THE CHEMISTRY & MANUFACTURE OF INDIAN DAIRY PRODUCTS

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FOREWORD

Messrs. K. T. Achaya and K. S. Rangappa have rendered a valuable service to the Dairy Industry of India by writing this book on the Chemistry and Manufacture of Indian Dairy Products. These products are fundamentally different in nature from the dairy products of Europe and America. In India we drink boiled milk, and sour milk as *dahi* or *lassi*, and consume ghee and khoa; while in Europe they take pasteurised milk, cheese and butter. The problems of manufacture and storage of Indian dairy products are thus very different from what they are in the West. They have not till recently received much attention; the published knowledge on this subject is also not easily accessible. The authors have collected all the information that is scattered in the literature pertaining to milk and dairy products in India and have incorporated them in this monograph with critical insight. A good deal of work on this subject has been done in the Indian Institute of Science, and in the Imperial Dairy Institute at Bangalore, which has not yet been published. The monograph has been brought up-to-date by the inclusion of this material.

This book is intended primarily for use by the scientific workers and the students of Dairy Industry in India. It is a matter for satisfaction that their number is increasing rapidly. It will also be useful to the public, the businessmen and rural welfare workers who are interested in the Dairy Industry. The value of milk and dairy products in India has been estimated to be Rs. 183 crores according to pre-war price index. The importance of this industry for the economic betterment of the rural population is obvious. It is hoped that early steps will be taken to reorganise and improve the methods of processing milk and preserving milk products in our country in the light of the information given in this book.

The authors are enthusiasts whose enterprise in bringing out this publication is commendable.

Indian Institute of Science,
Bangalore,
29th October 1945.

J. C. GHOSH.

PREFACE

Milk and its products form, as an indispensable supplement to the staple food, an integral part of civilised existence. During the ages, each country has developed its dairy industry by the usual method of trial and error, on lines suited to its environment. Thus while milk, butter and cheese are the popular dairy products in cold western climes, the taste for dehydrated butter, and fermented milk-beverages like curd and butter-milk are necessitated by the high temperatures prevailing in tropical countries.

With the progress of civilisation, organised social effort tends to improve and perfect the products of its industry. In India however, civilisation, particularly in the intellectual fields of dialectics and metaphysics, early reached a high state of perfection in world history, leading to complacency and stagnation in practical fields of human endeavour. The Indian dairy industry was thus one of the many victims of such an attitude. It has needed the violent impact of a secular civilisation to wake up the nation from centuries of somnolence, during which the rest of the world progressed like the proverbial turtle. Only during the last fifty years attempts are being made in India to solve the many problems of the dairy industry by rational methods of investigation.

The last three decades in particular, have witnessed the publication of a fair volume of work on Indian dairy products, both from the chemical and technological points of view. The work has been carried out chiefly by the Imperial Dairy Department, scholars in agricultural colleges, research institutions and municipal laboratories, and by a few provincial analysts scattered over the country. But hardly anything is being done, unlike in Europe and America, by private agencies. Therefore, the information available on indigenous products is far from complete. This is perhaps to be expected considering the vastness of the country, the variety of dairy products, the paucity of institutions for dairy research, the backwardness of the Indian farmer and the general want of encouragement to the industry as a whole.

So far even the available information has not been collated and critically presented. The presentation of the results obtained on the subject in a cogent and comprehensive form, it may be appreciated, is necessary for the proper planning of future work without duplication.

It has been thought desirable, therefore, to compile in one volume the scientific data on Indian dairy products which have been scattered till now in a large number of Indian and foreign periodicals. This would not only provide the analyst and the trader, who are cut away from the results of scientific investigation in the form of research papers, with a useful work of reference, but also give the research worker an idea of the ground already covered and a glimpse of the path that lies ahead. Many books have been published in Western countries on the chemistry and technology of dairy products with similar objects in view; but no such attempt appears to have been made in India till now. It is hoped that this volume will, at least partially, bridge the gap between the need and fulfilment. No attempt has been made, however, to write a practical handbook on the manufacture of Indian Dairy Products. For much more necessary information on the physico-chemical and technological aspects of industry is lacking. But the practical methods of preparation are discussed in relevant places in the course of the monograph.

The book has been divided into three parts dealing respectively with milk and unfermented milk products, fermented milk products, and ghee. As far as possible work done in India under Indian conditions is described here; but where comparisons were thought justified, reference has been made to researches carried out in other parts of the world as well. It may also be mentioned that some of the descriptions have been made longer than were perhaps necessary in view of the relative ignorance and misconceptions regarding Indian dairy products of many a dairy scientist overseas.

We are thankful to the authors and editors of the scientific books and periodicals from which most of the information presented here has been drawn. The following books and journals have been freely consulted.

The Chemistry of Milk, and Indian Indigenous Milk Products by W. L. Davies.

Dairy Chemistry by D. Richmond.

Fundamentals of Dairy Science by the Associates of Rogers.

Butter-fat (*Ghee*) by N. N. Godbole and Sadgopal.

The Problem of Milk in Indian Cities by L. L. Joshi.

Indian Journal of Veterinary Science and Animal Husbandry.

