conversion of ethyl alcohol into acetic acid is, above all, an oxidation process, thus necessitating an ample supply of oxygen. A sufficient number of holes should, therefore, be bored in each end of the barrel so that the openings come just beneath the bottom of the rack holding the shavings. No iron nails or any other metal should be used in the construction of the rack or its supports.

For initial acidification a vinegar of good quality which has not been pasteurized is allowed to run over the shavings and the inside of the barrel, so that the whole is thoroughly wetted. Fermented orange juice, preferably strained, is then run into the barrel while all the holes are tightly closed with wooden stoppers. The barrel is filled up to the holes on each side. The wooden stoppers are then replaced by plugs made of ordinary raw cotton to keep away flies and to allow free circulation of air. Several times each day these cotton plugs are removed and replaced with the wooden stoppers, and the barrel is turned over with the bung at the bottom and shaken several times so that the juice in the barrel will come thoroughly in contact with the

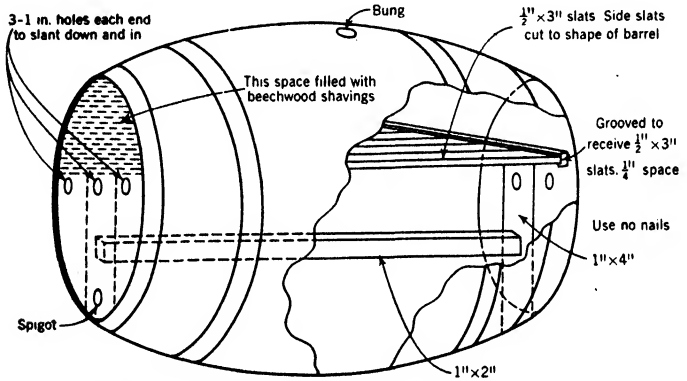


Fig. 95. Barrel generator for manufacture of vinegar by the "roller process."

beechwood shavings or the corn cobs. The barrel is then rolled back into its original position and the stoppers are replaced by the cotton plugs.

When the barrel generator is to be used for the first time, or after it has been standing idle for any length of time, it must be thoroughly scalded by blowing live steam through it.

The roller process takes from 2 to 3 months; if the treatment is kept up daily as prescribed an excellent vinegar will result. The most advantageous thermal conditions for active fermentation in vinegar manufacture are between 15 and 34° C, the optimum lying probably in the region between 25 and 30° C. Because the temperature during this process is lower than in the rapid generator method and therefore there is less evaporation in alcohol, the final product is higher in acidity and more nearly the theoretical yield.

(2) RAPID GENERATOR PROCESS

This method, also known as the "German process," consists in allowing the wine to trickle down through a tall receptacle containing loosely packed pomace or beechwood shavings, thus making the operation continuous. Any type of porous material may be introduced to obtain efficient contact between the organisms and the air.

The simplest form of a generator of this type is shown in Figure 96. It consists of a wooden vat 2 m high and 1 m in diameter, having two perforated discs placed about 150 cm from the top and bottom, the space between the discs being filled with beechwood shavings. An

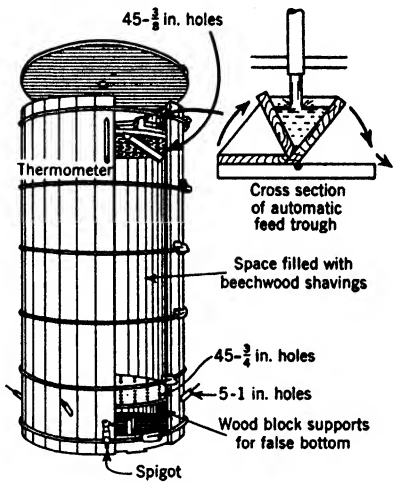


Fig. 96. Continuous generator for vinegar manufacture.

automatic distributor dumps regular amounts of the wine over the top disc, evenly distributing the flow of fresh fermented juice; at the very bottom of the vat is a spigot for drawing off the vinegar. Before starting the flow the generator must be seeded with good vinegar which has not been previously pasteurized. The temperature should be watched closely: any deviation from the normal can be corrected by either increasing or decreasing the flow of juice through the generator. If the drawn-off vinegar has not been completely acetified the product can be mixed back with the entering fermented juice.

Orange vinegar obtained in this way is then filtered, bottled, and finally pasteurized. The resulting product will have very good keeping qualities and will not cloud up after the bottle is opened.

Orange vinegar prepared by the rapid process requires aging just as is the custom with wines, for some changes occur during the storage period which very favorably affect both flavor and "bouquet" of the vinegar. The disappearance of the harsh flavor of fresh vinegars and the creation of a specific pleasant aroma is probably explained by the combination of ethyl alcohol with the acetic acid to create an ester, ethyl acetate, as follows:

$$C_2H_5OH + CH_3COOH \rightleftharpoons CH_3COOC_2H_5 + H_2O$$

When vinegar is prepared by the slow "roller process" aging is usually not required because esterification takes place during the slow vinegar formation.

In order to insure a gradual formation of esters and to improve the keeping qualities of the orange vinegar it is considered best to leave about 0.3 to 0.4% of alcohol.

(c) Summary

To sum up, orange vinegar properly prepared can easily compete, as far as quality and flavor are concerned, with the best brands of vinegar made from other sugar-containing materials. It is noteworthy that statements which have often been made that the use of vinegar in diet is harmful have been proved fallacious. Crawford and Ward,[26] who have carried out a comprehensive study on three generations of albino rats fed with vinegar to determine its effect on the circulating blood cells and hemoglobin, have revealed, as a result of numerous tests, that no anemia or any other pathologic change takes place.

4. Other Uses of Fermented Citrus Juices

During World War I, when acetone was scarce and in great demand, it was proposed to ferment citrus juices into acetic acid by neutralization with lime into calcium acetate and final distillation of the acetate to obtain acetone. One ton of oranges would probably yield as much as 15 to 20 kg of acetone. In the meantime, however, many other processes for manufacturing acetone from much cheaper sources have been devised, including the well-known process of fermenting sugar-containing materials by means of a special baceterium. *Clostridium acetobutyricum* Weizman, of which mention was made on page 87. During World War II acetone was produced in Palestine from citrus juices and peels by this method.

[26] Crawford, S. L., and J. M. Ward, "Effect of Vinegar on Circulating Blood Cells." *Fruit Prod. J.*, **12**, 266 (May, 1933).

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CHAPTER VIII

MISCELLANEOUS CITRUS PRODUCTS

A. JAMS, JELLIES, AND MARMALADES

1. General

Jams in their various forms are probably the earliest by-products made of citrus fruits. Even today a greater quantity of fruit is used by housewives for home-made jams than is utilized for this purpose in industrial enterprises; and, as in the case of housewives' "recipes," there are almost as many ways to make jams and marmalades in industry as there are manufacturers. In general, however, three types are distinguished:

(1) *Jams* are prepared by boiling fruit pulp (or whole fruit) with sugar to a thick or jelly-like consistency without retaining the shape of the fruit. This designation usually does not apply to citrus fruits.

(2) *Jellies* are prepared by substituting the fruit pulp by expressed and strained juices which jellify on cooling after having been boiled with sugar and concentrated to the proper consistency.

(3) *Marmalades* are clear jellies in which slices of fruit or peel are embedded in suspension.

It is common knowledge that, when boiled with sugar, fruits owe their property of forming jellies to pectin, a substance described in detail on pages 88–96. However, only some 25 years ago was it clearly recognized that the presence of pectin alone is not sufficient for the formation of fruit jellies. Equally important is the acidity of the fruit extracts, the jelly formation resulting only over a definite equilibrium range of the system "pectin-acid-sugar." In the manufacture of jam and marmalade without any extra addition of pectin and acid, only such fruits can be used in which the content of these two ingredients is sufficiently high for the formation of a firm jelly with the added sugar. Citrus fruits, especially when not overripe, fully comply with these requirements and are, therefore, very suitable for jam and marmalade manufacture. Sour apples, red currants,

quinces, or similar fruits are suitable for the same reason, while sweet apples or figs, for instance, which are poor in acid although rich in pectin, will not gel. Likewise, rhubarb or tomatoes are unsuitable; although rich in acid, their pectin content is too low.

2. Pectin-Acid-Sugar Ratio

The practical implication from numerous researches in this field is to adjust the amount of pectin and of acidity in such a way as to save sugar. For instance, an increase in acidity from 0.1 to 1.7% results in a saving of nearly 20% of sugar. The same is true about pectin: within certain limits (0.5 to 1.5% of pectin content) the higher the percentage of pectin in the juice the lower the amount of sugar required to form a jelly.

Comprehensive investigations made by Tarr (1923)* have shown that there is no direct relation between *total* acidity and jelly formation and that while the total amount of acid necessary for jelly formation may vary over a wide range, the hydrogen-ion concentration is practically constant for a particular juice and varies only slightly for different juices. This definite limit for hydrogen-ion concentration found by Tarr is a *p*H value of 3.46, which gives rise to a tender, delicate jelly. Jelly increases in stability with lower *p*H value (down to 3.1–3.2); with still lower *p*H values, syneresis occurs, i.e., the jelly exudes liquid on standing. In the opinion of some investigators citric acid is the least and tartaric acid is the most efficient, while malic acid occupies an intermediate position.

It should be noted that prolonged boiling weakens the jellying capacity of pectin, especially in acids such as citrus juices. It has been shown previously that in acid solutions pectin hydrolyzes quickly into pectinic acids with lower jellying capacity and finally into degradation products which have no jellying power at all. This means that pectin should be added as late as possible in the process of jam making. Prolonged boiling in acid media causes also the inversion of sucrose into invert sugar. It has, however, been found that inversion has no ill effect on the strength of the jelly; on the other hand, the inversion of sucrose prevents the crystallization of the sugar in marmalades during storage.

The actual technique of making citrus marmalades is, as stated, very diversified. Only very general lines can be mentioned here, indicating the principal operations involved.

* Tarr, L. W., "A Study of the Factors Affecting the Jellying of Fruits," *Univ. Delaware Agr. Expt. Sta. Bull.*, **133** (1922).

3. Manufacture

(a) Preparation of the Peel

In the main only sour or bitter oranges (of bigaradier type) are utilized, although in some places sweet oranges also are used, sometimes with the admixture of a certain proportion of grapefruit or lemons. The washed fruits are peeled by hand or by specially designed machines (Fig. 97). The peel (of which only a quarter to a half is utilized in proportion to the total weight of fruit) is then cut by special machines into very thin slices. Before being boiled with sugar these slices need to be softened, otherwise they subsequently

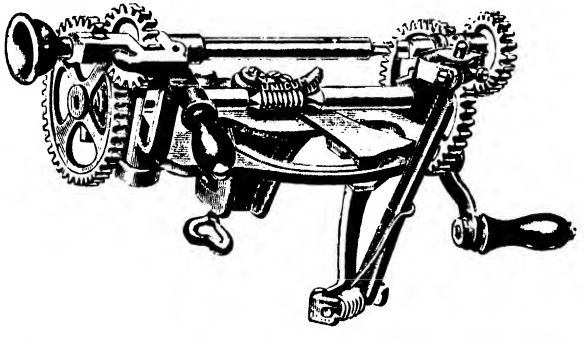

Fig. 97. Hand-driven machine for peeling citrus fruit.

would become very hard and tough. Softening is effected in one of several ways. The slices may be covered with water and cooked until tender, the water being changed at least twice during cooking. The same heating with water can be done in autoclaves under pressure of about 1 atm. More rapid softening is attained by boiling the peel in a solution of carbonate of soda or ammonium hydroxide, which remove from the cell wall certain substances which otherwise render the peel tough after boiling with sugar. The exact nature of the action of these chemicals is not yet established, but they are, however, entirely harmless.

Ammonium hydroxide is preferable to carbonate of soda, the action of the latter being somewhat more drastic. To avoid handling the peel after it has been softened, Morris (1933) suggests placing the peel intended for softening in perforated baskets of noncorrosible metal, each containing sufficient peel for one batch of marmalade.

After having been cooked in ammonium hydroxide or soda, the

peels may be recooked for a short time with a weak solution of citric acid to remove any traces of the hydroxide. It is recommended by Sedky, Fellers, and Esselen[1] that the peel be cooked in a 0.1–0.2% citric acid solution without any previous treatment.

(b) Preparation of the Juice

The peeled oranges are put through a meat grinder, or crushed between wooden rollers and cut into small pieces; they are then covered with about 1½ volumes of water and boiled gently in a glass-lined kettle or in wooden vats until the pieces are thoroughly disintegrated. The resulting cooked pulp is pressed through a coarse filter cloth or a finisher (screening machine) to remove pips and the coarser parts. During this boiling the necessary pectin is extracted from the white (albedo) portion, the protopectin being hydrolyzed into soluble pectin. The strained pulp contains also the fruit acid. At this stage the pulp should be adjusted in regard to both pectin (about 0.5%) and acidity (from 0.5 to 0.7%) contents. Acidity is best corrected by adding some lemon juice.

Comparing the relative viscosities of various fruit juices, Baker (1935) worked out a general equation for the calculation of sugar requirements and jelly yields. The viscosities of the extracted juice are measured by a modified Ostwald viscometer consisting of a bulb with a straight capillary pipet attached. When the fruit juice is brought into satisfactory *p*H range for the jellying condition prior to measuring the viscosity, the general equation to be used is then:

$$\log y = ax + b$$

where y equals the relative viscosity at 80° F (27° C) and x equals the parts of sugar per part of juice by weight which must be added to an extraction of viscosity y. The values of the constants a and b (for apple juice, for instance) are 0.675 and 0.236, respectively. For citrus marmalade the value of a becomes 1.2.

(c) Cooking the Marmalade

The actual boiling of the marmalade should be carried out as quickly as possible. This last stage of the manufacture is, therefore, performed in small shallow steam-jacketed vessels made of tinned copper or, better, of aluminum or stainless steel, capable of being tilted (Fig. 98). The smaller the cooking pans the quicker the "boil" and the better the quality of the final marmalade. Equal weights of

[1] Sedky, A., C. R. Fellers, and W. B. Esselen, Jr., "An Improved Orange Marmalade of High Vitamin C Content," *Fruit Prod. J.*, **21**, 170 (1942).

the pulp extract and pure cane sugar are then placed in the pan together with the previously prepared peel and the stock is evaporated as rapidly as possible to the jellying point. Usually only about 10 min are sufficient; during boiling the marmalade should be thoroughly skimmed with a long-handled, flat aluminum spoon or "skimmer." This spoon is also useful for testing the end point of the boiling. The end point is attained when the mass in the pan shows a tendency to "flake off" clean from the edge of the spoon. At this moment the

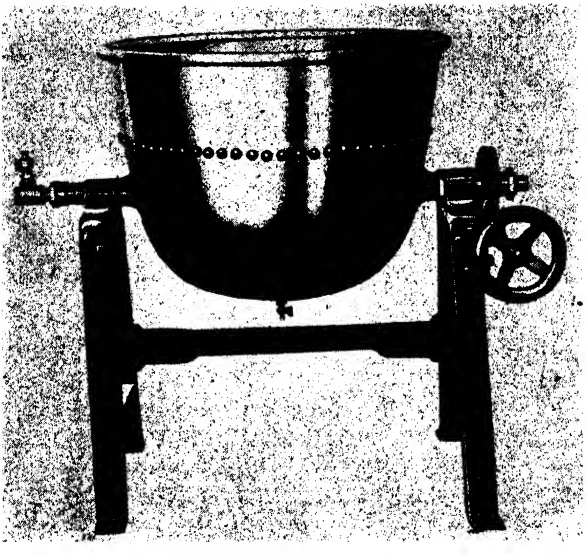

Fig. 98. Steam-jacketed kettle for boiling marmalade (courtesy Food Machinery Corporation, Hopeston, Illinois).

temperature of the batch rises usually to 220–230° F (104–110° C). The end point can also be easily determined by means of the pocket refractometer described on page 173. Boiling is thereafter discontinued and the contents are emptied by tilting into another kettle where the marmalade is cooled down to about 156° F (70° C); the whole is then stirred gently to avoid either the rising or the settling of the peel in the finished product. The marmalade is then ready for filling into containers, either glass or tin, where the jelly sets on cooling.

Some factories use the vacum-pan method whereby the marmalade is boiled in specially designed pans under reduced pressure and, naturally, at lower temperature.

As has been shown above, the factors affecting jelly formation are the proper proportions of the three essential ingredients—pectin, acid, and sugar. If these are correct, a jelly can be made in the cold, thereby eliminating the necessity of batch operation. Baker and Pheteplace[1a] proposed, therefore, a conversion of the batch operation in jelly-making to a continuous process and outlined a plan whereby correct and carefully controlled quantities of concentrated juices were mixed with sugar syrups applying only as much heat as necessary to assure perfect mixing and blending.

According to the accepted standards,[2] marmalades made of citrus fruit should contain 68.5% of total solids. Assuming that about 3.5% are represented by the total solids derived from the fruit, the remaining 65% are added sugar. Some manufacturers advise using, together with the sucrose, some dextrose in proportions not exceeding 25% of the total sugar.

A survey of commercial orange marmalades made by Sedky *et al.*[3] showed most of them to be relatively low in vitamin C, varying from 2.1 to 6.3 mg per 100 g of jam. They suggest, therefore, the use of an orange concentrate in making marmalade and claim that in such a product the vitamin C is as high as 17.5 to 29.2 mg ascorbic acid per 100 g, or a retention of 80% of the original vitamin C in the juice.

A dried citrus-fruit paste in the form of leatherlike sheets and suitable for the manufacture of marmalade can be prepared from comminuted citrus fruit including the albedo and the endocarpium, by adjusting the mass to *p*H between 2.5 and 4.5 followed by spreading into thin layers and drying at comparatively low temperature.[3a] This product has been invented and manufactured in Palestine.

B. CANNING "HEARTS"

1. Survey of Process

In preceding sections the discussion of the utilization of the endocarp has been limited to the extraction and processing of the juice, whether straight unfermented, concentrated, frozen, sweetened, or

[1a] Baker, G. L., and W. D. Pheteplace, Jr., "Making Jelly by Continuous Process," *Food Ind.*, July, 1940.