Agriculture and Livestock in India.

Indian Journal of Agriculture.

Indian Medical Gazette.

Indian Journal of Medical Research.

Analyst.

Dairy Science Abstracts.

American Chemical Abstracts.

Journal of Dairy Science.

The Journal of Dairy Research.

Various bulletins, circulars, pamphlets, reports of the Imperial Dairy Department, the Imperial Council of Agricultural Research and the Departments of Agriculture in India and the U.S.A.

We wish to acknowledge with gratitude the kindness and encouragement of Prof. V. Subrahmanyan, D.Sc. (Lond.), F.R.I.C., F.N.I., and the keen interest of Mr. B. N. Banerjee, M.Sc., in this work.

We appreciate very much the kindness of Sir J. C. Ghosh, Kt., D.Sc., F.N.I., Director, Indian Institute of Science, in contributing the Foreword to this book. And we offer our grateful thanks to the Council of the Indian Institute of Science for a generous grant towards the publication of this volume.

Our thanks are also due to Dr. G. T. Kale, D.Sc., Librarian, Indian Institute of Science, for the facilities he provided us with in the preparation of the monograph, to the late *Rajacharitavisharada*, *Rao Bahadur* C. Hayavadana Rao for his kind efforts towards publication, and to Messrs. The Bangalore Press for undertaking it in these difficult times. Finally, we are indebted to Mrs. P. Rangappa, B.Sc., for undertaking the trying task of proof correction and the final preparation of the indices.

Indian Institute of Science,
Bangalore,
5th August 1948.

K. S. R.
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PART I

MILK AND UNFERMENTED MILK PRODUCTS

K. S. RANGAPPA

Chapter I
MILK

Introduction

Milk has long been recognised as the most complete single food available in nature for the maintenance of health and promotion of growth of the mammal. It is not known exactly when in the history of the world man decided to utilise the milk of other animals for his own benefit. It is, all the same, an established fact that Aryans were the first people to domesticate cattle, use them for tilling land, and use their mammary secretion as food. This was at least as far ago as 1500 to 2000 B.C. Long after this date, about 600 B.C., the Greeks and the Romans used butter merely for medicinal and sacrificial purposes. The Aryans, on the other hand, prized the milk of the cow so much more than its meat that they forbade the killing of the cow, created legends about it and worshipped it as a goddess. To this day, the same practice is religiously followed all over India. It is to be mentioned to the credit of the ancient Hindus that they appraised a cow according to its yield of butterfat. And it may not be altogether impossible that the cause for the richer milk of the Indian cow lies in this wise tradition. Again, it is only the Middle East which has developed the buffalo as a milch animal. This has succeeded so well that to-day more than half the total production of milk in India is made up by the buffalo.

Requirements of Milk

The completeness of milk as food will be appreciated when it is seen that it has to serve in the new-born animal as the sole substitute, at least for a period, for the pre-natal nourishment that the fœtus was receiving through the placental circulation. In addition to the maintenance of the off-spring, the mammary secretion is called upon to supply the energy required for the newly-started functions of respiration, digestion, and even perhaps in some species, locomotion. Such energy is derived from the fat, protein and carbohydrate constituents of the milk, the proportions of which vary considerably with the species, degree of development of the off-spring, environmental conditions and other factors, known and unknown. Besides, the proximate principles in milk are presented to the infant system in a form most easily digested, next only perhaps to that in the amniotic fluids, and are supplemented by vitamins and mineral salts so indispensable to life and growth.

No less valuable is the immunising property possessed by the mammary secretion which enables the new-born to resist infections to which it is suddenly exposed with little preparation. Smith[1] has recently isolated from colostrum, drawn one hour post-partum, an immune lactoglobulin (euglobulin + pseudoglobulin) constituting more than half of the total proteins. And it is of significance that the high choline and vitamin content[2] of initial colostrum puts up the content of these in the calf's blood in the first week of its birth.[3] Although the interrelationship between the known immunological factors and the constituents present in large proportions in the post-partum secretion remains to be elucidated, it may be safe to surmise that the peculiar make up of colostrum is naturally aimed at satisfying the exacting requirements needed for the well-being of the off-spring. Even normal milk is known for its active protective property, however slight. Of all glandular secretions, milk contains the largest proportion of anti-toxins.[4] Morris[5] has shown that a bactericidal substance specific for coliform organisms is present in raw milk which is destroyed on pasteurisation.

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[1] *J. Biol. Chem.*, 1946, **164**, 345; *Ibid.*, **165**, 665.

[2] Pearson and Darnell, *J. Nutrit.*, 1946, **31**, 51.

[3] Waugh, Hauge and King, *J. Dairy Sc.*, 1947, **30**, 457.

[4] Kolle and Hetsch, "Experimental Bacteriology," 1940, Allen & Unwin, Ltd., London.

[5] *Dairy Ind.*, 1945, **10**, 180.

Milk as Human Food

The importance of milk in the human dietary cannot be stressed too much. The perfect composition of milk not only recommends itself for the growing organism, but it is also suited to satisfy the energy and vitamin requirements of the adult system. In India especially, where the menu is principally vegetarian, the fat and the essential vitamin needs are to be made up with milk and milk products. The indispensability of an adequate supply of milk and milk products in the human diet is shown by the fact that those nations are, in general, healthiest whose average consumption of dairy products are greatest. This is further supported by the fact that even in those countries, the poorer classes which consume a lower average of these products tend to be less robust.

Breeds and Yields of Cattle in India

The more important breeds of dairy cattle in India are the Sindhi, Tharparkar, Gir, Hariana, Kankrej, Ongole and Sahiwal cows, and the Murrah, Jaffarbadi, Surti, Mehsana and Nagpuri buffaloes. Official herd books have been recently opened for some of these breeds[6]; and private owners are encouraged to maintain records of yield, breed, length of lactation, etc., of their animals. It will therefore be some years before data are available regarding animals brought up under village conditions. Among the principal dairy animals of the country that are not yet included in the official registers are some of the breeds of the Montgomery and Kathiawar districts, Nili, Surti and Ongole buffaloes, and the goats of the Punjab, North-West Frontier Province and Bihar.

The only reliable data available on the milk-yields of cattle managed under village conditions are given in a report of the Imperial Council of Agricultural Research.[7] Daily

[6] *Misc. Bull. Imp. Coun. Agric. Res.*, No. 27, Delhi: Manager of Publications, 1939.

[7] *Ibid.*, 1939, No. 22.

milk yields were recorded for a week in 370 villages spread over seven principal dairy tracts of India. Total yields per lactation were calculated on the basis of these recordings and the approximate stage and average length of lactation as estimated by the producers. Table I gives the results of the village enquiry.

TABLE I

DAILY AND LACTATIONAL YIELDS OF VILLAGE CATTLE

Tract	Cows			Buffaloes		
	Milk yield per day	Length of Lactation	Lactational yield	Milk yield per day	Length of Lactation	Lactational yield
	lb.	(months)	lb.	lb.	(months)	lb.
Montgomery ..	4·72	10·43	1,344	8·24	11·24	2,470
Hariana ..	4·46	7·62	986	11·21	8·65	2,575
Kosi ..	3·89	8·25	865	7·06	9·29	1,768
Deltaic (Bihar) ..	2·74	8·14	651	5·46	8·82	1,255
Malvi (C.P.) ..	1·67	8·94	411	5·44	10·41	1,573
Kankrej ..	3·90	7·90	920	7·97	9·14	1,890
Ongole ..	4·64	9·54	1,236	6·53	11·30	1 903
Average ..	3·74	8·81	943	7·82	9·98	2,160

The report further adds that the average over-all daily yield per cow was 1·7 lb. and 4·0 lb. per buffalo. Although these figures do not represent the average of an exhaustive statistical enquiry, they clearly indicate the depressing trend of production among Indian cattle. This is supported by the authoritative data given in the International Year Book of Statistics (1938), wherein is mentioned the average annual milk yields of cattle in different countries of the world. Table II gives comparative figures for population and average production of cattle in Europe and India.

TABLE II

POPULATION AND PRODUCTION OF INDIAN AND EUROPEAN CATTLE

Country	Approximate No. of milch animals (lakhs)	Annual milk production (lakh mds.)	Average fat content of milk (per cent.)	Approximate milk yield per animal (lb.)
Total for Europe (Cows)[8]	477·85	26,900	3·8	4,460
INDIA[9]:—				
(*a*) Cows ..	490	2,898	5·0	487
(*b*) Buffaloes ..	214	3,203	7·0	1,229
(*c*) Goats ..	620	192	4 to 5	..

Considering the higher average fat content of the Indian dairy animals, their milk yields have been computed by the Marketing Board to be proportionately greater on the basis of butter-fat content. But it should be pointed out that the computation is defective as it leaves out of account the valuable solids-not-fat of milk. All the same, the average yield of the village cow in India falls far short of the European average.

The cause of this has been definitely attributed to under-feeding and unscientific management of dairy animals. Cattle brought from villages and maintained in Military and Government Dairy Farms under modern methods of dairying have proved the great potentialities of Indian cattle.

The data given in the following Table (Table III) indicate clearly that purchased village stock maintained in a modern commercial farm yield 3–7 times more than the same cattle (*cf.* Table I) managed under poor village conditions. Considering the generally higher yield of Farm-bred stock[10] (averaged over a much larger number of lactations) over those of purchased stock, it is easily seen

[8] *Rep. Marketing of Milk in India and Burma*, 1943, p. 296.

[9] *Ibid.*, p. 2.

[10] Chaudhuri and Rathore, *Indian Farming*, 1943, 4, 369.