[2] "Standards for Jams," *Analyst*, 55, 695 (1930).

[3] *Op. cit.*

[3a] Samisch, Z., "Dried Citrus-Fruit Paste," U.S. Patent No. 2,422,588 (June 17, 1947).

fermented into wine and vinegar. This section will be concerned with the whole endocarp, in the form of unbroken cells.

The first successful attempt at canning citrus fruit was made in Puerto Rico in 1918, where grapefruit "hearts" were canned in tins. Since then, this phase of the citrus products industry has expanded enormously. Although some other varieties of citrus fruit, such as oranges or tangerines, are canned to some extent (mainly in Japan), grapefruit is by far the most popular citrus fruit used for this purpose; for this reason, the following pages will be devoted, in particular, to grapefruit.

Before describing the process of canning grapefruit hearts, it may be worthwhile to enumerate the sequence of operations involved:

(1) washing, grading, and sorting the fruit;
(2) scalding and peeling or lye-treatment;
(3) cutting and sectioning the segments;
(4) filling the cans;
(5) syruping;
(6) exhausting;
(7) lidding and sealing the cans;
(8) sterilization;
(9) cooling;
(10) storing for control;
(11) casing.

2. Steps in Processing

(a) Grading and Washing

The preliminary treatment of fruit, such as the selection or grading and the proper washing, have been described in some detail on pages 172–182; naturally, they apply with full rigor also in this case. In grapefruit canning it is the general custom to use 54's, 64's, and 70's for No. 2 cans. Other sizes are used for correspondingly larger or smaller cans. It is, therefore, preferable to sort out the various sizes into at least three general canning groups by means of a mechanical sizer (described on page 181).

(b) Scalding and Peeling

The fruit must now be peeled in such a way that the segments are not injured; this operation, as well as the next one—the sectioning—is best performed by hand, for no method of mechanical peeling can

leave the fruit undamaged. However, to facilitate hand peeling, the whole fruit is first scalded by hot water or steam. A conveyor takes the fruit through a water bath kept at a temperature just below boiling point, or through a steam chamber, and is so timed that the fruit remains there long enough for the heat to penetrate only through the flavedo and albedo but not into the main body of the endocarp itself. Usually 3 min are sufficient to expand and loosen the peel from the membrane covering the outer ends of the juice carpels and to form an air chamber between this membrane and the peel, which can then be easily removed after scoring it in quarters.

Thereafter the membrane of albedo still adhering to the peeled fruit must be removed, for, as has been stated on page 96, the bitter principle—naringine—of the grapefruit, as well as the other glucosides, are concentrated mainly in this particular part of the fruit—namely, in the region between the albedo and the carpellary membranes of the segments. If these substances are not removed the canned product will be exceedingly bitter. Peeling can be done by hand with an appropriate knife; however, some canners prefer the so-called *lye-peeling* method. This consists in passing the fruit, placed in wire baskets, through a bath containing a $1\frac{1}{2}$ to $2\frac{1}{2}\%$ hot solution of caustic soda. The lye solution, which is applied for about 12 sec, is then washed off by fresh water sprays sufficiently strong to remove also the disintegrated white albedo remnants. The whole procedure is carried out in the so-called lye-peeler, an iron tank through which the baskets with the peeled fruit are conveyed on two endless cables. The lye-peeler consists of two sections: a lye section where the hot solution of caustic soda is circulated by pump and applied in sprays, and a second fresh-water section where peel and lye are thoroughly washed off.

Lye-peeling is, of course, much more economical than hand-peeling and is also more complete. It has, however, disadvantages of its own, one of them being that the high temperature of the caustic soda tends to expand the carpels and often to burst them, causing the so-called "bleeding." It does, however, leave a little fibrous cord or "backbone" down the back of the section, which helps to strengthen the segment and hold it together, but causes inconvenience to the trade because when the segment is consumed the cord is very hard to cut with a spoon. The opinion of some canners is that the lye-peeling method does not give as good a product as the knife-peeling one; other canners claim that the strongest point in favor of lye-peeling lies in the

fact that caustic soda neutralizes a small portion of the citric acid, thus helping to make the fruit sweeter.

(c) Sectioning the Fruit

The completely peeled fruit is placed in round wire baskets and is then ready for sectioning. The main purpose is to remove the carpellary membranes from the segments without breaking the segments or injuring the juice cells on their sides, otherwise the procedure becomes very costly. Segmenting is done by hand and requires a considerable amount of skill. It is usually started at the place where the vertex of the sections meets the core of the bloom end. To facilitate the process it is best to tear the fruit into two halves. The knife is then inserted between the membrane and the juice cells, pushed clear through the fruit, and subsequently pulled out with a vertical stroke. The knife is then inserted alongside the next membrane and the clear section is carefully separated and placed on aluminum trays. Broken sections should be collected separately and packed as second grade. Peeled sections are placed on the trays one section deep; piling up should be avoided to prevent breakage of the sections.

(d) Filling the Tins

Particular care must be taken in filling the tins, which is done by hand. By packing the sections carefully into the can so that they lie close to one another a solid and attractive pack can be formed. A properly packed tin when inverted can be easily removed from the segments, which retain the form of the container so well that the tin can be easily slipped over the mould again without injury to any of the segments. A No. 2 can holds approximately 475 g of fruit. After the segments are packed the can is filled with a hot sugar syrup of a strength of 40 to 60% (Brix), according to the specific fruit used, although some packers use no sugar at all. A few canners prefer to add dry sugar to the liquid syrup in the belief that the juice which bleeds from the cells, together with a little added water, will make up sufficient liquor to dissolve the sugar. The syrup is best added to the tin before the sections are packed; however, most of the packers make the final addition of syrup after the can has been filled.

A very important consideration is the amount of headspace which is to be left in the can: insufficient filling gives rise to oxidation and also loosens the pack; overfilling may cause the cans to swell and even to burst. This may be prevented by filling the cans with hot syrup and

by subsequent exhaustion; when the closed can cools down a vacuum is created within it. In modern practice a headspace of about 7 to 9 mm left below the top of the can before it is sealed is generally considered satisfactory.

(e) Exhausting

Exhausting, which is somewhat similar to the process of deaeration, is the next stage in canning grapefruit "hearts"; its purpose is to eliminate as much as possible of the air entrapped between the fruit

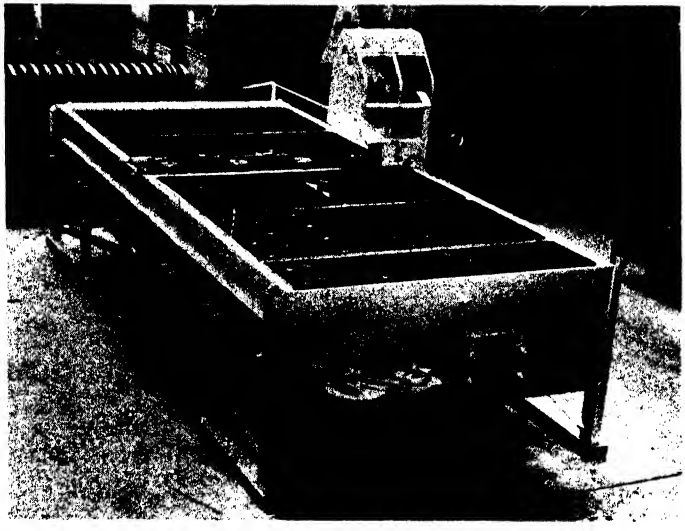

Fig. 99. Large-capacity exhausting bath with 12-inch disks (clad with stainless-steel teeth). Courtesy Food Machinery Corporation, San Jose, California.

sacs which otherwise would cause corrosion of the tin. The open cans are immersed in a hot-water bath to within an inch of their tops for 28 min (for No. 2 cans), causing the air bubbles to rise to top of the can. The usual procedure is to let the cans travel at a regulated speed through the exhaust box by some mechanical means—either a conveyor made of perforated steel slats or a series of large metal disks fitted with cog gears which mesh together (Fig. 99). In the latter arrangement the cans travel down one row of disks and back the next through the exhaust box, guided by curved iron rods fixed above the geared disks. The exhaust box is filled with water and has an overflow so adjusted as to keep the cans fully immersed within a reasonable

distance from their tops. Steam coils are provided to maintain a uniform temperature of 180° F (82° C) in the exhaust box.

Another way of exhausting the filled cans is to close them in a special vacuum-sealing machine operated so that the contents of the tin are subjected to a vacuum of about 25 in (650 mm). This method is mostly used when the fruit is packed in glass instead of tins.

(f) Sealing the Cans

If the cans are exhausted by the "heat-exhaust" method and not by vacuum they are conveyed from the exhaust box to a closing machine where the lids are placed in position on top of the cans and the cans

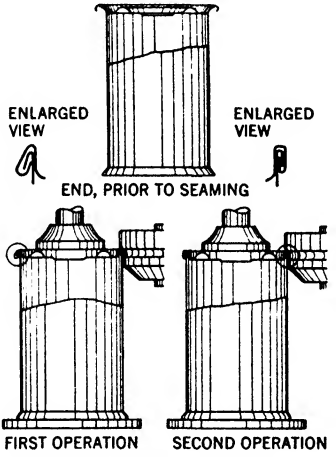

Fig. 100. Seaming operations.

are sealed by a double-seaming operation, as shown in Figure 100. Seaming is performed in two stages: in the first, rollers, specially grooved for the purpose, curl the cover flange; in the second stage other rollers compress the seam tightly. At this point special care must be taken, for the success of the canning process depends entirely on obtaining a perfect seal. For details of all these operations the reader is referred to special treatises on the subject (see bibliography at end of this chapter).

(g) Sterilization

After sealing, the cans are inverted and conveyed to the processing bath where they are sterilized at a uniform temperature of 180° F (82° C) for 25 to 28 min (for No. 2 cans). Immediately upon leaving

the sterilization bath the cans should pass to a cooling bath with running water or sprays and should be allowed to remain there until the temperature of the tin is sufficiently lowered to enable easy handling. This is best achieved by constantly rotating the cans during cooling so that the center of the can is cooled simultaneously. The cans should, however, remain slightly warm to the touch to enable the remaining water to evaporate quickly after the cans have been taken from the cooler. Upon emerging from the cooler the cans are stacked on their sides for not less than 15 days, after which they may be labeled and packed in wooden cases or cartons.

If the cans are supplied by modern plants fully equipped with automatic machinery, they are usually tested individually for any possible leakage or improper seal. In that case the cooled and dry cans may be packed immediately.

3. Spoilage

Inefficient pasteurization may cause fermentation of the contents and swell the cans; this may be caused also by an imperfect seal. However, some entirely different causes of spoilage exist.

The tin plate to be used in making the can must be of the very best quality, i.e., it should be uniformly coated with a sufficient layer of tin. (Cold-reduced plate is generally accepted as being more resistant than that made by the hot-rolling process.) Nevertheless, owing to normal imperfections existing in tin coating or owing to damage sustained during the can-making process, microscopic areas of iron are exposed to the action of the acid fruit. An electrocouple, consisting of the two metals, iron and tin, in contact with an electrolyte, is then formed. The internal corrosion of the tin plate thus takes place with the formation of hydrogen, which causes so-called "*hydrogen swells.*" A number of factors, including the efficiency of the exhaust, the size of headspace in the can, the temperature at which the product is stored, etc., are responsible in varying degrees for hydrogen swells.

Here again, oxygen plays the decisive role. In its absence the rate of attack is much slower, because the electrolyte—in this case the fruit juice—which contains such anions as citrates, is able to form with the tin stable complexes in which the tin is anodic. The attack is then confined more or less to the tin surface alone, while the iron base remains protected. If such tin complexes are not formed, however, the tin remains cathodic and the iron is freely attacked. Furthermore, when the hydrogen is displaced as a gas by the tin its evolution is very

slow unless oxygen acts as a depolarizer. In the absence of oxygen the hydrogen is not quickly evolved and it then exerts a back electromotive force which opposes further dissolution of the iron.

Evidently the main factor influencing the rate of corrosion of tin plate is the polarity of the two metals in the electrocouple. For this reason, several different enamels or coatings which have constantly been tried on tin have turned out to be entirely unsuccessful. The same applies also to glass packing.

Canned grapefruit when stored at ordinary temperature gradually becomes slightly yellow due to oxidation. When packed in plain cans, however, the reducing action of the tin, as explained above, has also a bleaching effect which retards color changes. In the enameled can or in a glass the color change is more rapid. The best containers in which to pack grapefruit hearts are, therefore, plain cans, which should be further protected by storage at low temperatures.

4. Recent Developments

In order to eliminate the tedious manual labor involved in the separation of grapefruit segments, one plant in Florida has recently patented[4] a process of canning grapefruit cells detached from their segments. This product is a heavy suspension of grapefruit cells in sweetened juice.

By this process the washed fruit is cored by a sharpened steel tube in a vertical position, cut in two halves which are then turned inside out. The everted fruit "collar" is then depulped in a special machine consisting of a metal wheel 7 inches in diameter and about 1¼ inches wide at the periphery, the wheel carrying a number of wire loops (about 1 inch in diameter) parallel to its axis. The fruit cells are scraped by the wire loops when the everted grapefruit halves are pressed against them, the wheel revolving at a peripheral speed of about 250 ft per min. The cells are then separated by a vibrating screen, inspected for seeds and pieces of rag which are removed, and, after remixing with the sweetened juice, are flash pasteurized and filled into cans.

C. MANUFACTURE OF CITRIC ACID

1. Source Fruits

All citrus varieties contain, to a greater or less extent, organic acids, particularly citric acid. However, only fruits of high acid content,

[4] Information published by Bruce's Juices, Inc., in *Food Ind.*, 19, 47 (1947).

such as lemons, limes, and bergamots, are used in the manufacture of citric acid. The acid is usually made from inferior fruit which is unfit for the preparation of juice to be used in beverages.

In Italy lemons have mainly been used for making citric acid for a long time; also, the pulp of bergamots has been utilized to a smaller extent. In the West Indies only limes, which are generally somewhat more acid than the lemons, are used.

The principle on which the present process of manufacture is based is that of Scheele, described on page 106. The citric acid is precipitated from the expressed juice by milk of lime in the form of calcium citrate, which is then treated back by H_2SO_4 and reconverted into citric acid.

2. Direct Crystallization of Citric Acid

The reader may justly ask why the transition through calcium citrate should be made when citric acid is found in the juice in its natural state; and why the acid cannot be crystallized from the juice directly. Indeed, many attempts have been made in this direction, but without results. The reason lies in the fact that the juice contains a considerable amount of colloidal matter, sugars, and inorganic salts (about 0.5 to 0.7%) which, on concentration, hinder the crystallization of pure citric acid.

Peratoner and Scarlata in 1910 suggested concentrating the juice in vacuo to one-tenth of its original weight, then treating the resulting syrup with a mixture of alcohol and ether in which all the citric acid is soluble, while many impurities remain insoluble. The alcohol and ether are then recovered by distillation and the citric acid is recrystallized from the residue. This process, however, has never been applied in practice.

Poore[5] attempted to solve the problem by dialyzing lemon juice using collodion sacs, and came to the conclusion that satisfactory crystallization is not affected by the small quantity of colloids (such as pectin, etc.) present in the juice; but is prevented by the presence of a high quantity of mineral ash. In the presence of this ash impurity, only a small amount of citric acid crystals is obtained in needle and leaflet modifications.

Ten years later (in 1932) Ajon proposed another scheme for direct crystallization. According to his process hot lemon juice is treated with tannic acid and decolorizing carbon, then filtered and set to

[5] Poore, H. D., "Effect of Dialysis on Direct Crystallization of Citric Acid from Lemon Juice," *J. Ind. Eng. Chem.*, **15**, 775 (1923).

ferment to eliminate all carbohydrate material. The juice is then concentrated in vacuo, and a partial demineralization is performed by treating it with alcohol with the addition of ammonium oxalate and tartaric acid (the latter being recovered in the form of potassium bitartrate). After filtration the alcohol is recovered and the resulting liquid is concentrated and set for crystallization. This complicated and extremely costly process has never attained practical application.

Recently, good results have been reported from the application of a novel method of demineralization—namely, the ion-exchange method. Although, as far as the writer is aware, this method has not yet been applied commercially, it is probable that in the very near future Scheele's classical method of citric acid manufacture will be entirely abandoned, as far as citrus fruits are concerned, and will be replaced by the ion-exchange method.

3. Ion-Exchange Method

For almost a hundred years it has been known that water fed to boilers can be softened by removing a substantial part of its salts, such as calcium and magnesium bicarbonates, by precipitation with lime and soda. Later it was found that certain natural and synthetic siliceous materials, called *zeolites,* are capable of reducing the permanent hardness of water by exchanging the Ca^{++} ion for a soluble Na^{+} ion. One of the most significant recent developments in the field of synthetic resins was the discovery by Adams and Holmes[6] in England that certain synthetic resins, when correctly formulated and prepared, can function very efficiently as cation or anion exchangers. There are now on the market two types of such exchangers, one type comprising the carbonaceous and resinous cation exchangers and the second the organic acid adsorbents. The soluble salts in the water or in the solution being treated are converted to their corresponding acids by passage through the hydrogen exchanger, and the acids thus produced are removed by the acid adsorbent. The reactions involved may be represented by the following equations (in which R denotes resins, M metal, X and Y acidic residues):

Sodium cycle:	$NaR + MX \rightleftharpoons NaX + MR$
Hydrogen cycle:	$HR + MX \rightleftharpoons MR + HX$
Acid adsorption:	$RNH_2 + HX \rightarrow RNH_2 \cdot HX$
Anion exchange:	$RNH_2 \cdot HX + HY \rightleftharpoons RNH_2 \cdot HY + HX$

[6] Adams, B. A., and E. L. Holmes, "Adsorptive Properties of Synthetic Resins," *J. Soc. Chem. Ind.,* **54**, 1–6T (1935).

Theoretically, then, there are two ways of preparing citric acid from citrus juice. The first is to demineralize the juice by a cation exchanger so that subsequently the acid will easily crystallize. The second way is to absorb the citric acid by a resinous acid adsorbent and finally to recover the pure acid solution by substituting another cheaper mineral acid for citric acid in an anion exchange.