TABLE III

MILK YIELDS OF INDIAN BREEDS IN ACCREDITED FARMS [11]

Breed	Purchased Stock		Farm-bred Stock		
Cow—	No. of Lactations averaged	Yield per Lactation lb.	No. of Lactations averaged	Yield per Lactation lb.	Rise (+) or fall (−) in yield of farm-bred stock
Deoni ..	5	2,943	2	2,673	−
Gir ..	5	2,753	..	..	..
Hariana ..	36	3,400	101	3,459	+
Kankrej ..	2	3,407	54	3,232	−
Malvi ..	4	2,863	4	2,469	−
Ongole ..	..	..	10	2,938	..
Sahiwal ..	65	3,735	127	4,269	+
Sindhi—					
(a) All types ..	15	3,534	85	3,604	+
(b) Military Dairies ..	100	4,150	..	..	..
Tharparkar ..	44	3,438	89	4,056	+
BUFFALO—					
Murrah—					
(a) All types ..	35	4,427	97	4,113	−
(b) Military Dairies (N. Circle)	861	3,771	..	..	..
*(c) Military Dairies (S. Circle)	228	4 230	262	4,643	+
Nili ..	2	5,212	..	..	..
Surti ..	5	3,576	4	4,359	+

* Calves are not suckled at the Military Dairy Farms.

that India is a long way off yet from the limit of her production capacity.

In support of the great capabilities of Indian cattle, the compilers of the Report on the Marketing of Milk in India (1943) state, " that it is possible to increase readily, through proper feeding and management, the yields of Indian cattle by nearly 50 per cent. The first progeny of these cows show a further improvement of 10 to 15 per cent. in their milk yields over and above the increase recorded by their dams.

[11] *Rep. Marketing of Milk in India and Burma*, 1943, p. 7.

The long dry periods are also shortened considerably, which proportionately reduces the cost of production of milk. These encouraging features and the fact that yields of several herds have actually been trebled in a period of less than twenty years show the immense potentialities of Indian cows in matters of improving the milk yields through better feeding, selective breeding and efficient management."

Concurrently with this procedure of proper feeding, grading up of low-yielding breeds with indigenous high yielders may be extensively planned as a long-range method of permanent increase of milk production in the country. In this connection the use of European dairy breeds is generally deprecated[12] as the process leads to lowered resistance of the progeny to tropical diseases. Schneider,[13] however, feels that crossing with imported breeds is wrongly discredited on the ground that grading the first cross up to the foreign breeds results in cattle unsuited to Indian conditions, and interbreeding the first cross leads to excessive variation. He advocates, therefore, the policy of crossing with imported sires carefully selected for similarity to Indian dams in every respect except milk yield in order to introduce genes for high yield, and then back-crossing to good sires of Indian breed. It is evident that if this plan succeeds milk production in India can be stepped up in a much shorter time than is otherwise possible.

Milk Consumption in India

The *per capita* consumption of milk and milk products in India has been estimated to be hardly 5·8 oz. in India and less than 2 oz. in Ceylon.[14] This is indeed very low, not only when compared to the high averages of 30 to 40 oz. obtaining in Western countries, but to the minimum of 21 oz. required from the standpoint of balanced nutrition.[15]

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[12] Habargoda, *Trop. Agriculturist*, 1944, **100**, 147.

[13] *Allahabad Farmer*, 1944, **18**, 2.

[14] *Rep. Milk Committee*, Ceylon Govt. Press, 1944.

[15] *United Nations Conference*, Hotsprings, U.S.A., 1943.

Slightly more than a fourth of the total production of milk is consumed as fluid, the major part of which is made up of cow's milk, amounting to half the annual yield from the cow. One reason for such a partiality to cow milk is that it more nearly approximates in composition to human milk than does the buffalo's. This fact is illustrated in Table IV.

TABLE IV

APPROXIMATE COMPOSITION OF MILK OF VARIOUS MAMMALS[16] (PER CENT.)

Species	Water	Casein	Albumin	Fat	Lactose	Ash
Human ..	87·41	0·91	1·23	3·76	6·29	0·31
Cow ..	87·27	2·95	0·52	3·66	4·91	0·69
Goat ..	84·14	3·04	0·99	6·00	5·02	0·81
Buffalo ..	82·14	4·29	0·49	7·44	4·81	0·83
Ass ..	89·88	0·73	1·31	1·50	6·09	0·49
Mare ..	90·68	1·27	0·75	1·17	5·77	0·36
Monkey[17] ..	..	Protein	2·1	..	5·90	0·26

As might be expected, monkey's milk is the nearest in composition to human milk; although, among quadrupeds, equine milk resembles human milk very closely. All the same, ass's or mare's milk is not commonly used as a substitute. In this connection it is of interest that, in its quick passage through the stomach and gastric digestion, ass's milk compares more favourably with human milk than does cow milk.[18] This perhaps explains, at least partially, why it is sometimes used medicinally for infants in this country.

The more important reason for preferring cow milk for fluid consumption is that buffalo milk, in view of its higher fat content, lends itself to better economic exploitation in the form of milk products.

[16] Associates of Rogers, "Fundamentals of Dairy Science," 1927, A.C.S. 41, The Chemical Catalog Co., Inc., N.Y., p. 17.

[17] Wagenen, Himwich and Catchpole, *Proc. Exp. Biol.*, N.Y., 1941, **48**, 133.

[18] Gaucher, *Compt. Rend.*, 1909, **148**, 361.

Of the total annual production milk that is not used directly as fluid is converted into ghee, curd, butter, khoa and other milk products of indigenous type. Table V gives the proportion of milk used for each of these products in India.