The first method has recently been very successfully applied in sugar technology, where the exchange process apparently represents a real advance. There, also, the nonsugar constituents of the juice retard the crystallization of sucrose with subsequent loss in yield. After a prolonged study during which many means of removing these nonsugar impurities were tried, a resinous exchanger was developed which treats the partially purified sugar juice prior to evaporation and crystallization. This process has shown as high as 98% removal of inorganic salts and very considerable elimination of organic impurities, color, and colloidal substances. Although the application of deionizing methods to an industrial process of this kind is complicated by the numerous varieties of impurities present, there is no reason why such a treatment should not be developed for the manufacture of citric acid from lemons, etc. The application of this principle has been the subject of a recent patent,[6a] which deals with the purification of aqueous solutions containing high concentrations of citric acid in conjunction with metallic ions as impurities.

On the other hand, citric acid may probably be adsorbed from the juices by a resinous acid adsorbent (anion exchanger) and regenerated with Na_2CO_3. After the resin has taken up as much citric acid as possible, it is treated with a 10% solution of H_2SO_4, which displaces the citric acid from the resin–citric acid salt to form resin–H_2SO_4. The resin is then regenerated with Na_2CO_3, according to the following formula:

$$RX \cdot H_2SO_4 + Na_2CO_3 \rightarrow RX + Na_2SO_4 + H_2O + CO_2$$

The ion-exchange method may be used with success also in the manufacture of other citrus products, such as pectin and ascorbic acid (see page 366).

Two firms in the United States prepare a series of ion-exchange resins: *Amberlites,* by The Resinous Products and Chemical Company of Philadelphia, and *Zeo-Karb* and *De-Acidite,* by Permutit Company of New York.

[6a] Cole, G. M., "Manufacture of Fruit Acids," U.S. Patent No. 2,253,061 (August 19, 1941).

4. Scheele Method

Although the chemistry of the old classical method of citric acid production from lemons, etc., is very simple, its manufacture is difficult and is associated with many rather complicated phases (Fig. 101).

(a) Expression and Purification of the Juices

The juices must first be properly prepared before the citrate of lime can be precipitated. For this purpose lime and lemon juices may best be extracted by means of a powerful screw press after the fruit has been sufficiently crushed.

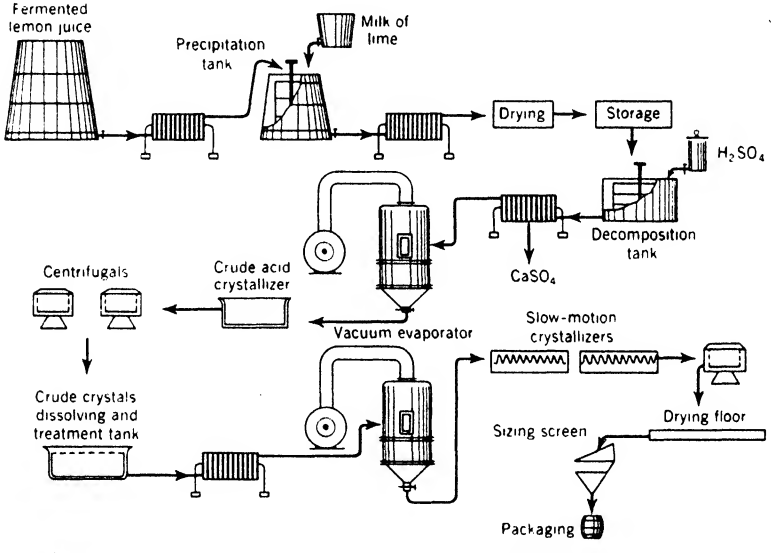

Fig. 101. Flow sheet showing the process of citric acid manufacture.

In Italy and other countries where lemon oil is extracted by the primitive sponge method, the resulting endocarp (after the rastrello knife has been used) is crushed between two wooden rollers to break the partitions and cells. The crushed pulp is placed into large straw-woven filtering mats and packed down as tightly as possible, one above the other, in upright presses. The expressed juices from both the crusher and the presses are collected in large wooden tanks. In modern industries, where the oil is expressed by mechanical rasping machines, the fruits are coarsely torn by a series of circular cutting knives and thoroughly bruised by a set of wooden roller-crushers; at

that stage some juice is expressed. The crushed fruit then enters a continuous screw press where most of the juice is removed (so-called "first pressing"). Subsequently the residual pulp is passed through two similar screw presses, after being previously soaked and saturated with fresh water ("second and third pressings"); the juice from the third pressing is used as maceration water for the first soaking. The combined extracts are stored together for further treatment.

To express the juices more efficiently and to eliminate the difficulties in filtering the juices owing to the very many impurities caused by the mucilaginous character of the pulp, A. H. Bennett, in collaboration with Dr. A. Ricevuto, has recently announced an important discovery forming the subject of Italian and United States patents.[7] They found that if the crushed and minced pulp receives an addition of not more than one-fourth to one-fifth of the quantity of lime necessary for complete neutralization and is left for some time before pressing (about two hours), the mucilaginous mass soon forms a more or less hard gel as a result of the formation of calcium pectate. A syneresis then occurs, with spontaneous separation of a clear liquid containing citric acid partly neutralized by the lime. The remaining mass is no longer sticky and is very easily pressed either in a screw press or in a hydraulic frame press. The solution thus obtained is remarkably free from impurities and yields a very pure calcium citrate. This method has been adopted, after many successful trials, by the Italian Camera Agrumaria; the authors claim that their process should render it possible for citrate made of citrus to compete with the biological product described later in this section.

The expressed juices must next be properly purified in order to yield citrate of lime of high quality. The general practice is to let the juices undergo fermentation to remove completely the carbohydrates, to liquefy some of the mucilaginous, slimy constituents, and to coagulate the colloidal matter. The fermentation of sugars causes hardly any loss of citric acid; however, when using the Bennett-Ricevuto process, fermentation can be avoided. After fermentation, which takes some 4 to 5 days, the juices are put through a suitable filter (a continuous Oliver-type filter or any plate-and-frame filter press may be used) after the addition of a filter aid, such as kieselguhr, fuller's earth, or Filter-Cel. The present practice is to filter the whole juice after boiling it with about 12 to 20 kg of Filter-Cel for each 1000 liters of juice.

[7] Bennett, A. H., and A. Ricevuto, "Nuovo Metodo di Preparazione del Citrato di Calcio," *Riv. Ital. Essenze*, 19 (No. 2), 44 (1937).

(b) Precipitation of Calcium Citrate

The second phase of citric acid manufacture—namely, the precipitation of citrate of lime—is probably the most important phase of the entire production and, unless care is taken at each step, considerable losses may occur or the final product may be of a very poor quality.

The citric acid contained in the juice is precipitated by milk of lime while very hot, because calcium citrate is considerably more soluble in cold water than in hot water. Therefore, all the operations comprised in this phase of production must necessarily be performed while the solutions are near the boiling point. Accordingly, a given charge of the juice is measured into a wooden tank, where it is heated with direct steam to at least 80° C. If preferred, the juice may be heated by closed steam coils; however, these should be made of stainless steel or at least of block tin, to avoid incorporation of heavy metals such as iron or copper. The amount of calcium required to precipitate the citric acid is calculated following a laboratory test of the juice.

For each 100 kg citric acid add:

45 kg CaO
or 57 " $Ca(OH)_2$
or 80 " $CaCO_3$

TABLE XXX

Necessary Quantities of Calcium for Precipitation of Calcium Citrate*

Citric acid	$CaCO_3$	$Ca(OH)_2$	CaO
1	0.715	0.529	0.401
2	1.429	1.058	0.801
3	2.144	1.588	1.202
4	2.859	2.117	1.602
5	3.574	2.646	2.003
6	4.288	3.175	2.403
7	5.003	3.704	2.804
8	5.718	4.234	3.204
9	6.433	4.763	3.605
10	7.147	5.291	4.006

* Parts by weight. For any other quantity of citric acid, multiples of these amounts can be taken.

Ninety per cent of the required amount, in the form of milk of lime, is then added slowly to the juice with constant stirring. The milk of lime should be prepared from hydrated lime of very high purity and brought to a boil before being run into the juice. The

hydrated lime should contain no iron or magnesia; in the presence of magnesia, citric acid will be lost, because of the fact that magnesium citrate is soluble. The stirring may be done by a mechanical agitator (not iron) or, better, by compressed air. The remaining 10% of the acid is neutralized by the addition of sufficient calcium carbonate; it is even desirable to have a slight excess of the carbonate. The reason for completing neutralization with calcium carbonate instead of with milk of lime lies in the fact that if any iron is present it will remain in solution as long as it is acid, while with milk of lime at an alkaline *p*H value it will go into the precipitate. If this very important point is neglected the calcium citrate will be of very poor quality. Juices completely neutralized with calcium hydroxide give rise to dark-colored compounds which are difficult, if not impossible, to wash out. On the other hand, however great an excess of calcium carbonate is added to the juice, there is always a small residual acidity, varying from 0.08 to 0.20% and depending on the acidity of the original juice.

To ascertain whether precipitation is complete a measured portion is titrated against 0.1*N* NaOH, with phenolphthalein as indicator. A fairly correct judgment can be made by adding a little $CaCO_3$ to a sample drawn from the batch: if no effervescence is produced the reaction is complete.

When the precipitation is completed the whole is heated for a few minutes nearly to boiling point; this causes the citrate of lime to become crystalline and subsequently to settle rapidly in the tank, leaving a clear yellowish supernatant liquid, which is immediately siphoned off. The residual citrate is then washed with boiling water and pumped through a plate-and-frame filter press and again thoroughly washed with water at as near 100° C as possible.

If worked directly into citric acid the still wet citrate of lime is transferred to the decomposition tank. When, however, it is not worked immediately the precipitate should be thoroughly dried at this point. Failure to do so may completely destroy the citrate of lime, which has a great tendency to ferment, whereby the citrate is converted into a carbonate. It has been demonstrated that fermentation readily takes place if more than 12% of water is present, and that unless the citrate of lime is dried to below 5% water content it is exceedingly difficult to preserve the citrate without deterioration. Efforts should, therefore, be made to secure thorough drying by means of a drying chamber with sufficient ventilation or in a suitable continuous drier where heat is supplied by closed steam coils at a temperature above 100° C, preferably at 120–150° C.

It may be worthwhile to summarize the important points in the preparation of calcium citrate: (*1*) thoroughly filtering the juices; (*2*) keeping both juice and milk of lime as hot as possible; (*3*) using only highly pure lime, free of Fe and Mg; (*4*) neutralizing 90% of the acid by milk of lime, the remaining Ca being added in the form of a carbonate; (*5*) washing the precipitate thoroughly with boiling water; (*6*) drying the calcium citrate quickly and thoroughly; (*7*) avoiding contact with iron throughout the work.

If citric acid is not manufactured on the spot, the dry citrate of lime is packed in bags or hogsheads and is marketed usually as a product of standard quality, containing 64% or more of citric acid. The citrate must not contain over 2% of free chalk. Commercial citrate of lime of exactly 64% strength signifies a content of 86.892% of pure calcium citrate with 4 moles of H_2O and 13.108% of impurities, including humidity and excess calcium; the product also contains 18.324% of calcium combined with the citric acid.

Citrate of lime procured for the manufacture of citric acid often arrives from sources which are not very reliable—mainly from countries where it is still made under primitive conditions. In such cases the calcium citrate must be especially purified before it is further treated for the production of citric acid. Some British patents[8] deal with the purification of calcium citrate by treating it with a solution of an alkali bisulfate, such as $NaHSO_4$, or with an aqueous solution of SO_2, the resulting solution of $CaH_2(SO_3)_2$ and citric acid then being boiled, after removing organic matter by filtration, to drive off the SO_2 and re-form the calcium citrate.

More modern methods make use of ion exchange, as previously described, mainly for the elimination of Fe by using a carbonaceous hydrogen zeolite.[9] This treatment is made, of course, after the decomposition of the commercial citrate of lime with H_2SO_4.

(c) Decomposition of Calcium Citrate and First Crystallization

To decompose calcium citrate and to reconvert it into citric acid sulfuric acid is used, the reaction being represented as follows:

$$Ca_3(C_6H_5O_7)_2 + 3\,H_2SO_4 \rightarrow 2\,C_6H_8O_7 + 3\,CaSO_4$$

A container of suitable size—a tin-lined wooden or iron tank or, better still, a glass-lined iron tank provided with a slow-moving

[8] Mellersh-Jackson, W. J., British Patent No. 137,396 (Jan. 30, 1919). Golding, H. D., J. Raschen, and United Alkali Co., British Patent No. 136,979 (Mar. 20, 1919).

[9] Cole, G. M., "Use of Carbonaceous Hydrogen Zeolite in Manufacture of Fruit Acids," U.S. Patent No. 2,253,061 (Aug. 19, 1941).

stirrer—is used. The weighed quantity of citrate of lime is suspended in water or in a dilute citric acid liquor obtained from previous operations (mainly after washing a previous batch of gypsum). To the suspended citrate, heated to boiling point and continuously stirred, 30° Bé sulfuric acid is slowly added; the mixture is finally boiled and stirred for another 30 min. The decomposition of the citrate is usually completed in about 3 hours.

The transformation of calcium citrate into calcium sulfate is deemed to be completed when the following crude test holds: to a sample of the filtered mixture 5 cc of a 45% $CaCl_2$ solution is added in equal parts; only a faint precipitate of calcium sulfate should be noticeable after warming the mixture for a few minutes, indicating

TABLE XXXI

Special Gravities of Citric Acid Solutions (According to Gerlach)

(Percentages at 15° C.)

% Citric acid	Specific gravity	% Citric acid	Specific gravity	% Citric acid	Specific gravity	% Citric acid	Specific gravity	% Citric acid	Specific gravity
1	1.0037	14	1.0549	27	1.1106	40	1.1709	53	1.2358
2	1.0074	15	1.0591	28	1.1152	41	1.1761	54	1.2410
3	1.0111	16	1.0632	29	1.1198	42	1.1814	55	1.2462
4	1.0149	17	1.0675	30	1.1244	43	1.1856	56	1.2514
5	1.0188	18	1.0718	31	1.1288	44	1.1899	57	1.2575
6	1.0227	19	1.0761	32	1.1333	45	1.1948	58	1.2627
7	1.0268	20	1.0805	33	1.1378	46	1.1998	59	1.2683
8	1.0309	21	1.0847	34	1.1422	47	1.2051	60	1.2738
9	1.0350	22	1.0889	35	1.1438	48	1.2103	61	1.2794
10	1.0392	23	1.0931	36	1.1515	49	1.2153	62	1.2848
11	1.0431	24	1.0972	37	1.1563	50	1.2204	63	1.2904
12	1.0470	25	1.1016	38	1.1612	51	1.2256	64	1.2960
13	1.0509	26	1.1060	39	1.1661	52	1.2307	65	1.3015
								66	1.3071

an excess of not more than 0.2% H_2SO_4. The gypsum is then separated from the dilute citric acid solution by filtration through a continuous Oliver filter or through a wooden plate-and-frame filter press and finally washed with *cold* water by the countercurrent principle, the first wash waters being united with the solution of citric acid. Wash waters with less than 5° Bé are used for suspending new batches of citrate. For the convenience of the operator two tables are presented, the first (Table XXXI) showing specific gravities of pure citric acid solutions, the second (Table XXXII) showing Baumé concentrations

The solution of citric acid must then be concentrated, to be subsequently set for crystallization. Concentration is effected in two stages

TABLE XXXII

Baumé of Citric Acid Solutions at 15° C (According to Roux)

Bé	% Citric acid	Bé	% Citric acid	Bé	% Citric acid
1	2	13	24	24	46
2	4	14	26	25	48
3	6	15	28	26	50
4	8	16	30	27	52
5	10	17	32	28	54
6	12	18	34	29	56
7	14	19	36	30	58
8.5	16	20	38	31	60
9.5	18	21	40	32	62
10.5	20	22	42	33	64
12	22	23	44	34	66

in either lead- or glass-lined vacuum evaporators of suitable type. The evaporating equipment described on pages 278–281 and used for the concentration of citrus juices may be used for the purpose, provided the recipient includes a so-called "salting-box" from which the heavy concentrate containing crystals may be pumped out. The first step consists in concentrating the liquors to about 30° Bé, at which stage the greater part of the still-dissolved $CaSO_4$ precipitates and (after cooling) is subsequently filtered off in a filter press, the residue being thoroughly washed again. After filtrating the citric acid liquor the second stage of the vacuum concentration takes place in the same or in a second similar evaporator. This time, concentration is carried on until a sample shows a concentration of 40 to 42° Bé. The thick liquor is then delivered to wooden- or lead-lined crystallizers in which a good crop of crystals is set after 3 to 5 days. The crystals are then transferred to a basket centrifugal. (A standard centrifugal of the Weston type, such as used in the crystallization of sugar, is about 30 in. in diameter; for citric acid the basket should be made of stainless steel or monel metal, and the curbs should be lined with lead.) During centrifugation the crystals are washed with a spray of cold water. Centrifugation of each batch takes about one hour.

(d) Purification of Citric Acid Crystals

The crude citric acid obtained by the foregoing process still contains some impurities, mainly organic color, heavy metals (such as lead, copper, tin, and iron), some occluded sulfuric acid and mineral ash, and in particular, calcium sulfate. To eliminate these foreign substances the crude crystals are dissolved in warm water by placing them in a perforated lead basket suspended at the top of a tank; the

heavy solution goes to the bottom while the most dilute solution always touches the surface where the crystals are continually dissolved. The solution is then treated with 1 to 2% of very active carbon to remove the color (Norit superneutral being the best for this purpose), the liquor being slowly warmed to about 70° C. The completion of the decolorization is tested by filtering a small sample.

Before filtering the liquor it should be suitably treated for the removal of heavy metals. Lead, copper, and tin can easily be removed by precipitation as sulfides. Iron, on the other hand, can be precipitated by calcium ferrocyanide; the required amount of this chemical is calculated by a careful test in the laboratory. Only 90 to 95% of the iron should be precipitated as Prussian blue, care being taken that no excess of calcium ferrocyanide is introduced into the liquor. The best procedure, however, for removal of iron is the use of the ion-exchange method previously described in this section.