TABLE V

UTILISATION OF MILK IN INDIA[19]

Type of Products		Quantity of Milk used	Percentage to total production	Percentage to total quantity converted into products
		(lakh mds.)		
Consumed as fluid	..	1,762·18	28·0	..
Converted into Ghee	..	3,589·14	57·0	79·2
" Khoa	..	311·67	5·0	6·9
" Curd	..	327·95	5·2	7·2
" Butter	.	107·56	1·7	2·4
" Ice-cream	..	21·35	0·3	0·5
" Cream	..	23·62	0·4	0·5
" Other products		149·44	2·4	3·3
TOTAL	..	6,292·91	100·0	100·0

It is clear from the above data that ghee (clarified butter) forms the most important milk product manufactured and marketed in India. This is not surprising in view of its exceptional storage property.

Another potential source of milk supply in India is the goat. The majority of the goat population is found to exist in the United Provinces, Madras and Bengal.[20] The breeds of the Punjab, North-West Frontier Province and Bihar are remarkably heavy milkers. The Jamna Pari and Bar Bari are noted among these. Their annual average of 440 lb.[21] exceeds the normal yield of cows in areas like Assam,

[19] *Rep. Marketing of Milk in India and Burma*, 1943, p. 66.
[20] *Indian Livestock Census*, 1940.
[21] *Rep. Marketing of Milk in India and Burma*, 1943, p. 10.

Mysore and Hyderabad. Sen[22] has described a few more breeds of Indian goats with good promise as dairy animals. The only drawback to the use of goat milk is its strange flavour which has been differently attributed to the leafy feed, lack of cleanliness of the animals and the herding of the males and females together. But it is likely that people could get used to it with practice, and the flavour itself may perhaps be lightened by stall feeding and proper management. The large deficiency of milk supply in India may therefore be made up to an appreciable extent by an intensive process of goat breeding all over the country.

[22] *Indian Farming*, 1944, 5, 356.

Chapter II

COMPOSITION OF MILK

Definition

Milk is a heterogenous product in which fat, proteins, sugar, vitamins and mineral salts are held in solution or colloidal suspension in the major constituent, water. In the form of microscopic globules fat is dispersed in the aqueous phase, and it holds in solution carotinoid pigments, fat-soluble vitamins and minor ingredients of milk like lecithin, cephalin, cholesterol and ergosterol. Protein is present mostly as its calcium salt. It is maintained in the colloidal state by the lactose and soluble salts and is coagulated under the influences of pressure and acid fermentation.[1] Only a small fraction of the proteins—lactalbumin—is water-soluble. Lactose, mineral salts, a few of the vitamins and non-protein nitrogenous constituents exist in solution in the aqueous phase. These general observations are common to all milks which differ mainly in the proportions of the constituents and therefore in some of their physical properties, such as viscosity, freezing point, specific gravity, etc.

General Composition of Milk

The following chart presents in fairly detailed fashion the various constituents of milk.

CHART I

Milk Fat	Proteins	Non-Protein Nitrogenous Compounds
Lecithin	Casein	Lactochrome
Cephalin	Lactalbumin	Creatine
Carotene	Lactoglobulin	Creatinine
Xanthophylls	Fibrin	Urea and Uric acid
Cholesterol	Traces of other proteins separable by various solutes	Trimethylamine
Ergosterol		Ammonia

[1] Lindet, *J. Agric. Prat.*, 1915, **28**, 277, 294.

Milk Sugar	Inorganic Constituents	
Lactose	Calcium	Traces of :
Glucose (?)	Phosphorus	Copper
	Potassium	Iron
	Sodium	Zinc
	Sulphur	Manganese
	Chlorine	Aluminium
	Flourine	Silicon
	Carbon dioxide	

Citric acid and iodine are also present combined with minerals.

VITAMINS

A—Growth promoting, anti-xerophthalmic.

B_1—Anti-beriberi.

B_2—Anti-pellagra, Anti-neuritic, Anti-dermatitis.

Nicotinic acid—Pellagra-preventing.

Pantothenic acid—Anti-chick dermatitis.

Inositol—Mice anti-alopecia.

Biotin—" Egg white injury "-preventing.

C—Anti-scorbutic.

D—Anti-rachitic.

E—Reproductive.

K—Blood-clotting.

ENZYMES

Amylase, catalase, diastase, galactase, lipase, peroxidase, phosphotase, reductase.

Average Chemical Composition of Milk

It has already been observed that the chemical composition of milk varies with the species and is characteristic of it. Apart from these gross variations, minor differences are noticeable in the proportion of the constituents of milk of animals maintained in different parts of the world. Table VI illustrates the composition of the average sample of milk of the cow and the buffalo in different countries. The average for the Indian cow milk is drawn from analyses of composite samples of herds made up of various breeds of Indian cows. No mention is made, however, of the breeds of buffaloes.

TABLE VI

AVERAGE COMPOSITION OF MILK OF COW AND BUFFALO IN DIFFERENT PARTS OF THE WORLD (PER CENT.)