After the preceding treatments have been completed the liquor is thoroughly filtered through a plate-and-frame filter press and returned to the vacuum pan for a two-stage evaporation, as described on page 278. Calcium sulfate is then eliminated by an additional filtration immediately upon withdrawal of the liquor from the vacuum evaporator, after the desired degree of concentration has been reached. The clear filtered liquor, free of impurities, is then finally concentrated in vacuo and crystallized, preferably in shallow wooden or aluminum tanks. After 3 to 5 days the crystals are removed, transferred to the centrifuge, washed with chilled water, and spread on a clean floor to evaporate surface moisture. In wet weather it is necessary to use artificial drying; in that case, the best device is a vacuum shelve-drier.

The mother liquors are concentrated again up to 40–42° Bé and further crops of crystals are obtained. When the mother liquors become enriched with impurities they are again precipitated with calcium into citrate of lime, which again enters the cycle of operations.

When small granular crystals are desired the liquor set for crystallization is kept in constant slow motion by means of a small air jet or mechanical agitators. Citric acid crystals are usually graded by means of a series of vibrating screens and are then packed in wooden kegs of about one hundredweight.

5. Mycological Method

Before World War I, Italy was the chief producer of citrate of lime, supplying 90% of the world's requirements. During that war

and immediately afterward the new method of producing citric acid by fermenting sugar-containing materials was commercially developed in many European countries and in the United States. An international citric acid agreement has since been reached between England, France, Italy, Belgium, and Czechoslovakia to regulate exports and to maintain prices of citric acid produced from lemons as well as that processed mycologically.

The mycological method has been only very briefly mentioned on page 106; however, in spite of the great amount of research carried out on this subject, the true mechanism of the formation of citric acid by fungi is still obscure, and no one theory presented to date satisfactorily explains all the observed facts.

Various sugar-containing materials have been used for fermentation by fungi into citric acid; however, sucrose solutions, such as molasses, are mainly used as the starting material. One patented process,[10] with a citric acid yield as high as 65%, makes use of glucose.

The actual commercial practice is still closely guarded as a trade secret, because the producers have devoted considerable time and money to develop strains and to study the conditions most suitable for production. The results published in scientific and technical literature have been obtained mostly on a laboratory scale and are often contradictory, owing to the fact that many investigators have used strains of *Aspergillus niger* which were not satisfactory to the particular medium.

In commercial practice solutions containing from 15 to 20% of sugar are used. More highly concentrated solutions are unsuitable for the growth of *Aspergillus*. The solutions must be thoroughly sterilized before inoculation with the selected strain, to avoid the growth of other undesirable microorganisms. To the sugar solution an appropriate amount of some nutrient salts are now added, according to the particular strain of fungi and to the carbohydrate source used. In general, the addition of nitrogen, phosphate, and magnesium is necessary. Nitrogen is supplied in quantities of 0.01 to 0.08% by adding ammonium salts or nitrates. Low nitrogen content apparently favors the production of acid, while a high nitrogen percentage results in abundant growth of the mycelia with low acid content. Phosphorus is usually supplied by potassium acid phosphate (about 0.15 to 1.0 g per l) and magnesium is added in the sulfate form (about 0.15 to 0.2 g per l).

It is now necessary to add the organisms. Although the early re-

[10] Channey Chemical Corp., British Patent No. 491,534 (Sept. 5, 1938).

searches on the subject were made with various species of *Citromyces*, today certain strains of *Aspergillus niger* are mostly used which, as already mentioned, are usually kept secret. However, by careful selection it is not very difficult to adapt an appropriate strain.

Since the fermentation process for the conversion of sugar into citric acid requires an abundant supply of air, it is customary to use shallow aluminum pans about 5 to 7 cm deep with a surface of one square meter. If fermentation takes place in a closed system air must be blown slowly through it, but care should be taken to avoid quick evaporation of the fermenting liquid; in other words, the rooms in which fermentation takes place should be kept at a relative humidity as near 100% as possible. Attempts to produce citric acid in a closed system by submerged cultures have so far failed, the reason not being definitely known. It is obvious that submerged fermentation would give an enormous saving in space, compared to the shallow pan system.

The submerged culture fermentation method as used, for instance, in the production of penicillin has been recently applied for citric acid on a laboratory and semipilot-plant scale.[10a] A strain of *Aspergillus wentii* was found to produce, under definite balance of minerals in the medium and under highly aerobic conditions, citric acid with complete absence of oxalic acid. When a portion of the citric acid in the process of production was neutralized with calcium carbonate, higher yields resulted.

It is further thought that some derangement in the enzyme system may be responsible for the rather long duration of the fermentation process, which usually takes some 8 to 10 days. One may assume that the accumulation of the acid formed retards further progress of fermentation. A high *p*H of the substrate also favors the production of oxalic acid and encourages contamination by foreign organisms. It is possible, therefore, to carry out the fermentation process continually in very long channels in which, at one end, the fresh sugar solution is added drop by drop, while at the other end, using a constant-level arrangement, the acid is likewise collected. The arrangement has to be calculated so that the rate of travel of the substrate through the entire system will take several days. It is most probable that such an arrangement will considerably shorten the period of fermentation, since the converted substrate is in this manner continually eliminated from the system. A somewhat similar arrangement has been

[10a] Karow, E. O., and S. A. Waksman, "Production of Citric Acid in Submerged Culture," *Ind. Eng. Chem.*, **39**, 821 (1947).

described by Frey,[11] who uses an apparatus consisting of superimposed trays which communicate by pipes, a point near the top of each tray connecting with a point near the bottom of the tray below.

Fermentation, in commercial practice, is carried out at a temperature between 25 and 35° C, depending mainly upon the particular strain of organism used. At any rate, the temperature must be constant during the entire process which, for this reason, has to be carried out in large rooms subjected to automatic thermostatic control.

While some of the investigators working under laboratory conditions report yields as high as 90 to 100%, based on the sugar consumed, the present commercial process attains a yield of only about 60%.

The necessity of using a great number of trays for fermentation makes the process rather cumbersome and, for this reason, means have been sought to ferment sugar-containing materials without previously extracting the sugar in the form of a solution. This rather unusual method has been developed by Cahn,[12] who makes use of finely shredded carbohydrate-containing materials such as sugar beets, or impregnates cane bagasse with blackstrap molasses. Stern[13] uses broken carob (locust-bean pods) from which the kernels have been eliminated, or minced citrus peels after the extraction of both essential oils and juice.

Citric acid fermentation on solid materials takes only two to three days. This fact is of great importance, for during so short a period no foreign organisms can grow on the material in question, as the acid which develops on the first day suppresses the growth of other bacteria. This precludes the necessity of sterilizing the mass prior to inoculation with the fungi. The shredded mass is mixed with a selected strain of *Aspergillus niger,* spread on wire screens to a thickness of 5 cm (the screens being preferably bakelite-varnished), and set for fermentation. The easy access of air causes the fungi to grow inside the cake; thus, the fermentation is completed sometimes in as little as 48 hours. The fermented mass is then leached out with water and pressed in a continuous screw press. If bagasse or the solid residue of carobs is used as an absorbent for molasses it can be used over again several times. Such use of the spent solid material has a number of advantages: a certain amount of sugar remains undecomposed,

[11] Frey, A., German Patent No. 567,071 (Mar. 30, 1933).

[12] Cahn, F. J., "Citric Acid Fermentation on Solid Materials," *Ind. Eng. Chem.,* 27, 201 (1935).

[13] Stern, F., and Jaf-Ora Ltd., Palestine Patent No. 3349 (Feb. 10, 1946).

which thus has a chance of being utilized during the next working cycle, and the residue is full of the active fermenting agent, which accelerates the next fermentation cycle.

Whatever method is used for fermentation, the resulting acid liquor is treated in exactly the same way as lemon juice—namely, heated to boiling and the citric acid precipitated as citrate of lime.

It is very important to remember that failure to obtain a proper fermentation and a satisfactory yield often depends on metallic impurities contained in the sugar solutions. In such cases it is strongly advised to investigate the reason and to select an ion-exchange base for the removal of the cation which hinders the fermentation. By careful experimentation in this direction excellent results may be obtained with the mycological process of citric acid fermentation.

6. Lactic Acid

In recent years attention has been called to the possibility of producing lactic acid from citrus surpluses. A method has been worked out[14] for preparing calcium lactate and lactic acid from cull grapefruit unsuitable for canning or as fresh fruit. It consists in fermenting the pressed and screened juice by a lactic acid bacterium of the *Lactobacillus delbruckii* type, normally present on the fruit. The citric acid present in the juice is previously neutralized by adding a calculated amount of calcium carbonate, thus producing calcium citrate which can be conveniently allowed to remain in the juice during fermentation. This is carried out at 50° C (122° F) and the lactic acid produced is periodically neutralized by calcium carbonate because the activity of the lactobacilli is inhibited when over 1.5% of lactic acid is set free. The fermentation requires from 6 to 8 days, the yields of lactic acid ranging from 71 to 84% based on sugar converted. The calcium citrate is then filtered off hot, the filtrate concentrated to 25° Bé, and the calcium lactate crystallized from the concentrated liquor.

D. UTILIZATION OF CITRUS PEELS

1. Problems of Disposal of Exhausted Peels

In previous sections the extraction of citrus oils from the epicarp and of citrus juices from the endocarp has been described. There now remain the exhausted peels, which in many existing plants form a

[14] Nolte, A. J., and H. W. von Loesecke, "Possibilities of Preparing Lactic Acid from Grapefruit Juice." *Fruit Products J.*, 19, 204 (1940).

troublesome waste very difficult to dispose of. Huge quantities of peels accumulate in the yard; soon they begin to rot, creating a serious nuisance to the vicinity and a real danger to the sanitation of the plant itself, unless they are removed daily. However, transportation of the waste to remote unpopulated areas is an expensive and difficult undertaking.

2. Dehydrated Peels

When the essential oil is not extracted before the juice, a certain quantity of peels may be dehydrated and sold for various purposes—for medicines, for cooking, and for confectionery. The so-called *Corlex aurantium,* sold by pharmacists since remote times, is an example.

To dry orange or lemon peels for this purpose, they must be cleaned and separated from the inner tough membrane which separates the albedo from the segments. This operation can be done only by hand. The peel, cleaned in this way, is then cut into suitable pieces with a plain knife or by means of a sharp die made in various forms: stars, half-moons, or circles. (These are commonly used, after impregnation with sugar syrup and drying, to prepare the so-called *Arancini.*) The market often requires the peel to be cut into strips, which can be easily obtained by peeling the whole fruit by special machines (Fig. 97). Regardless of the way the peel is cut, it is then dried in the sun or spread on trays in a tunnel, as in the case of vegetables. The temperature of such tunnels should not exceed 60° C, if a satisfactory product is expected.

On the island of Curaçao (Dutch West Indies) the peels of the bigaradier (*Citrus aurantium amarum*) are dried in the sun and used to prepare the famous Curaçao liqueur.

3. Peels in Brine

Certain varieties of citrus fruit, in particular the citron, are specially cured and preserved in brine. Citrons, which have a delicious fragrance when ripe, are entirely unfit for food in their natural state. In the Mediterranean region citron peels are extensively packed in the following manner, as practiced on the island of Corsica.

When the fruit is full grown, but still has a greenish tinge, it is cleaned and cut lengthwise in halves (Fig. 102), and the seeds and pulp removed with the rastrello knife (Fig. 37). Soon after this the peels are placed in casks full of sea water. Mediterranean sea water contains some 3.7 to 4% of salts and is best suited for this process.

Sea water may, however, be replaced by a solution of the following composition (g per liter):

sodium chloride (NaCl)	27.0
potassium chloride (KCl)	0.75
magnesium chloride ($MgCl_2$)	3.65
magnesium sulfate ($MgSO_4$)	2.30
calcium sulfate ($CaSO_4$)	1.40

Immersed in brine, the citron peels begin to ferment and give off CO_2. It has been suggested[15] that this fermentation is caused by the

Fig. 102. Longitudinal section of citron.

action of both yeasts and bacteria, the specific yeast being *Saccharomyces citri medicae* and the bacteria, *Bacillus citri medicae.* After the fifteenth day the water in the casks is changed, the entire process taking some 30 to 40 days until completion.

In a very similar way orange and grapefruit peels can be cured. The desired result in curing or pickling the peels is to obtain a firm yet tender rind which becomes transparent while its bitterness dis-

[15] Chadefaux, S., "La Fermentation des Cédrats," *Rev. Hort. Algerie,* **28**, 214 (1924).

appears. When the curing comes to an end, 7 to 10% of salt is added to the sea water and in this brine the peels are shipped to the factories for the candying process.

Before candying, the peels are removed from the brine and washed in flowing water. They are then gently boiled until the rind becomes sufficiently tender. To restore their crispness the peels are then kept for 24 hours in cold water and finally impregnated with sugar by long immersion in syrup of successively increasing density. Starting with a syrup of 18° Brix, the peels are usually taken out of a syrup which is very thick when cold (80 to 84° Brix). The candied peels are then dried and in this form can be preserved for a very long time.

4. Ensilage of Citrus Peels

The foregoing uses for exhausted citrus peels, although remunerative, can absorb only a very small portion of the peels discarded by an average plant and only a negligible portion of those of a large factory. Obviously, the methods described above cannot solve the problem of disposal of huge quantities of peels resulting from the daily operations of a citrus products plant. The only solution for the utilization of this surplus is the production of citrus meal to be used as cattle fodder.

Extensive experiments have been carried out with some success in Palestine for the ensilage of fresh citrus peels. The peels are finely cut and then ensilaged in pits in alternate layers of peel and straw. They are then covered with straw and earth and stored in this way for several months. In these pits the fresh peels undergo a lactic acid fermentation, following which they can be fed to cattle. To insure the success of the ensilage it is necessary to add some inorganic acid. During fermentation the *p*H value of the pulp increases while the amount of pectin slightly declines. The ensilage when ready contains considerable amounts of lactic acid but is nearly free of butyric acid. According to Bondi,[16] the nitrogen-free extract and sugar of both orange and grapefruit peels ensilaged for 8 months showed a decline and the ensilaged pulp was free of spore-forming rods. The digestibility coefficient for N-free extracts exceeded 90%. The feeding value of such pulp is about 16% higher than that of whole fruit, and, according to Bondi, compares well with that of clover, corn, alfalfa, and beets.

[16] Bondi, A., "The Ensilage of Citrus Fruit Pulp," *Empire J. Exptl. Agr.*, **10**, 89 (1942). Bondi, A., and J. Meyer, "The Digestibility of Citrus Feeds," *ibid.*, **10**, 93 (1943).

5. Dry Citrus Meal

(a) Processing

The common method of utilizing exhausted citrus peels is to dry them for the preparation of dry citrus meal. The use of dried citrus

Fig. 103. Rietz disintegrator for mincing peels (courtesy Joseph Wagner Manufacturing Co.).

peels for feeding livestock was originally suggested by F. A. McDermott, who held a Florida Citrus Exchange fellowship at the Mellon Institute in 1916. The first commercial dehydration of citrus peels was achieved by a comparatively small plant in Los Angeles,

California; it brought tribulation to the owners, for although the process of drying citrus peels is ostensibly a simple one, actually it is rather complicated, both chemically and physically. It is now successfully practiced in the United States and Israel.

The peels are first crushed or minced in powerful disintegrators of suitable size (Fig. 103). Various types of machinery are used for this purpose, such as large-capacity meat mincers, hammer mills, or any other suitable cutters. The finely cut peel forms a very sticky and wet mass and cannot be directly dried. It contains an appreciable amount of pectin which makes it sticky; if dried in this form case-hardening would result—i.e., a product dried only superficially, the inside remaining wet. Such a product is worthless, for the occluded humidity will soon begin to transfuse through the outside dry crust. To destroy this effect of the pectin the reduced peels must be properly treated with alkalies or alkaline earths, such as calcium or magnesium. This process is often called "liming" and has been patented in various countries at different times by a number of authors.[17] Usually milk of lime (or powdered lime) is added continuously to the crushed slimy citrus waste, which, after standing for a couple of hours, gives rise to syneresis; thus, the peel becomes easy to press. The whole is now passed through a continuous screw press where the so-called *peel juice* is separated from the pressed peel. This effluent, which is neutral or slightly alkaline, contains some 9% of sugars and can either be fermented into alcohol or concentrated to a syrupy consistency in the form of so-called "citrus molasses."

Before proceeding further, it may be worthwhile at this juncture to draw up a balance of the main constituents of the peel in relation to the whole fruit.

100 kg of fruit deliver, on the average:

35 kg juice for beverage
35 kg effluent from peels
30 kg still-wet peel which, when dried, results in 9 kg dried fodder

Thus, it is necessary to remove, by drying, some 20 kg of water for each 100 kg of original fruit. On the other hand, it is obvious that

[17] Bennett, A. H., and A. Ricevuto, "Nuovo Metodo di Preparazione del Citrato di Calcio," *Riv. Ital. Essenze,* 19 (No. 2), 44 (1937); also U.S. Patent No. 2,072,530 (Mar. 2, 1937). Braverman, J. S., and Jaf-Ora Ltd., "Improvements in or Relating to the Drying of Citrus Fruit Peel," Palestine Patent No. 1471 (Feb. 13, 1939). Lissauer, A. W., and Julius Credo, "Citrus Feed," U.S. Patent No. 2,362,014 (Nov. 7, 1944).

the dry meal still contains some 30% of sugars. If properly treated the proccessed peel will, in fact, yield the results indicated above.

The pressed peel still contains, therefore, some 60–65% of water (calculated on the basis of press-cake) which has to be removed by heat. The water is best removed in a rotary drier consisting of a revolving iron cylinder about 12–14 meters long and $1\frac{1}{2}$ to 2 meters in diameter (Fig. 104). Such driers are usually horizontal although sometimes they may be slightly inclined. The wet peel is fed at the upper end, at the point where the hot furnace gases enter the drier; owing to the rotation of the drier, the peel advances progressively to the lower end where it is discharged continuously. In these direct-

Fig. 104. Rotary drier as used for drying citrus meal.

heat revolving driers the flue gases, mixed with sufficient amount of air, are drawn through the cylinder (parallel to the flow of the material) by powerful exhausters placed at the far end of the drier. The most common form of rotary driers have, in addition, longitudinal shelves which carry the material halfway around the circumference and drop it in the central part of the cylinder, where the air is hottest and is least saturated with moisture. The rate of feed and the speed of rotation must be regulated in accordance with the length and the diameter of the drier, so that the peel is dried just before discharge. A convenient speed of rotation is about 6 to 8 rpm. At the end of the drier a sufficiently large dust collector is placed to receive all light particles carried away by the blower. The rate of drying citrus peels differs with driers of various sizes and types of construction, but usually it takes not more than 20 minutes from the time the peel enters the drier. Use is made also of driers indirectly heated by steam.

The dried citrus meal is now ready for bagging.

(b) Feeding Value

The first investigations concerning the feeding value and digestibility of dried citrus pulp were undertaken by the University of California in 1925. Further systematic tests conducted by experiment stations in Texas, California, Florida, and Palestine[17a] have proved that the feeding value of dried citrus peel and other cannery waste is much the same as that of dried beet pulp, and that it compares favorably in heat units (called therms) and in net energy with shelled corn. Table XXXIII presents comparative values in detail.

TABLE XXXIII

Content of Citrus Pulp Compared with Various Carbohydrate Feeds (Percentages)

	Net energy	Total digestible nutrients	Protein	Fat	Fiber	Nitrogen-free extract
No. 2 shelled corn	80.0	80.6	9.4	3.9	2.2	68.4
Citrus pulp	79.0	75.8	4.9	1.1	11.9	69.6
Threshed Kaffir	75.2	80.1	11.2	3.0	2.3	70.3
Threshed milo	75.2	79.9	11.2	2.9	2.2	71.2
Oats for dairy cattle	71.3	71.5	12.0	4.7	10.6	60.2
Beet pulp	70.5	71.8	9.0	.8	18.8	59.9
Gluten feed	69.4	77.6	25.8	2.5	7.3	48.9
Wheat bran for dairy cattle	66.0	70.2	15.8	5.0	9.5	54.3
Brewers' dried grains	62.0	65.3	25.6	6.7	14.8	42.0
Rice bran	60.0	67.7	13.4	12.8	13.0	41.1

Chemical analyses of dried citrus peels show that they have low protein and fat values, while their carbohydrate content is very high. They contain, on the average, 6% of crude protein, a little over 1% of fat, about 10–15% of cellulose, and 65% of nitrogen-free extract, over 30% of which are sugars. Table XXXIV gives a comparison of chemical analyses of citrus meal from various sources.

The minerals present are mainly phosphorus, potash, and lime. According to a determination by the Armsby and Fries method, the net energy value is 91.59 therms, in contrast with beet pulp 74.57 therms, wheat bran 50.91 therms, and alfalfa hay 36.92 therms. Although the pulp and meal are employed for general animal-feeding, their largest outlet is for dairy feeding, in which sphere they have given excellent results in milk production and in conditioning dairy cows. The most notable attractions of citrus meal and pulp for dairy cows are palatability, digestibility, energy value, and laxative qualities.

[17a] See bibliography at the end of this chapter.

TABLE XXXIV

Comparison of Chemical Analyses of Dried Citrus Peels (Percentages)

	Florida		Texas	California		Palestine	
	Oranges	Grapefruit	Grapefruit	Oranges	Lemons	Oranges	Oranges*
Crude protein	5.84	4.94	6.0	7.70	6.39	5.38	5.79
Crude fat	0.69	1.06	4.8	1.68	1.23	1.11	1.19
Water	13.95	8.23	—	12.50	7.10	7.05	—
Ash	4.13	4.23	—	3.35	5.04	7.61	8.19
Cellulose	10.64	11.94	10.5	7.81	15.00	16.34	17.58
Nitrogen-free extract	64.74	69.60	62.0	66.96	65.24	62.51	67.25
Total sugars	—	—	—	—	—	31.35	33.73
Glucose	—	—	—	—	—	24.75	26.63
Invert sugar	—	—	—	—	—	6.60	7.10

* Calculated on the basis of dry matter.

Of still greater interest are the digestibility trials carried out in Florida. These showed that high nitrogen-free extract is 88 to 92% digestible. The total digestible nutrients per hundred pounds of dry matter are 82.80 and 80.82 pounds for grapefruit and orange refuse, respectively. Table XXXV gives a concise résumé of the Florida digestibility trials, showing the coefficients of digestibility and the digestible nutrients.

TABLE XXXV

Composition, Coefficients of Digestibility, and Digestible Nutrients (Per Cent) of Citrus Refuse

	Dry matter	Crude protein	Crude fiber	N-free extract	Crude fat	Ash	Total digestible nutrients
Dried orange pulp							
Composition	86.05	5.84	10.64	64.74	0.69	4.13	—
Coefficients of digestibility (average)	—	36.57	93.91	88.51	6.59	—	—
Digestible nutrients	—	2.14	9.99	57.30	0.05	—	69.55
Dried grapefruit refuse							
Composition	91.77	4.94	11.94	69.60	1.06	4.23	—
Coefficients of digestibility	—	24.83	71.52	92.43	79.37	—	—
Digestible nutrients	—	1.23	8.54	64.33	0.84	—	75.99

Summing up the conclusions of these investigations, the workers of the Florida Agricultural Experiment Station say: "The results of the digestion trials placed these feeds in the class of high carbohydrate concentrates. . . . Dried grapefruit and orange cannery refuses have a laxative action when fed as a large proportion of the ration. General effects of the dried grapefruit refuse were favorable as indicated by thrifty appearance, gloss of the coat of hair, and improvement in thickness of flesh."

6. Dried Citrus Pomace

Citrus peels and especially those of grapefruit are a rich source of pectin, which fact can be utilized by drying the peel from cannery waste and converting it into a pomace. Such pomace can be easily stored and can, therefore, be used by pectin manufacturers or directly by producers of jams and jellies. It can be manufactured quite easily if only certain precautions are taken; first, to remove as far as possible the bitter glucosides naturally occurring in the peel and, secondly, to inactivate the natural pectolytic enzymes which otherwise will degrade the pectin on storage and finally destroy its jellifying properties.

Any seeds or pulp adhering to the peels should be removed by means of a shaker. The peels are then minced to shreds approximately 6 mm (¼ in) in diameter in order to facilitate the extraction of the bitter principle. The best way to inactivate the pectolytic enzymes is to steam thoroughly the shredded peel for several minutes. However, it can also be plunged, immediately after mincing, into boiling water for five min and stirred continuously. The water is then drained off and replaced at once by cold water, and these operations of successive boiling and hot water are repeated for two further treatments (2 min each). During the final washing, 0.1% of aluminum sulfate, $Al_2(SO_4)_3$, may be added to facilitate the pressing of the washed peel.[17b] (In this case the finished dry pomace should not contain over 1% of aluminum sulfate.) The object of repeated treatments of the peel with successive batches of hot and cold water is to complete the removal of most of the water-soluble substances, among them also the bitter substances, and to render the peel crisp. The pressed peel is then dried by indirect steam in a drum drier at a temperature not exceeding 150° F (65° C) to a moisture content of about 6%. The pomace is then ground to pass completely through a 20-mesh-to-the-inch sieve.

The pectin may be extracted from this raw material by means of hot acid solutions which hydrolyze the protopectin to pectin and can be used by jam manufacturers in this form.

7. Utilization of Waste Juice (Citrus Molasses, Alcohol, Dry Yeast)

As mentioned earlier in this section, the minced peel, after having been treated with an alkaline calcium or magnesium compound, gives off upon syneresis and after pressing a considerable volume of

[17b] Pulley, G. N., E. L. Moore, and C. D. Atkins, "Grapefruit Cannery Waste Yields Crude Citrus Pectin," *Food Ind.*, April, 1944, p. 285.

effluent, very similar in composition to the peel itself, except for rag. This liberated bound water has some 10 to 12% of total solids, of which 8 to 10% are sugars.

The waste juice can be utilized in various ways. In the United States it is usually concentrated in a vacuum evaporator to a thick syrup, called *citrus molasses*. The syrup can then be added to the dried citrus meal to increase its feeding value. Evaporators for this purpose may be of the same type as described on pages 278–281 for concentration of citrus juices, but they need not necessarily be built of stainless steel, for the effluent contains no acid; evaporators made of iron are suitable. The best system to be used in concentrating citrus molasses is, however, a triple-effect evaporator, which gives a considerable saving in steam—and also there is no danger of the product being overheated.

Analysis of citrus molasses showed[17c] the following approximate composition (in per cent):

Dry matter	67 - 75
Crude protein	3.4–5.0
N-Free extract	58 - 66
Ash	3.5–5.3
Reducing sugars	21 - 32
Nonreducing sugars	15 - 20

No fiber and only a little fat was found. Dairy cows found citrus molasses highly palatable if mixed into the feed at 5 to 10% levels. Citrus molasses can, of course, be also sent to the distilleries for alcohol production.

In one plant in Florida and in Palestine the waste juice of citrus peels, mainly from oranges and grapefruit, is fermented by ordinary methods to alcohol, which is then distilled in a standard distillation column to give an industrial alcohol of 96% strength. The methods of fermenting the waste juice are similar to those described on pages 309–313 for citrus wines, except that the waste juice, being alkaline, should be slightly acidified with a mineral acid. Under no circumstances should H_2SO_4 be used for this acidification, because the lime present in the waste juice combines with sulfuric acid to create gypsum ($CaSO_4$), which will subsequently deposit on the walls and inside the tubes of the still with detrimental effects on the equipment. Nutrient salts, such as ammonium phosphate, are often added to the fermenting mash to expedite fermentation, which is usually completed in about 20 hours.

[17c] Becker, R. B., P. T. Dix Arnold, G. K. Davis, and E. L. Fotus, "Citrus Molasses—a New Feed," *J. Dairy Sci.*, 27, 269 (1944).

The resulting spirits contain rather large amounts of peel oil and fusel, which can be conveniently separated in a special decanter, shown schematically in Figure 105. Peel oil, mainly limonene, which is volatile with water vapors and is not soluble in weak alcohol, will separate first and can easily be removed with the "heads."

As explained previously in the discussion on fermentation, ethyl alcohol made from citrus peels contains a small amount of methyl

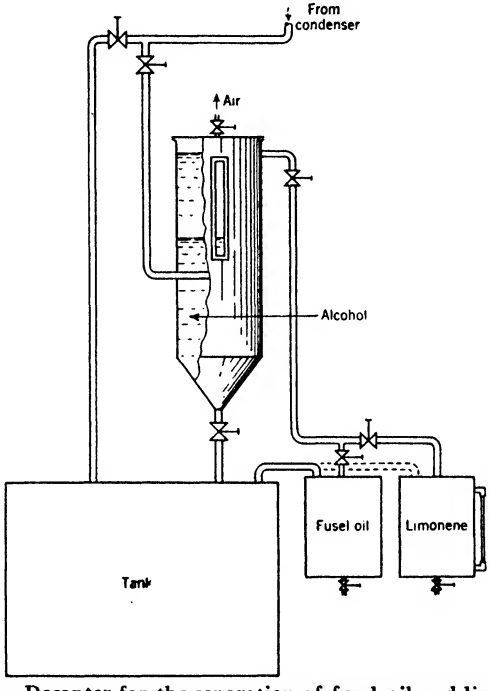

Fig. 105. Decanter for the separation of fusel oil and limonene.

alcohol which results from splitting off methoxyl groups from the pectin present in the peel juice. The rectification of citrus alcohol and the elimination of the undesirable methanol is a rather difficult problem and requires specialized apparatus. However, citrus alcohol is very well adapted for use as fuel spirits and for a number of industrial purposes.

Since one ton of fruit utilized in the plant gives, on the average, some 350 kg of peel juice with about 8% of fermentable sugars, the quantity of alcohol obtainable will amount to about 14 kg; in other words, to produce one ton of 96% alcohol about 25 tons of waste juice are required. In any event, by careful operation alcohol can be

obtained from the press juice with an average yield of over 90% of the theoretical. Considering the conditions under which such waste juice is obtained, the raw-material cost for the production of citrus alcohol is less than half that of blackstrap molasses.

Recently a process[18] has been developed for a continuous alcoholic fermentation in which sugar-containing mashes can be fermented to completion in a 5- to 7-hour cycle, yeast inoculation being necessary only at the beginning of a run. One of the principal values resulting from this process is the extensive reduction in equipment requirements.

Instead of alcohol, feed yeast can, of course, be similarly produced from the effluent of citrus peels.

The process of producing yeast consists of diluting a part of the press liquor to give a sugar concentration of about 0.5%. A suitable quantity of yeast inoculum is added, the *p*H and temperature are adjusted to 5 and 30° C, respectively, and the mass is aerated vigorously in a tank. The aeration induces the yeast to grow and produce additional cells rather than alcohol, as would be the case if there were no aeration and the sugar concentration comparatively high. As growth proceeds, undiluted juice is added to replace the sugar as fast as it is used up, thus maintaining an available supply at low concentration. Ammonia is also added gradually to supply the necessary nitrogen and at the same time to maintain the *p*H at or near 5. Phosphate, potash, calcium, magnesium, iron, and other inorganic constituents are essential for the nutrition of the yeast. If not already present in adequate amounts, these must be supplied during the culturing period. Yeast propagation continues until the working capacity of the tank is reached or until the maximum concentration of the yeast cells is reached. At this point the aeration may be stopped and the whole mass of liquid centrifuged to recover the yeast, or the fermentation may be converted into a continuous process by withdrawing liquid from the tank at the same rate that fresh liquid is added, while continuing aeration. Centrifuging yields a yeast "cream" of about 10 to 15% solids which may be dried by means of a drum drier, or a spray drier, or other suitable methods.

Yeast contains about 50% protein and is considered the richest natural source of the B group of vitamins. Because it is rich in protein and vitamins, yeast is of considerable value as a feed supplement.

[18] Bilford, H. R., R. E. Scalf, W. H. Stark, and P. J. Kolachov, "Continuous Process for Alcoholic Fermentation of Molasses," *Ind. Eng. Chem.*, **34**, 1406 (1942).

A method has recently been described[19] for the production of feed yeast with an average yield of 46% of dry yeast, based on the total sugar content of the press juice. In this method *Torula utilis* has been found to be the most suitable of the yeasts tried for this protein synthesis.

Waste juice from lemon peels can be utilized also for the recovery of some of the citric acid contained in the pulp and waste residues. The effluent resulting from liming, with *p*H 7.0 to 7.6, should first be filtered cold to remove any insoluble matter. The clear filtrate is then heated to boiling to precipitate the citrate of lime, which is insoluble in hot water. In this way an appreciable amount of citrate can be recovered.[20]

Neither the production of yeast nor of alcohol can entirely solve the sewerage problem in the citrus products plants, but the effluents obtained in these two processes have a lower B.O.D. (biological oxygen demand) than the original press juice. For methods of disposing of waste waters see Appendix I.

E. MANUFACTURE OF CITRUS PECTIN

1. General Considerations

The chemistry of pectin has been described on pages 88–96, in the discussion of the constituents of citrus albedo. Although all citrus peels contain comparatively large quantities of pectin, it would be unwise to consider that the entire peel-waste of a citrus products plant could be profitably converted into this valuable material. In the first place it has been shown that only citrus fruits not fully ripe are suitable for this purpose because as the season advances the quantity of pectin in the peel diminishes. Furthermore, the amount of pectin varies greatly with the different varieties of citrus fruits. The most suitable varieties are grapefruit, pomelo, and lemon. The manufacture of pectin cannot, therefore, be regarded as a means of getting rid of the peels; on the contrary, being rather complicated, it should be regarded as a special enterprise.

In commercial practice the resulting product is not a pure substance; its degree of purity, or rather its evaluation, depends largely on the methods of manufacture, the molecular size of the pectous sub-

[19] Nolte, A. J., H. W. von Loesecke, and G. N. Pulley, "Feed Yeast and Industrial Alcohol from Citrus Waste Press Juice," *Ind. Eng. Chem.*, **34**, 670 (1942).

[20] Leo, H. T., and C. C. Taylor, "Recovering Citric Acid from Citric Products," U.S. Patent No. 2,362,906 (Nov. 14, 1944).

stances, the degree of esterification, and the amount of accompanying ballast material present in the final product.

2. Manufacturing Procedure

Pectin, which is present in citrus peel in the form of protopectin, is insoluble in alcohol and acetone; for this reason it can be precipitated by them from an aqueous solution. The necessary steps in the manufacture of pectin are, therefore, as follows:

Preparation of materials
Removal of ballast
Acid hydrolysis of protopectin and dissolution of pectin
Precipitation of pectin
Purification
Drying of the end product

(a) Preparation of Material and Leaching

It would be advantageous to free the albedo from its flavedo. However, unless the yellow portion is removed by hand or by peeling machines such as described for the preparation of marmalades, both albedo and flavedo are used for the manufacture of pectin after the extraction of essential oils and juices. The peel is finely comminuted in a mincer and subsequently subjected to a thorough process for removing such foreign substances as coloring matter, bitter principles, residual acids, sugars, etc. Their removal can be effected in two different ways: (*1*) by thoroughly leaching the minced peel in cold water; (*2*) by means of alcohol.

When the peel is washed by water, some of the soluble pectins present also are removed. This loss, however, is of no great concern to the manufacturer because the soluble pectins are of very low jellifying power and are considered a ballast to the main portion of pectins, which at this stage are still in the form of protopectin. Cold water, therefore, does not dissolve any appreciable amount of pectin. The wash water should be continuously changed and the whole mass constantly stirred. The leaching operation is one of the most important steps in the entire process, the quality of the final product being largely dependent upon the leaching efficiency. If the sugars, for instance, are not entirely removed they may cause undesirable jellification of the concentrated pectin syrups or may cause the powdered pectin to become hygroscopic. Leaching is considered to be completed when the wash waters contain no more soluble solids from the peel.

The peel is now ready for hydrolysis, unless it is desired to store it

for a long time or to market it as raw material for the manufacture of pectin. In that case the peel is pressed in a hydraulic press and is subjected to dehydration at a low temperature (see page 357).