Type of Cattle	No. of Samples examined	Specific gravity	Water	Total Solids	Fat	Non-fatty Solids	Ash
Indian Cow[2]	500	1·0295	86·37	13·63	5·03	8·60	0·70
European Cow[3]	..	1·0320	87·36	12·64	3·75	8·89	0·75
Indian Buffalo[2]	500	1·030	82·13	17·87	8·47	9·40	0·81
Egyptian Buffalo[4]	..	1·0324	83·09	17·91	7·95	9·95	0·78
European Buffalo[5]	..	1·0333	81·94	18·04	9·07	8·97	0·883
Chinese Buffalo[6]	10 samples extending over many months	..	76·98	23·08	12·46	10·62	0·88

The difference between the average composition of the Indian and European cow milk is seen to be more appreciable than in the case of the buffalo. The average fat content of Indian cow milk is distinctly higher than that of the European cow, although the solids-not-fat per cent. is a little lower. The specific gravity of Indian cow milk naturally tends to be lower in view of its higher fat, and lower non-fatty-solids content.

Of all buffaloes, the Chinese buffalo appears to yield the richest milk. The fat as well as the total-solids, and non-fatty-solids contents of the milk of this animal exceed even those of the Indian buffalo. Naturally, therefore, the specific gravity is also higher.

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[2] Bruce, *Analyst*, 1922, **47**, 288, 273.
[3] Richmond, *Ibid.*, 1927, **47**, 287.
[4] Lappel and Hogan, *Ibid.*, 291.
[5] Tartler, *Bull. Agric. Intelligence*, 1919, **10**, 345.
[6] Levine, *Phil. J.Sc.*, 1919, **15**, 92.

It is to be regretted that more detailed and comprehensive data, including the percentages of lactose, total proteins and non-nitrogenous matter in composite samples from Indian herds of the various breeds of cows and of buffaloes are not available. Those that are available, however, are either of individuals or, at best, of particular breeds of cattle.

Meggitt and Mann[7] have analysed bulk samples of the cow and buffalo herds of the civil dairy at Poona. The herd of cows was chiefly composed of animals of the "Gir" and "Sindhi" breeds. The buffalo herd consisted almost entirely of animals of the "Surti" breed with only a few of the Delhi, Deccani and Jaffarbadi breeds among them. Figures in the following table are averaged from the monthly averages extending over seven months.

TABLE VII

DETAILED ANALYSES OF BULK SAMPLES OF INDIAN COW AND BUFFALO MILK (PER CENT.)

Constituents	Indian cow[7]	Indian buffalo[7]	European cow[8]
Water ..	85·28	81·74	87·40
Total Solids ..	14·72	18·26	12·60
Solids-not-fat ..	9·05	10·15	8·93
Fat ..	5·67	8·11	3·67
Proteins ..	3·60	4·33	3·42
Lactose ..	4·69	5·00	4·78
Ash ..	0·76	0·82	0·73

It may be seen that the difference in the amounts of dry matter in the milks of the Indian and European cows is mostly made up of butter-fat. The proteins in Indian cow milk are also slightly in excess, but the lactose and ash contents are very nearly the same. (Data supplied by Bruce

[7] *Mem. Dept. Agric. India* (Chem. Ser.), 2, 1, 195.

[8] Davies, W. L., "The Chemistry of Milk," Chapman & Hall, London, 1939, p. 9.

also point to the same conclusion.) It must be mentioned, however, that the fat content, referring mainly to the Sindhi and Gir breeds of cows, is much above the average (*vide* Table VI) for bulk samples of a mixed breed of Indian cows. The reason for this is partly that the figure for fat relates to the milk drawn after the calf has sucked an unknown quality of the fore (poorer in fat) milk of the cow's udder.[9]

The composition of the buffalo milk, however, is fairly representative, as it is not subject to the same degree of variation from breed to breed as is cow milk. The total dry matter as well as its individual constituents in buffalo milk appreciably exceed those of cow milk, Indian and foreign. But the disparity is greatest in the fat content. These facts lead to the conclusion that there is no reason why the buffalo should not entirely replace the cow in the dairy unless it be to satisfy the differences, if any, between the two types of milk in their physiological, therapeutic, and perhaps, psychological functions. To recommend this further is the large immunity to tuberculosis which the buffalo enjoys and to which disease even the Indian cow is greatly susceptible.

Levine[6] reports that the allopathic physicians (American and European) in China prefer buffalo milk to cow milk for infant feeding, where modified milk is required. This is because of the ease with which it lends itself to modification. He states that 100 grams of (Chinese) buffalo milk with 18 grams of sugar made up to 300 grams with water, brings the composition of the modified milk very near to that of human milk.

Taking the mean figures into consideration Leather[10] has stated that the relation existing between the specific gravity, solids-not-fat and fat agree well in all cases with those which Richmond[11] and others have found for English cows. The

[9] Leather and Dobbs, *Mem. Dept. Agric. India* (Chem. Ser.), **3**, 148.

[10] *Analyst*, 1901, **26**, 40.

[11] Richmond's formula for the determination of total solids (T.S.) from the fat content (F) and specific gravity (L, Lactometer reading) of milk.

$$T.S. = 1{\cdot}2\,F + 0{\cdot}25\,L + 0{\cdot}14.$$

same relationship has been found to fit in equally well for the buffalo milk. Richmond's formula, therefore, provides an easy method of estimating with a reliable degree of accuracy the solids-not-fat in both cow and buffalo milk by means of the specific gravity and fat content of the milk. This has been supported by Meggitt and Mann[7]; But the Indian Council of Agricultural Research has recommended a slightly revised formula (T.S. = 1.21 F + 0.25 × L+0.66) which is in closer agreement with estimated values. But Bruce[2] and Bunce[12] found, however, though only in a few cases, a difference between the results calculated by Richmond's formula and those obtained by actual estimation.

The relationship between the lactose, proteins and ash in Indian cow milk agrees well with the proportions 13:9:2, of milk from English cows. On the other hand, the ratio between these three ingredients in buffalo milk is not the same as for cow milk. Taking the mean figures Leather[10] arrives at the approximate relationship, confirmed by Meggitt and Mann,[7] Lactose: Protein: Ash::6:5:1, which is the same as for the Egyptian buffalo[7] milk also. The same author[10] believes it probable that the proportion of proteins is necessarily higher in a milk containing so much butterfat in order that the albuminoid ratio may be maintained.

We have so far been dealing with the milk of cattle maintained in Government or Military Dairy Farms under systems of scientific feeding and management. Considering the very limited number of such institutions in India and the fact that practically 99 per cent. of the cattle are owned and reared by farmers ignorant of any system of management, it is very necessary to examine the composition of milk under the poor conditions of village management.

Here also the literature on the milk composition of country-bred cattle in India is none tco exhaustive. Indeed it would be an indispensable addition to our knowledge of Indian

[12] *Analyst*, 1932, 57, 450.

dairy products to examine in detail milk and its derivatives in different parts of the country, and study their variations under differing conditions of breed, feed, season and management.

Ghosh and Datta Roy[13] have analysed a number of genuine samples of market milk along with those of a government dairy farm. But it has not been possible to sift the data and distinguish the market samples of milk from those of the dairy farm. Out of 231 samples of buffalo milk in Assam, they found that 15·15 per cent. contained 5 per cent. and less (up to a limit of 2 per cent.) of fat, and 77·49 per cent. of the samples contained 6 per cent. and more (up to a limit of 12 per cent.) of fat. The common legal standard for buffalo milk in different parts of the country fixes the fat content at a minimum of 5–6 per cent., and solids-not-fat content at a minimum of 8·5–9 per cent., the standards varying slightly from province to province.[14]

Ghosh and Datta Roy[13] have found that less than 1 per cent. of the samples of buffalo milk contained solids-not-fat below the legal minimum, the upper limit for the same constituents being as high as 15 per cent.

The lactose contents varied between 4·0 and 5·3 per cent., the samples containing higher than 4·9 per cent. lactose tending towards low fat contents of 6 per cent. or less. Ghosh and Datta Roy[13] observe that lactose in buffalo and cow milk is subject practically to the same range of variations. They suggest that the inverse relationship between fat and lactose opens a way to the detection of adulteration of buffalo milk diluted to conform to the standards of cow milk.

Working on bulk and individual samples of cow and buffalo milk in Burma over a period of eleven months, Bunce[12] has given the following averages for milk composition.

[13] *Indian Med. Gaz.*, 1941, **76**, 279.

[14] See Appendix for Provincial Legal Standards for Milk Composition.

TABLE VIII

AVERAGE COMPOSITION OF MILK OF COUNTRY-BRED CATTLE IN BURMA (PER CENT.)

Type of Cattle	No. of samples examined	Sp. Gr.	Total Solids	Fat	Solids-not-fat	Ash
Cow[12] ..	290	1·0329	13·71	4·41	9·30	0·75
Buffalo[12] ..	276	1·0333	17·42	7·38	10·04	0·78
Cow[15] ..	640 in Mandalay, others in villages	..	14·53 to 15·13	5·10 to 5·72	8·43 to 9·41	..
Cow[16] ..	43	1·0247 to 1·0328 (20°C.)	11·20 to 15·22	2·15 to 6·6	8·28 to 9·31	..

Samples examined by Bunce include those of the poorest types of animal kept under very poor conditions. The examination also extends over all the seasons of the year. In the absence of similar data in India, basing on the trend of figures obtained in Indian dairy farms, it may be safe to observe that country-bred cattle in India even under similar conditions of poor feeding and management can be expected to yield milk, richer in fat than the average European sample.

Extreme Variations in Proximate Composition

So far we have dealt with the average composition of bulk samples. Different workers in this field have found slightly different values for the highest fat and solids-not-fat content of samples, from individual or small groups of animals in a herd. But there is less extensive record of the lower limit in proximate composition of Indian milks.

[15] Warth, Bull. No. 15, Burma Dept. Agric., 1917 (cited in *Rep. on Marketing of Milk in India and Burma*, 1943).

[16] Rangappa (unpublished).

TABLE IX

EXTREME VARIATIONS IN THE PROXIMATE COMPOSITION OF MILK (PER CENT.)