The second method for the removal of the ballast material is to treat the minced peel first with cold and then with boiling 95% alcohol and finally to remove the alcohol by ether, pressing the residue and thus obtaining a practically dry peel. This method is, of course, much more efficient for the removal of alcohol-soluble constituents, but undoubtedly it is much more expensive.

(b) Extraction of Pectin by Hydrolysis

After the peel has been leached it is brought quickly to boiling to destroy the pectolytic enzymes present, which have a tendency to bring about an undesirable hydrolysis of the pectin during the acid extraction. To convert the insoluble protopectin into a soluble pectin the peel is now boiled for about an hour in a weak acid. For this purpose hydrochloric acid is added to the water in which the peel was previously boiled to destroy the enzymes. The acidity of the batch is brought to about *p*H 2. Other mineral acids and sometimes tartaric or lactic acids (about 0.2% of the entire batch) are used for hydrolysis. A patented[21] process suggests the extraction of pectin from the ground peel at 80 to 90° C with water acidified with sulfur dioxide.

The solution now becomes more and more viscous, for the resulting pectin dissolves in the acidified water. Several extractions are necessary to extract the bulk of the pectin from the peel. Extraction is usually performed in wooden tanks fitted with steam coils. Some authors have suggested that hydrolysis be performed under pressure in autoclaves; however, most manufacturers proceed with hydrolysis under atmospheric pressure and at a temperature below 100° C. Extraction having been completed, the pectin solutions are drawn off and the residual peel is pressed very slowly in hydraulic presses, to prevent the bursting of the cloths.

The pectin solution so obtained is often treated with an extract of diastatic enzyme from a mold of the *Aspergillus group* (Taka diastase) to hydrolyze any starch present in the solution. About 50 g of such an enzyme preparation is usually sufficient for every 100 kg of pectin solution. The solution is then filtered through cloth while hot. Filtration of a viscous colloidal substance is, however, a tedious

[21] Jameson, E., F. N. Taylor, and C. P. Wilson, U.S. Patent No. 1,497,884 (June 17, 1924).

process and takes a long time. A much easier method consists in clarifying these liquors by means of a centrifugal separator. When it is not intended to obtain powdered pectin the clarified solution is evaporated in vacuo to a thick syrup, which, however, cannot contain much of the solid pectin because a 4% pectin solution is already exceedingly thick. The liquid pectin is filled in bottles, corked, and pasteurized for about 30 min at approximately 70° C.

(c) Dry Pectin

While a certain amount of pectin can be sold as a liquid, the bulk is now usually marketed in powdered form. Dry pectin powder can be produced by subjecting the concentrated pectin syrup to spray-drying. The principle of spray-drying has been described on page 290 in the discussion of the drying of citrus juices. In this case the pectin syrup is sprayed into large conical chambers through nozzles located near the top of the chamber. The current of hot air, which is preheated by specially constructed radiators, meets the sprayed liquid and removes the moisture from the tiny globules of the liquor; the instantly dehydrated solids fall to the floor of the chamber.

This method of preparing dry citrus pectin is most economical and quite satisfactory as far as quality of the final product is concerned. However, to obtain high-grade pectin use is made of the alcohol precipitation method.

By adding strong denatured ethyl or isobutyl alcohol or acetone to the concentrated pectin solution, the pectin will precipitate in stringy, fibrous, spongelike masses, as soon as the alcohol or the acetone contents exceed 60% of the whole. It is claimed that the more fibrous the precipitate seems to be, the higher the jelly strength of the pectin. The precipitated pectin is filtered off and pressed in thin layers so as to recover most of the alcohol. The pectin is then dried in the air or in a vacuum chamber, and subsequently pulverized in a suitable hammer mill to the desired degree of fineness. The main objection to this precipitation method is the difficulty of recovering all of the solvents (alcohol or acetone) used.

Recently a combination process of both spray-drying and alcohol precipitation has been patented[22] in the United States. According to this process the water extract of pectin, previously concentrated by evaporation in vacuo, is treated by adding Na_2SO_3 to reduce the *p*H to neutrality. It is then spray-dried, during which process SO_2 is set

[22] Leo, H. T., C. C. Taylor, and J. W. Lindsey, "Preparation of Dry Pectin," U.S. Patent No. 2,367,131 (Jan. 9, 1945).

free and driven off. The pectin so obtained is then added to the spent 60% alcohol obtained by precipitating another batch of pectin concentrate. The product is allowed to soak and is subsequently filtered. The filter cake, free of all nonpectinous matter soluble in water or alcohol, is again washed with a quantity of 90% alcohol and again filtered, hydraulically pressed, and dried in vacuo.

3. Various Other Methods

To avoid the use of such expensive solvents as alcohol and acetone a number of other methods have been suggested for the precipitation of pectin from its aqueous solutions. All these methods make use of the fact that various mineral salts, whose particles carry in their colloidal state an electric charge with a sign opposite to that of the positively charged pectin, are able to coagulate the pectin from its aqueous solutions. Several patents describe such procedures in detail; however, in actual practice these methods are made use of in only one or two plants, because it appears to be very difficult to remove the occluded salts completely.

In one plant in the United States the following procedure is used. After the extraction of juice and oil, citrus peels are subjected immediately to thorough steaming in order to insure total inactivation of pectic enzymes. The peel is then ground and kept in large storage tanks with the addition of SO_2 as a preservative.

From the storage tanks the peel is pumped and mixed with sufficient water, boiled to extract the pectin (the presence of SO_2 gives the material a sufficient acidity necessary for the pectin extraction), and screened over a series of vibrating screens with successively smaller openings, and finally mixed with a filtering medium and charcoal and filtered in large plate-and-frame filter presses until a quite clear and decolorized liquid is obtained.

To the filtered pectin solution, ammonia is added to bring its *p*H to 4 and sulfate of alumina is added. This operation is carried out in wooden tanks with false bottoms made of copper screens and provided with slowly moving agitators. When the precipitation of pectin has been completed, the free liquid is drained through the false bottom aided by the agitators, while the precipitated gel is collected on the screen and pressed between cloth. The pressed gel is then passed through a specially designed shredder. The pectin gel shreds are then boiled in long columns first with acidified alcohol–water solution, then with acidified isobutyl alcohol, and finally with strong water-free alcohol. The washed shreds are pressed, dried, and ground,

while the alcohol is regenerated by distillation. The grade of the pectin obtained is about 300.

Following the idea of Fellenberg (1914) and of Zoller (1918) who, in order to produce a satisfactory pectin product, recommended the use of alcohol to remove all soluble constituents such as coloring matter, essential oils, glucosides, acids, sugars, etc., P. Hirsch[23] combined this preliminary step with the hydrolysis step by proposing to use acidified alcohol, in which the hydrolysis of the protopectin into pectin proceeds freely. Thus, Hirsch combines practically all operations into one step by placing the minced peel in a long column and allowing acidified alcohol slowly to trickle through the whole mass. Most of the ballast material is thus washed away and meanwhile the protopectin is converted into pectin. The resulting product consists of practically pure cellulose and pectin. Such a product may be directly used in cooking jams, for a little more cellulose will do no harm. The commercial possibilities of such a product (it was manufactured in Palestine under the name "Cello-pect") depend entirely on how it will be accepted by the users of pectin.

The iron-exchange method, described on page 335 in connection with the manufacture of citric acid, has been reported successfully applied to the preparation of pectin, according to Myers and Rouse.[24] By this process ground citrus peel is thoroughly washed with water at 80° C to remove soluble salts, etc., about a quarter of its weight of carbonaceous zeolite (such as Zeo-Karb H) is added, and the whole is mixed and heated to 90° C to extract the pectin. The zeolite removes calcium, magnesium, potassium, etc., and the extract is separated from the ion exchanger and the peel by centrifuging. On drying the extract a dry 100-grade pectin is obtained, corresponding to some 5% of the original peel taken. The zeolite and peel are further separated by screening and backwashing. The zeolite is then regenerated with H_2SO_4 and rinsed; it can be used again.

The medical profession has lately displayed considerable interest in galacturonic acid and certain of its derivatives. A method of preparing D-galacturonic acid directly from pectin solutions, instead of from pectic acid, using the commercial enzyme (Pectinol 46 AP) has been described recently.[24a] The reaction takes about ten days and yields of 14 to 80% are obtainable.

[23] Hirsch, P., British Patent No. 453,877 (May 26, 1937); U.S. Patent No. 2,008,999; Palestine Patent No. 1376.

[24] Myers, F. J., and Rouse, U.S. Patent No. 2,323,483.

[24a] Rietz, E., and W. D. Maclay, "Preparation of D-Galacturonic Acid from Pectin," *J. Am. Chem. Soc.*, 65, 1242 (1943).

The preparation of dried citrus pomace as a source of pectin was described in the previous chapter.

4. Low-Ester Pectinic Acids

The production of high-methoxyl pectins has always been an expensive process. Whatever method is used, it necessitates the recovery of large quantities of the solvent, or removal by heat *in vacuo* of large quantities of water. All this requires large expenditures either in equipment and/or energy. In recent years much attention has, therefore, been given to developing products of greater versatility than ordinary high-methoxyl pectins, which can be produced by cheaper means.

It has been found that low-ester pectins, which are obtained from partially de-esterified pectin solutions, form gel structures through reaction with calcium or other polyvalent cations. Such de-esterification can be attained by one of three methods: by use of an alkaline base, by acid catalysis, or by enzymes (pectin esterase).

A method involving direct precipitation of low-ester pectinic acids has been recently described[24b] and it appears that appreciable concentration of such low-methoxyl pectins can be accomplished merely by mechanical means. Pectin solutions obtained from citrus waste are cooled to 13° C, followed by the addition of ammonia solution, and kept at 15° C for over three hours. The whole is then poured into about an equal volume of a 4% sulfuric acid solution. The resulting mixture with a *p*H of about 1.3 is slowly stirred to break up lumps and to ensure complete precipitation of the pectinic acid. The acid gel is then drained from the excess liquid first by screening and then by pressing in a hydraulic press to a solids content of 30%. The press cake is suspended in water and thoroughly stirred to wash away the excess acid and ammonium salts, the draining and pressing being repeated twice. The resulting pectinic acids may be dried *in vacuo* at 60° C or upon trays in a current of air at 80° C, and subsequently ground in a hammer mill to pass a 100-mesh screen. The yield is about 90%.

5. Evaluation of Pectin

Since pectin is only a generic name applied to various pectic substances with quite different degrees of jellification, the grade of a commercial pectin is expressed, therefore, as the number of parts of

[24b] McCready, R. M., H. S. Owens, A. D. Shepherd, and W. D. Maclay, "Acidic Isolation of Low-Ester Pectinic Acids," *Ind. Eng. Chem.*, **38**, 1254 (1946)

sugar one part of pectin will gel to standard firmness when the jelly is made by a definite standardized procedure.

The grade is thus determined by actually making 65% sucrose jellies with the finished pectin. A series of uniform glasses is taken for making the trial jellies. An aluminum saucepan is tared together with a suitable wooden spoon on a quick-acting torsion balance. Sufficient sucrose is weighed out on separate sheets of paper to make up several standard jelly glasses full of 65% sugar jellies. To each portion of the sugar, accurately weighed portions of the tested pectin are added in the aluminum saucepan. Sufficient water is then added, while the saucepan is on the balance, to provide for a 65% sugar jelly as well as for the loss during the quick cooking on a gas plate. The whole is thoroughly stirred and 1 cc of a 20% citric or tartaric acid is poured in. After adjusting the weight again on the balance, the syrup is poured into the jelly glass and set aside for congelation. The whole process is repeated with varying amounts of pectin, keeping all other ingredients constant. To judge the finished jellies they must be turned out of their glasses and compared. The correct consistency is tender yet crumbly: if tough and hard, too much pectin was used; if watery and having a tendency to syneresis, too little pectin was used.

To measure the percentage of deformation within the elastic limit of the jelly due to the compressive stress of its own weight, an apparatus has recently been developed by Cox and Higby.[25]

6. Uses of Pectin

The main use of pectin is in jam-making, which has been discussed in more detail on page 322. However, the use of pectin is rapidly increasing in a variety of other applications.

Pectin serves as an excellent emulsifier for various oils in water: for instance, for essential oils in flavoring extracts, for castor oil and mineral oil (liquid petrolatum) in medicinal use, and for tree-spray emulsions. Pectin as an emulsifying agent has been compared[25a] with gums of tragacanth, Karaya, and acacia, and has been found to be slightly better for cottonseed oil and equal to the above for olive oil. As an emulsifying agent for mineral oil, pectin has been shown to be clearly superior to other emulsifying agents.

Pectin is extensively used in modern food industry for a variety of

[25] Baier, W. E., "How the Citrus Industry Measures the Pectin Jelly Grade," *Calif. Citrograph*, **30**, 202 (1945).

[25a] Lotzkar, H., and W. D. Maclay, "Pectin as an Emulsifying Agent," *Ind. Eng. Chem.*, **35**, 1294 (1943).

confections, salad dressings, and for ice creams. Glue and mucilages are often also made of pectin. Viscous citrus pectin and pectates have recently been shown[26] to be excellent creaming agents for rubber latex, as well as for hardening steel. Finally, pectin has been found to be a good blood agglutinant and is used in the treatment of intestinal hemorrhages. Sullivan and Manville[27] have found that galacturonic acid, the main constituent of the pectin molecule, is necessary in the diet for a normal self-regulatory defense mechanism. They have been very successful in the treatment of diarrhea or dysentery with pectin or pectin-containing fruit.

Pharmaceutical pastes and ointment bases can be stabilized with pectin and various medicaments incorporated. Experiments showed[27a] that such pastes and bases are quite stable at room temperature but at 55° C they become discolored and gell after five months' storage.

7. Preparation of Naringin

Citrus peels contain, in addition to pectin, appreciable amounts of glucosides, some of them being of considerable commercial importance. In this category, for instance, are grapefruit residues, the complete analysis of which shows that together with some 3 to 4.5% of pectin they contain about 0.75% of naringin.

Methods have been developed by Poore[28] to recover naringin concurrently with pectin. According to these methods, the finely ground peel is heated with four parts of water at 90° C for 5 min, filtered, pressed, and again similarly heated with 2 parts of water. The combined extracts are boiled with 1% filter aid, filtered, and evaporated in a vacuum pan to one-ninth of their original volume. After cooling, the concentrate is seeded with a few naringin crystals and set for crystallization for two or three days. The resulting crystals can be recrystallized after treatment with neutral lead acetate and hydrogen sulfide in the usual manner.

After the removal of the naringin, the residue of the peels presents an excellent source for the manufacture of pectin in the manner described above.

[26] Wilson, C. W., *Rubber Age* (New York), **51**, 121 (1942); also U.S. Patent No. 2,132,064.

[27] Sullivan, J. T., and Manville, *Am. J. Health*, **27**, 1108 (1937).

[27a] Maclay, W. D., A. D. Shepherd, and H. Lotzkar, "Use of Pectin in Pharmaceutical Pastes and Ointments," *J. Am. Pharm. Assoc.*, **33**, 113 (1944).

[28] Poore, H. D., "Recovery of Naringin and Pectin from Grapefruit Residue," *Ind. Eng. Chem.*, **26**, 637 (1934).

Another method for the recovery of flavanone glucosides in general and that of naringin in particular, patented recently,[28a] consists in treating comminuted citrus peel with sufficient lime (10 lb of slaked lime and 200 gal of water) to coagulate slimy plant components, and then adjusting the alkalinity with a 25% caustic soda solution to a *p*H of about 9 to 11. After agitating the slurry for 30 min, the pulp is pressed and the extracted liquor is adjusted by addition of hydrochloric acid to a *p*H 4 to 5 and permitted to stand until crystallization of the glucoside is completed.

[28a] Higby, R. H., "Methods of Recovery of Flavanone Glucosides," U.S. Patent No. 2,421,061 (May 27, 1947); and "Method of Recovery of Naringin," U.S. Patent No. 2,421,062 (May 27, 1947).

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CHAPTER IX

CONCLUSIONS. NEW LINES OF APPROACH

Throughout the previous discussions the author has often allowed himself to make suggestions of a technical or scientific nature and even to advance theories which in many cases were probably not sufficiently warranted. The reason for doing so lies in the author's belief that in writing a book for technical men it is not sufficient merely to report various scientific and technical data, accompanied by an expression of an opposite opinion and an indication of their source. Such a procedure may be correct from the point of view of the research scientist, but it will not satisfy the practical engineer. Based on long personal experience, the writer has taken the liberty to refer to some data more or less critically. It may be said that a critical approach reflects one's personal attitude toward various methods; in fact, it *is* personal and should be so regarded.

Following this line of thought, the author desires to conclude this book with a note—again a personal one—on future developments in the citrus products industry.

It is generally conceded that at plants fully utilizing all component parts of citrus fruits, only a very slight proportion of the really valuable components has so far been fully turned to profitable use; as the saying goes, more finds its way through the back door of the factory than is really taken advantage of.

1. Vitamin C from Peel Effluent

Consider, for instance, the situation with respect to vitamin C. Citrus juices are generally regarded as a very convenient source of vitamins C and P. However, we now know that the peel of citrus fruits is much richer in these vitamins than their juices. Only 31% of total ascorbic acid is obtained by reaming the juice without the peel.[1] Furthermore, in seeking to obtain a maximum concentration of these valuable components we try to concentrate and to preserve the juices

[1] Lampit, L. H., and L. C. Baker, "Ascorbic Acid in Oranges," *Nature*, **149**, 271 (1942).

by various methods. It is, however, obvious that if the total solids of an original orange juice, for instance, are somewhere around 11 or 12% and its vitamin C content is, say, 50 mg per 100 g, the maximum concentration obtainable in practice for such a juice will be 7:1, or about 75 to 80% of total solids, with a vitamin C content of not more than 300 mg per 100 g. This concentration cannot be increased, owing to the presence of sugars. If, however, the sugars were, for instance, to be eliminated by fermentation, the concentration could then obviously be brought up probably to 20:1 or so, and in that case a product could be obtained containing 1000 mg of vitamin C per 100 g, or even more. Taking into account the fact that the peel has an even higher vitamin C content than the juice, it seems that the slop left after the distillation of alcohol from fermented peels should constitute an outstanding source of vitamin C and probably also of vitamin P.

2. Utilization of Peel

This is an example of what can be done in one particular direction, but many other possibilities present themselves, taking into account only the peel. For purposes of illustration a scheme drawn up by Dr. F. Stern (of Rehovoth, Israel) is shown, in which a number of products—some of them commercially feasible and others probably only illusory—are indicated (Fig. 106). This scheme for the utilization of citrus peels differs from the process in actual use by the introduction of the additional recovery of essential oils, lost in previous operations, as well as by further exploitation of the residue and the slop left after distillation of ethyl alcohol. The reader is invited to judge this scheme on its merits.

One can easily calculate the quantities of products that can be obtained per ton of peel:

Essential oil	1.5	kg
Terpenes	0.5	kg
Aldehydes	20	g
Methanol	1	kg
Ethanol	15	kg
Vitamin C	20	g
Citric acid	1.5–3	kg
Glucosides	1	kg

Recently Heid[2] described the possible and actual by-products

[2] Heid, J. L., "The Utilization and Disposal of Citrus-processing Wastes," *Citrus Industry*, 26, 11 (1943).

which can be produced in view of new developments and prospects in the utilization of citrus fruits. These include: ascorbic acid, vitamin P, provitamin A, oils, pectin, galacturonic acid, hesperidin, naringin, *p*-coumaric acid, rhamnose, phloroglucinol, yeast, ensilage, fuels, and fertilizers.

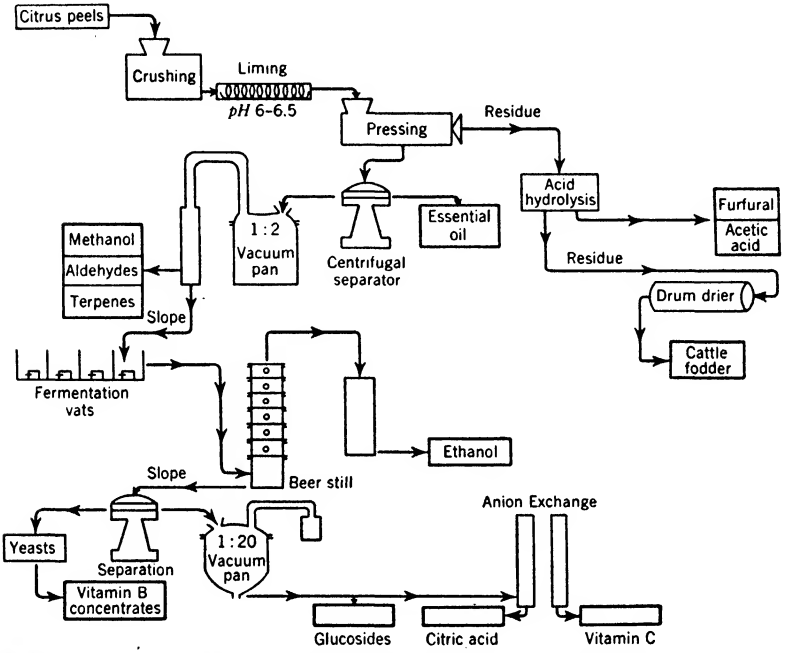


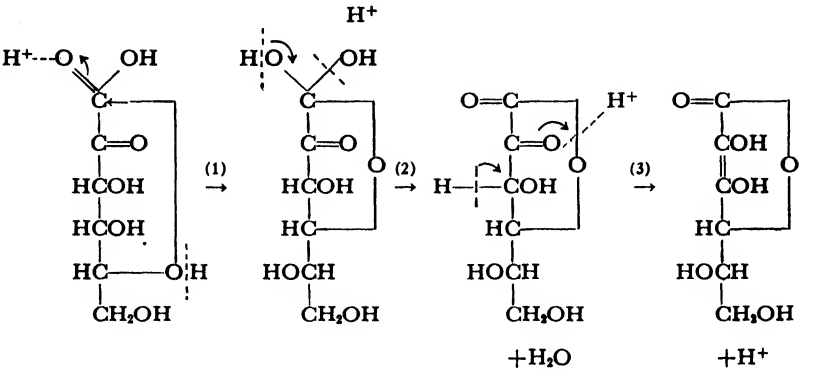
Fig. 106. Flow sheet showing possible methods for the utilization of citrus peels.

3. Converting Pectin into Vitamin C

Quite recently Isbell[2a] proposed a new synthesis of ascorbic acid from galacturonic acid. Although the starting material in this synthesis was beet pulp, it can no doubt be made from citrus peel rich in pectin. Isbell hydrolyzed beet pulp into Na-Ca-galacturonate obtaining 15 to 20% of the weight of dry pulp. Further hydrogenation yielded over 90% of sodium-*l*-galactonate and calcium-*l*-galactonate and subsequently *l*-galactono-γ-lactone. When oxidized, this latter compound yielded 25 to 30% of 2-keto-*l*-galactonic acid which was esterified into methyl 2-keto-*l*-galactonate. Finally, by lactonization and enolization, ascorbic acid was prepared with a yield of over

[2a] Isbell, H. S., *J. Research Natl. Bur. Standards*, **33**, 45 (1944).

90%. For this last step, Isbell gives the following electronic interpretation:



This most exquisite synthesis may cause wasted citrus peel to become a most important source for synthetic vitamin C in quantities six times greater than the amounts of natural ascorbic acid contained in the peel.

4. Drying by Sublimation

However, the foregoing enumeration represents only one side of the picture. Additional by-products, interesting as they may be, can be economically produced only when they are recoverable in sufficiently large quantities to warrant the investment which their production necessitates. Another rational approach is to seek new ways of improving the quality of citrus products now universally produced and primarily of citrus juices.

During previous discussions attention has often been directed to the defects of existing methods and the general striving towards the production of an ideal juice. Quite recently, novel methods—for the time being on only a laboratory or semicommercial scale—have been developed for desiccation of juices from the frozen state (drying by sublimation), thereby preserving the original natural aroma and flavor.

One method of this type has been developed by Flosdorf[3] for the F. J. Stokes Machine Company of Philadelphia. The juices are frozen in trays and the trays are put in a chamber in which a vacuum of about 200 microns is maintained by Microvac vacuum pumps. The

[3] Flosdorf, Earl W., "Drying by Sublimation," *Food Industries*, **17**, 22-25, 98, 100, 102, 104, 106, 108 (Jan., 1945).

trays are heated by hot water so that the water is evaporated out of the frozen juice while the juice remains in the frozen state. The water sublimed from the frozen juice is condensed on a surface condenser, on which the vapors are collected in the form of ice. The system is batch-operated; when the juice contained in the trays has been dried into powder, the product is discharged and the ice is removed from the condensers by melting it.

The moisture content of the product is below 1%. At this moisture content it is claimed that vitamin C losses and deterioration of flavor are practically nil for a long period of time, even when stored at normal temperature.

A process somewhat similar to Flosdorf's method has been worked out by the National Research Corporation of Boston. It functions under an even higher vacuum (2–3 microns) and the temperature of the condensers is maintained at —72° C. This method is also batch-operated, but the evaporated ice is continuously removed by scrubbing the walls of the condenser.

The two main difficulties heretofore encountered in the process of drying by sublimation are, first, to make the process continuous instead of batch-operated, and secondly, to solve the problem of vapor disposal in a more rational way than is being achieved by the methods described above, in which large quantities of ice accumulate in the condensers.

Working on the same problem, H. Smith, Jr. (of the Chain Belt Company of Milwaukee), has attempted to solve these two difficulties. His pilot plant installation consists of an endless stainless-steel belt running within a vacuum chamber in which all motors and moving parts are also enclosed. The stainless-steel belt travels on a steel plate which can be steam-heated by three heating elements which are regulated separately. The liquid to be dried is sprayed in a thin layer on the belt at one end. Owing to the vacuum the juice, which is injected at normal temperatures, freezes as soon as it is sprayed on the belts. Rapid evaporation in a very thin spray draws off five hundred and sixty calories per liter from the surroundings, thereby creating a tremendous decrease in temperature which consequently causes immediate freezing of the juice. The belt carries the juice to the other end of the vacuum chamber; during the time the belt is traveling on the steel plate, heat is applied to the latter and thereby to the frozen juice. When the juice reaches the other end of the plate, which takes about 30 sec, it has been transformed into powder containing not more than 1–2% of moisture. The powder is scraped off the end

of the belt by a knife and is discharged and packed continuously. The vacuum maintained in the vacuum chamber is not higher than 1–1.5 mm and can be produced by ordinary vacuum pumps. No high vacuums such as are required in other processes are needed here, owing to the fact that the frozen juice forms a very thin layer.

The problem of the disposal of the vapors has been solved in Smith's plant as follows: Within the vacuum chamber and beneath the plate on which the stainless-steel belt is traveling, a tank has been fitted, of about the same length and width as the belt itself. Into this tank a solution of 30% lithium chloride is circulated and sprayed. The vapors evaporating from the juice, which is being dried on the belt, condense into the lithium chloride solution, which has a freezing temperature of about —90° F, and cannot freeze in the vacuum. As the solution is continuously diluted by the vapors condensing into it, about 5% of the solution is continuously regenerated by heating.

All these methods of drying by sublimation from a frozen state have been worked out during the recent war, when extensive research was carried out in the United Kingdom and the United States on problems connected with the preservation of blood plasma. There is no doubt whatever that in a very short time great improvements will be achieved in the preservation of citrus juices in various forms.

5. Frozen Jellies

Another domain in which novel methods are being tried is the manufacture of jams and marmalades. Housewives for many centuries, and numerous factories at present, have been cooking jams according to old-established formulas, as described on pages 321–326. Recently, in the course of studies on uses for frozen fruit purées, a spread unique among jam and jelly products has been developed. This spread is nothing more than uncooked, cold-pressed, gelled and frozen fruit. The new spread retains the natural color, flavor, and nutritive value of the ripe fruit, which is ordinarily lost to a great extent in the cooking of jams and jellies; it uses less sugar, yet it has the desired texture for spreading. This new method holds considerable and as yet unexplored possibilities.

6. High-Frequency and Infrared Dehydration

With the recent advancements in physics a number of new instruments have been developed in the field of high-frequency radio energy applied to industrial high-speed heat-treatment problems. Elec-

tronic heat, according to Sherman,[4] can remove 1 lb of water per kw-hr of energy at a very low cost. The moisture content of whole-milk powder is reduced from 3 to 1% in a very short time.

In a similar way, infrared light is now used for dehydrating fruits and vegetables. Instead of the prolonged time—some 5 to 7 hours—required in the early processes, dehydration by infrared radiation requires only some 20 min. The explanation of this phenomenon lies in the fact that while in the early processes drying has necessarily been slow to avoid "case-hardening," infrared radiation penetrates the substance to be dried; thus, the evaporation of the moisture is accomplished quickly from within the material.

Special high-speed electronic heating units are now commercially manufactured; there is no reason why they should not be tried in the dehydration of citrus products in one form or another.

7. Direct Crystallization of Citric Acid

New methods of attacking scientific and technical problems renew old hopes which sometimes have been entirely abandoned. For instance, the efforts to crystallize citric acid directly from lemon juice without the necessity of first converting it into citrate of lime—a problem on which a number of able chemists have been dwelling for many years—may be mentioned. The new methods of anion and cation exchange described on pages 335–336 now simplify the elimination of all the ballast, and the direct crystallization of citric acid from lemon juice has now, in fact, been reduced entirely to a question concerned with the economics of the process.

8. Sterilization by Ultraviolet and Ultrasonics

At one time the use of ultraviolet rays was proposed for processing citrus and other juices. Ultraviolet rays exert two kinds of action: The first is a "biologic" or stimulative effect which is exerted by ultraviolet rays extending from the limit of the visible spectrum to about 290 $m\mu$ (2900A); this corresponds closely to the ultraviolet component of solar radiation at the earth's surface. The other, the "abiotic" or lethal effect, is exhibited by ultraviolet rays of wavelengths shorter than 290 $m\mu$ (2900A) which are largely absorbed by the atmosphere. Living beings on the earth evidently have adapted themselves to exposure to the longer ultraviolet rays.

[4] Sherman, V. W., "Electronics in the Food Industry," *Proc. Inst. Food Techn.*, 87 (1944).

The bactericidal region for ultraviolet rays extends from 296 to 210 mμ, with a maximum effect between 280 and 250 mμ (2500A). The penetration of these rays into organic matter is extremely feeble; their action upon the bodies of the bacteria is direct and is a function of the intensity of the radiation and of the time during which it acts. It is not due to the formation of germicidal substances, but is chiefly, if not entirely, exerted upon the protein constituent of the bodies of the organisms, the aromatic amino acids being, most probably, those which are specifically attacked.

Sterilization by means of ultraviolet rays must be performed upon a very thin layer of the substance; special apparatus have been designed for this purpose. It is claimed that while color does not interfere with the process, turbidity of the liquid, on the other hand, greatly obstructs the sterilizing action.

The use of ultraviolet rays as a sterilizing agent has, of recent years, been somewhat on the decline. Further research is probably necessary in this direction, as well as in the direction of applying ultrasonics. Beckwith and Olson[5] have experimented on the effect of ultrasonic radiation (sound waves of over 20 kH, or 20,000 frequencies per second) on *Saccharomyces ellipsoideus* and have found that after a few minutes the microflora is reduced to practically total sterility. It appears, therefore, that ultrasonic radiation is an effective agent in killing yeasts.

[5] Beckwith, T. D., and A. R. Olson, "Ultrasonic Radiation and Yeast Cells," *Proc. Soc. Exp. Biol. and Med.*, **29**, 362 (1931).

APPENDIX I

FACTORY HYGIENE AND WASTE DISPOSAL

FACTORY HYGIENE

Spoilage in citrus juices can occur even when they have been processed with care and proper attention to pasteurization temperatures, etc.; in such a case spoilage is largely due to improper sanitary conditions in the plant which are too often overlooked. The importance of high standards of cleanliness and plant sanitation in the manufacture of citrus products—as in every other food processing industry—cannot be dismissed in a book of this kind, but space limits the discussion here to general considerations only.

Plant Design. First, careful consideration must be given to sanitary requirements while designing and building the plant as well as while choosing the equipment for processing. The building should be designed from the standpoint both of process requirements and of ease of maintenance of the entire plant in an excellent sanitary condition. This includes simplicity: avoiding corners or inaccessible places difficult to clean. Floors should be easy to clean and preferably of acid-resistant tiles or other suitable material resistant to corrosion by juice drippings. Asphalt floors are satisfactory; wooden floors should be avoided.

Choice of Equipment. Except for equipment used in fruit conveying, washing, and the utilization of wastes, almost all other machinery (juice extractors, strainers, pasteurizers, concentrators, tanks, piping, pumps, etc.) should be constructed of suitable stainless steel or aluminum, although the latter is less stable toward detergents. Because of the adverse effect of the cuprous ion on retention of vitamin C in citrus juices, parts made of copper must not be used. The following general principles should be considered when designing or choosing equipment: use of smooth surfaces, rounded corners and junctions; avoidance of dead ends; accessibility of parts; ease of dismantling; and protection against lubricants and dripping.

Cleaning Facilities. An ample water supply at a convenient pressure is one of the primary requirements for the establishment of a proper system of sanitary housekeeping in the plant. Suitable detergents should in each case be chosen separately and applied in proper proportions. Attention is especially drawn to the quaternary amonium salts. Facilities should be available for good channeling and the efficient eliminations of wastes that tend to disintegrate quickly by fermentation and bacterial spoilage.

Various Sources of Contamination. It has been generally accepted that the original source of spoilage organisms (bacteria, molds, yeasts), so far as infection within the factory is concerned, is the raw product—*the fruit*—specifically, as shown previously, the large number of microflora on the outside skin of citrus fruits brought to the factory. Because of the numerous cavities and depressions in the peel, the fruit can hardly be rendered sterile no matter how efficiently it is washed, and there is ample evidence that the raw product may carry into the line of production a sufficiently large bacterial load to be of subsequent danger.

Conveyor belts have a tendency to accumulate small amounts of solids that can serve as sources of food for bacterial growth; where drippings from these belts enter the juice line an increase in bacterial load results. This situation seems best remedied by installing sprays on the returning side of the conveyors and by continual washing of the belt during operations.

A serious source of spoilage may arise from the *lodging of citrus debris in various parts of the juice extractors or in pipes, unions, cocks, and pumps.* Such debris often accumulates together with large deposits of glucosides precipitating from the juice and, if left long enough, presents a perfect medium in which yeasts and bacteria can imbed. All these parts must be frequently disconnected, taken apart, and thoroughly cleaned and sterilized. A convenient arrangement is the insertion of short lengths of glass pipe into the pipe lines in order to check on the effectiveness of the cleaning operations. Tanks and evaporation vessels should be thoroughly scrubbed and flushed after each operating period. Before starting operation all machines, tanks, and pipes must be flushed with boiling water or steamed for at least five minutes. Elimination of deposits in pipes and difficultly accessible parts of machinery is best effected by pumping through the system a hot 0.5% solution of sodium hydroxide and by subsequent flushing with boiling water.

CITRUS WASTE DISPOSAL

This is the most serious problem confronting the manufacturer of citrus products, especially in the larger factories and in those having no adequate possibilities for disposal of their waste liquors through the city sewerage system. It is assumed here that the peels and rag are dried into cattle fodder and that the press liquors are either concentrated into citrus molasses, fermented into alcohol, or made into yeast products. But even then waters from plant washings, etc., carry with them considerable amounts of pulp and rag and all these liquors are most difficult to dispose of without becoming a public nuisance to the neighborhood. The liquids tend to putrefy rapidly and cause very objectionable odors.

All clean water, such as cooling waters and condensates, should be diverted separately. These sometimes represent the bulk (up to 90%) of the water used by a plant and can be easily disposed of. The remaining volume of waste waters, consisting of press liquors from the peel and wash water, should be handled separately. The biochemical oxygen demand (B.O.D.) of press liquors, e.g., is very high—about 40,000 ppm—and they are very unstable; but since the fermentation process reduces the amount of organic material and thus reduces the B.O.D. of the liquor by nearly 75%, the fermented liquor, being more stable, does not putrefy as readily as the unfermented.[1] Even ordinary wash waters and liquid wastes from a citrus canning plant engaged in the production of juices and canned grapefruit "hearts" has a B.O.D. of about 5000, and in a large plant, working some 2000 tons fruit per day, the waste disposal problem may be equivalent to that of a city with a population of 50,000.

The ways of solving such a difficult problem are of course based entirely upon the local conditions at each plant. When factories are situated in the vicinity of proportionally large lakes or rivers or near the seashore, the waste liquors may be well admixed with the large bodies of water; if not, they may give rise to objectionable odors (such as hydrogen sulfide in the case of salt water) and may cause the death of fish. Sand beds for filtering may be used, if available, provided they are frequently changed to allow complete absorption. It must be noted that citrus residues are likely to clog the soil quickly and to check its permeability and that periodic harrowing of the sur-

[1] McNary, R. R., "Industrial Wastes. Citrus Canning Industry," *Ind. Eng. Chem.*, **39**, 625 (1947).

face is necessary. Addition of nitrates, which supply oxygen to the bacteria and prevent anaerobic putrefaction, helps to eliminate objectionable odors.[2]

When no natural possibilities exist the waste liquors should be treated systematically. Effective screening should be the common practice with all such waste waters. A number of other methods are possible: chemical precipitation (mainly with lime); sedimentation; biological filtration; or lagooning.[3] The selection of one or more of all these methods depends on the degree of treatment necessary in each case. Satisfactory treatment could be obtained also by the use of trickling filters.[4]

[2] Sauborn, N. H., *Canner*, **92**, No. 16, 12 (1941); **102**, No. 14, 18 (1946).

[3] Eldridge, E. F., "Industrial Wastes. Canning Industry." *Ind. Eng. Chem.*, **39**, 619 (1947).

[4] von Loesecke, H. W., G. N. Pulley, A. J. Nolte, and H. E. Goresline, "Experimental Treatment of Citrus Cannery Effluent in Florida," *Sewage Works J.*, **13**, 115 (1941).

APPENDIX II

STANDARD SPECIFICATIONS

The following are *examples* of standards for straight juice and concentrates as accepted in different countries.

UNITED STATES STANDARDS FOR GRADES OF CANNED ORANGE JUICE
(Effective November 15, 1946)

Canned orange juice is the undiluted, unfermented juice obtained from the matured fresh fruit of the orange tree (*Citrus sinensis*) which fruit has been properly washed; may be packed with or without the addition of sugar; and is sufficiently processed by heat to assure preservation of the product in hermetically sealed containers.

Grades of Canned Orange Juice

U.S. Grade A or U.S. FANCY canned orange juice possesses a bright typical color; is practically free from defects, possesses a fine, distinct normal canned orange juice flavor; and scores not less than 85 points when scored in accordance with the scoring system outlined herein. Canned orange juice of this grade meets the following requirements:

Brix—Not less than 10.5 degrees Brix.

Acid—Not less than 0.75 g nor more than 1.4 g, calculated as anhydrous citric, per 100 ml of juice.

Recoverable Oil—Not more than 0.030% by volume of recoverable oil.

U.S. Grade C or U.S. STANDARD canned orange juice possesses a good typical color; is fairly free from defects; possesses a good, normal canned orange juice flavor; and scores not less than 70 points when scored in accordance with the scoring system outlined herein. Canned orange juice of this grade meets the following requirements:

Brix—Not less than 10.0 degrees Brix.

Acid—Not less than 0.65 g nor more than 1.6 g, calculated as anhydrous citric, per 100 ml of juice.

Recoverable Oil—Not more than 0.050% by volume of recoverable oil.

U.S. Grade D or SUBSTANDARD canned orange juice is orange juice that fails to meet the requirements of U.S. Grade C or U.S. STANDARD.

Canned orange juice of any of the foregoing grades may be considered "Sweetened" if sugar has been added and the juice tests not less than 13.5 degrees Brix.

Recommended Fill of Container

It is recommended that canned orange juice occupy not less than 90% of the volume capacity of the container.

Ascertaining the Grade

The grade of canned orange juice may be ascertained by considering, in addition to the foregoing requirements, the following factors: color, absence of defects, and flavor. The relative importance of each factor has been expressed numerically on a scale of 100. The maximum number of points that may be given for each factor is:

FACTOR	POINTS
I. Color	20
II. Absence of defects	40
III. Flavor	40
Total score	100

Ascertaining the Rating of Each Factor

The essential variations within each factor are so described that the value may be ascertained for each factor and expressed numerically. The numerical ranges within each factor are inclusive. For example, the range 17 to 20 means, 17, 18, 19, and 20.

I. COLOR

(A) Canned orange juice that possesses a bright typical color may be given a score of 17 to 20 points. "Bright typical color" means that the orange juice possesses a bright yellow to yellow-orange color typical of freshly extracted juice and is free from traces of browning due to scorching, oxidation, caramelization, or other causes.

(C) If the canned orange juice possesses a good typical color, a score of 14 to 16 points may be given. Canned orange juice that falls into this classification shall not be graded above U.S Grade C or U.S. STANDARD, regardless of the total score for the product. "Good typical color" means that the orange juice is slightly amber or very light in color but typical of canned orange juice and may show evidence of slight browning.

(D) If the canned orange juice is definitely dull, amber, or off-color for any reason, a score of 0 to 13 points may be given. Canned orange juice that falls into this classification shall not be graded above U.S. Grade D or SUBSTANDARD, regardless of the total score for the product.

II. ABSENCE OF DEFECTS. The factor of absence of defects refers to the degree of freedom from particles of membrane, core, skin, seeds and seed particles, "rag," recoverable oil, residue, similar substances, or other defects.

(A) Canned orange juice that is practically free from defects may be given a score of 34 to 40 points. Canned orange juice that shows coagulation shall not be scored in this classification. "Practically free from defects" means that there may be present not more than 0.030% by volume of recoverable oil when determined in accordance with the method outlined herein and that the juice contains no noticeable seeds particles, similar substances, nor other defects.

(C) If the canned orange juice is fairly free from defects, a score of 28 to 33 points may be given. Canned orange juice that shows more than a slight

coagulation shall not be scored in this classification. Canned orange juice that falls into this classification shall not be graded above U.S. Grade C or U.S. STANDARD, regardless of the total score for the product. "Fairly free from defects" means that there may be present not more than 0.050% by volume of recoverable oil when determined in accordance with the method outlined herein and that seed particles, similar substances, or other defects may be noticeable but not prominent.

(D) If the canned orange juice fails to meet the requirements of the foregoing paragraph (C), a score of 0 to 27 points may be given. Canned orange juice that falls into this classification shall not be graded above U.S. Grade D or SUBSTANDARD, regardless of the total score for the product.

III. FLAVOR

(A) Canned orange juice that possesses a fine, dintinct, normal canned orange juice flavor, free from traces of scorching, caramelization, oxidation, or terpene may be given a score of 34 to 40 points. To score in this classification canned orange juice shall meet the following additional requirements:

Brix—Not less than 10.5 degrees.

Acid—Not less than 0.75 g nor more than 1.4 g, calculated as anhydrous citric, per 100 ml of juice.

(C) If the canned orange juice possesses a good normal canned orange juice flavor, having a slightly caramelized or an oxidized flavor, but not an objectionable flavor, a score of 28 to 33 points may be given. Canned orange juice that falls into this classification shall not be graded above U.S. Grade C or U.S. STANDARD, regardless of the total score for the product. To score in this classification canned orange juice shall meet the following additional requirements:

Brix—Not less than 10.0 degrees.

Acid—Not less than 0.65 g nor more than 1.6 g, calculated as anhydrous citric, per 100 ml of juice.

(D) If the canned orange juice fails to meet the requirements of the foregoing paragraph (C), or if the canned orange juice has the flavor of green fruit, is off-flavor, or is distinctly unpalatable for any reason, a score of o to 27 points may be given. Canned orange juice that falls into this classification shall not be graded above U.S. Grade D or SUBSTANDARD, regardless of the total score for the product.

Explanation of Terms

10.5 Degrees Brix. The juice tests 10.5 degrees when tested with a Brix hydrometer, read at the proper temperature for the instrument used.

Normal Canned Orange Juice Flavor. The product is free from objectionable flavor or off-flavor of any kind.

Acid in orange juice is determined by titration with standard sodium hydroxide solution, using phenolphthalein as indicator. Acid is calculated as anhydrous citric acid.

Per Cent by Volume of Recoverable Oil in orange juice is determined by steam distilling a measured quantity of juice and collecting the distillate in a microburette, conveniently placed so as to read the oil phase.

Tolerance for Certification of Officially Drawn Samples

When certifying samples which have been officially drawn and which represent a specific lot of canned orange juice the grade will be determined by averaging the score of all containers, provided not more than one-sixth of the containers

fail in some respect to meet the requirements of the grade indicated by the average score.

However, none of the containers may fall more than 4 points below the minimum score for the grade indicated by the average score, and if one-sixth or less of the containers fail to meet the requirements of the indicated grade by reason of a limiting rule, the average score of all containers for the limiting factor must be within the range for the grade indicated by the average total score.

This tolerance does not apply if any container falls below any applicable standard of quality promulgated under the Federal Food, Drug, and Cosmetic Act.

UNITED KINGDOM, MINISTRY OF FOOD, CONCENTRATED ORANGE JUICE SPECIFICATION F.V.P. 8307 (November, 1946)

1. The concentrate shall be prepared by low temperature distillation in vacuo of clean, unfermented juice extracted from sound, properly matured, fresh oranges which have been thoroughly washed immediately prior to processing.

2. The process of concentration shall be carried to the stage where the packed concentrated juice gives a refractometer reading equivalent to 65° to 66° Brix at 20° C. uncorrected for acidity.

3. The concentrate shall be guaranteed to contain no additions of sugar, acid, preservative, colouring, flavouring or any other substance and it must possess a uniformly bright, typical colour free from traces of browning.

4. Upon arrival in the United Kingdom the concentrate shall:

(a) contain not less than 220 milligrams of naturally present Vitamin C (ascorbic acid) per 100 grams when tested by the method described in Appendix A attached to this specification;

(b) contain no viable yeasts when tested by the method described in Appendix B attached to this specification and be free from moulds and harmful bacteria;

(c) be free-flowing and of smooth consistency.

5. After dilution with cold water to approximately 12° Brix, the reconstituted concentrate shall:

(a) possess a good distinctive orange flavour, free from traces of scorching, caramelization, oxidation and bitterness;

(b) contain no specks of foreign matter or particles of core, skin or seeds;

(c) contain no particles of membrane larger than will pass freely through a standard A.S.T.M. No. 18 sieve.[1]

6. The concentrate shall be packed in fibreboard or wooden cases each containing six No. 10 (1 U.S. gallon) cans and the cases must be sufficiently strong to arrive in good condition at the U.K. port.

THE STANDARDS INSTITUTION OF ISRAEL SPECIFICATIONS FOR CONCENTRATED GRAPEFRUIT JUICE

Foreword

The production of citrus concentrates is of increasing importance to the economy of Israel and the need for specifying standards for these products is gen-

[1] American Society for Testing Materials, Standard E11-39. Sieves for testing purposes: mesh No. 18, aperture width 0.0394 inches. *Alternative:* British Standard 410 – 1943. Test sieves: mesh No. 16, aperture width 0.0395 inches.

erally recognized, as at present sales are based on the variable stated requirements of individual purchasers.

The present specification is intended to give a general technical basis for future sales of grapefruit concentrates produced in Israel. It has mainly been based upon the specifications issued in the U.S.A. by the Food Distribution Administration, but has taken into account also the U.K. requirements for such products.

The present specification is divided into two parts, Part 1 covering sterile concentrated juice, and Part 2 covering preserved concentrated juice.

The purpose of this specification is to facilitate the ordering and the supply of concentrated grapefruit juice. It lays down the technical standards necessary for the supply, but does not purport to cover the non-technical clauses of a contract.

Specification

Part 1. Sterile Concentrated Grapefruit Juice

Scope

101. This specification deals with sterile concentrated grapefruit juice prepared by freezing or evaporating unfermented juice obtained from the properly matured fresh fruit of the grapefruit tree (*Citrus grandis*) without any addition of foreign matters. Sterile concentrates are to be packed in hermetically closed containers.

Preservation

102. The sterile concentrated juice shall be preserved by Pasteurization only.

Absence of Defects

103. The juice reconstituted by addition of water to approximately 10° Brix shall not contain noticeable particles of seeds, membrane, core of the fruits, nor any other impurities.

Colour

104. The juice reconstituted as indicated in clause 103 shall present a bright colour typical of natural fresh grapefruit juice, free from traces of browning due to scorching, oxidation or caramelization. The addition of artificial coloring matters is forbidden.

Flavour

105. The juice reconstituted as indicated in clause 103 shall possess a good canned natural grapefruit juice flavour, free from traces of scorching, oxidation or caramelization.

Concentration

106. The degree of concentration shall be determined by the refractometric method. The Brix value and the specific gravity of the concentrated juice shall be taken from the table given in the Appendix to this Specification.[2]

Ascorbic Acid (Vitamin C)

107. The concentrated juice shall contain not less than 0.033 mg of ascorbic acid per gram, per degree Brix.

Citric Acid

108. 100 g of concentrated juice shall contain not less than 0.12 g nor more than 0.22 g acid, calculated as anhydrous citric acid, per each degree Brix.

[2] See page 288 of this book.

Recoverable Oil

109. 100 g of the concentrated juice shall contain not more than 0.003 ml of recoverable oil per each degree Brix.

Sterility Test

110. The concentrate shall contain no viable yeasts per 1 ml.

Supplementary Test

111. If required, the percentage of free and suspended pulp may be determined in accordance with the Standard Methods of Testing.

Selection of Representative Samples

112. The consignment shall be divided into lots of 1000 cans each, and from each lot a can shall be selected as representing the whole lot, for the purpose of testing by an authorized testing laboratory.

Retesting and Rejection

113. Should the sample selected in accordance with the provisions of clause 112 fail to meet all the requirements of the present specification, two further cans shall be selected from the same lot and tested. The retest shall apply only to those requirements the first sample failed to meet. Should any of these additional samples fail to pass the retests, the lot they represent shall be deemed as not conforming to the present specification.

Costs of Tests

114. Unless otherwise specified in the order, the cost of the tests shall be borne by the producer.

Method of Testing

115. The tests required in connection with this specification shall be carried out in the manner prescribed in the Tentative Method of Testing Concentrated Citrus Juice of the S.I.P. (P.S.S. 07T).

Part 2. Preserved Concentrated Grapefruit Juice

Scope

201. This specification deals with preserved concentrated grapefruit juice prepared by freezing or evaporated unfermented juice obtained from the properly matured fresh fruit of the grapefruit tree (*Citrus grandis*). No addition of foreign matters, other than specified preservatives (see clause 202) shall be allowed. Preserved concentrates are to be packed in containers lined with paraffine wax or with other suitable lining.

Preservation

202. The concentrated juice shall be preserved by the addition of preservative matters. Only sulphurous acid and its alkaline salts or benzoic acid and its alkaline salts may be used as preservatives. The quantity of preservative matters shall not exceed 2500 parts per million in the case of sulphorous acid expressed as SO_2) and 2000 parts per million in the case of benzoic acid (expressed as C_6H_5COOH).

Absence of Defects

203. The juice reconstituted by addition of water to approximately 10° Brix shall not contain noticeable particles of seeds, membrane, core of the fruits, nor any other impurities.

Colour

204. The juice reconstituted as indicated in clause 203 shall present a bright colour typical of natural fresh grapefruit juice, free from traces of browning due to scorching, oxidation or caramelization. The addition of artificial colouring matters is forbidden.

Flavour

205. The juice reconstituted as indicated in clause 203, shall possess a normal grapefruit juice flavour, free from traces of scorching, oxidation or caramelization.

If the concentrated juice is preserved by SO_2 or its alkaline salts, the testing of the flavour may be carried out after expulsion of the preservative by passing CO_2 through it.

Concentration

206. The degree of concentration shall be determined by the refractometric method. The Brix value and the specific gravity of the concentrated juice shall be taken from the table given in the Appendix to this specification.[8]

Ascorbic Acid (Vitamin C)

207. The concentrated juice shall contain not less than 0.025 mg of ascorbic acid per gram, per each degree Brix.

Citric Acid

208. 100 g of the concentrated juice shall contain not less than 0.12 g nor more than 0.22 g acid, calculated as anhydrous citric acid per each degree Brix.

Recoverable Oil

209. 100 g of concentrated juice shall contain not more than 0.003 ml of recoverable oil per each degree Brix.

Supplementary Tests

210. If required, the following tests may also be carried out: (a) percentage of free and suspended pulp; (b) free and combined SO_2.

Selection of Representative Samples

211. Specimens of not less than 0.5 liter shall be taken in the manner described in clause 1 of the Standard Method of Testing Concentrated Citrus Juices for the purpose of testing by an authorized laboratory.

From consignments comprising not more than 100 barrels, specimens shall be taken from at least three barrels.

Consignments comprising more than 100 barrels shall be divided into lots of 100 barrels each. From each lot of 100 barrels or fraction thereof, specimens shall be taken from at least three barrels.

The three specimens taken from any 100 barrels or fraction thereof shall be mixed into one representative sample.

No specimen shall be taken from any barrel showing visible signs of fermentation, and such barrels shall be discarded.

Retesting and Rejection

212. If the representative sample selected in accordance with the provisions of clause 211 does not comply with all the requirements of the present Specification, two new samples shall be taken from the lot following the same procedure. The retest shall apply only to those requirements the first sample failed to meet. Should any of these two new samples, in turn, fail to pass the retest, the lot or the

[8] See page 288 of this book.

consignment they represent shall be considered as not conforming to this Specification. Should they, however, pass the retest, those barrels from which the first representative sample had been taken shall be rejected.

Cost of Tests

213. Unless otherwise specified in the order, the cost of the tests shall be borne by the producer.

Method of Testing

214. The tests required in connection with this Specification shall be carried out in the manner prescribed in the Tentative Methods of Testing Concentrated Citrus Juices.

AUTHOR INDEX*

* *Italic numbers* refer to literature citations from the selected references at the end of each chapter.

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