Workers	Fat		Solids-not-fat	
	Maximum			
	Cow	Buffalo	Cow	Buffalo
Leather[7] ..	6	(10 nearly)	..	..
Meggitt and Mann[7,17]	6·8	9·7	9·75	10·3
Bruce[2] ..	..	12	..	15
Ghosh and Datta Roy[13]	..	12	..	15·5
Levine[18] ..	..	15	..	..
Rangappa[16]: Country ..	6·6	9·0	9·36	10·27
Farm ..	7·1	Ca 11·0	9·23	9·59
	Minimum			
Ghosh and Datta Roy	..	2·3	..	8·5
Bunce[12] ..	<3·0	<5·0	<8·5	..
Rangappa: Country ..	2·15	4·5	8·28	8·04
Farm ..	3·6	5·0	7·36	8·13

Among these, Ghosh and Datta Roy report a minimum of 2·3 per cent. fat only in one, and 8·5 per cent. S.N.F. in two samples of buffalo milk. The lowest fat content of buffalo milk observed by Bunce in Burma—3 out of 200 herd samples and 2 out of 76 individual samples—was a little less than 5 per cent. Among cows he found that only 2 out of 200 samples contained less than 8·5 per cent. solids-not-fat, and 17 contained less than 3 per cent. fat. The author[16] has observed that among nearly 100 dairy samples and 40 village samples each of cow and buffalo milk analysed throughout the year only 3 from cow had less than 3·5 per cent., one from buffalo 5 per cent., and 2 less than 5·5 per

[17] *Mem. Dept. Agric. India* (Chem. Ser.), **2**, 195.
[18] *China Med. J.*, 1918, **32**, 536.

cent. fat. But of these nearly 14 per cent. of cow and 8 per cent. of buffalo milk samples contained less than 8·5 per cent. fat-free solids, the current presumptive standard in this country and in Europe. Mehta[19] reports that among cows of Jodhpur State the fat varies from 2·0 to 6·9 and the total solids from 8·5 to 15·8 per cent., and among buffaloes the fat ranges from 3·7 to 12·0 and the total solids from 11 to 18 per cent. It is therefore clear that there should be many instances wherein the S.N.F. happens to be below the legal standard. Anantakrishnan and co-workers[20] also observed that the ash content of Indian cow milk does not always conform to the Western minimum of 0·7 per cent. The author found that 6 out of 60 samples of cow milk contained from 0·66 to 0·69 per cent. of ash. Brahmachari[21] reports a wider variation ranging from 0·4 to 0·85 per cent. of ash in cow milk.

Legal Standards

The lowering of S.N.F. and ash appear to occur especially during the dry months of the year. In many parts of India the summer months could indeed be very dry, bordering on drought conditions. It should therefore be clear that the current legal standards for fat and fat-free solids definitely call for a revision. And in fixing the standards seasonal and regional variations (which are to a large extent interdependent) demand special allowances. The available data, scanty as they are, indicate that the fat minimum could perhaps be slightly raised, while the S.N.F. standard (and also perhaps of ash) needs to be lowered particularly during the dry months of the year.

The highest fat content of Indian cow milk differs very little from that (6%) of the European cow. But the frequency of fat contents close to that figure is much higher

[19] *Proc. Indian Sc. Cong.*, 1947, *Sec.* 10, p. 2.

[20] Anantakrishnan, Dastur and Kothavalla, *Indian J. Vet. Sc. and Anim. Husb.*, 1943, **18**, 297.

[21] *Indian Med. Gaz.*, 1934, 69, 76.

among Indian animals. And this is characteristic of certain breeds, especially the Sindhi and the Gir. It is therefore obvious that by a process of selective and intensive breeding it is possible to build up dairy stock of cows yielding milk of an average richness fairly approximating to that of buffalo milk.

Physical Properties of Indian Milks

Two types of milch animals being involved in India, a comparison of their physical properties will be of considerable practical interest. The difference in colour and consistency are the most obvious. While cow milk is pale yellow in colour and rather thin in consistency, buffalo milk is snow white and slightly more viscous. Leather[8] remarks that the Indian cow milk, unlike the English milk which is deeper in colour, is very nearly white. Doctor and his co-workers[22] also conclude from their observations on the native and foreign breeds that the Indian cow is unable to transfer as much carotene and carotenoid pigments from its feed as the English cow. Again, since green feeds are richest in carotene, the fat-soluble pigment, cow milk produced in the rainy season is slightly more yellow than in the dry, hot months of the year.

It has already been noticed that the densities of the two types of milk differ only slightly and overlap over most of the range of variation. Density therefore fails to serve as a distinguishing test between the two milks. Different workers give slightly different average figures for the specific gravity of each milk. These are found in Table X along with the available values for the freezing point of the two milks.

It will be seen that specific gravity fails as a criterion of identification of the type of milk because the specific gravity of cow milk overlaps with that of buffalo milk.

[22] Doctor, Banerjee and Kothavalla, *Indian J. Vet. Sc. and Anim. Husb.*, 1940, **10**, 77.

TABLE X

SPECIFIC GRAVITY OF COW AND BUFFALO MILK

Worker	Specific Gravity at 15·5 °C.		Freezing point (° C)		
	Cow	Buffalo	Cow	Buffalo	European Cow (Sato)[25]
Meggitt and Mann[7]	1·029 to 1·030	1·031 to 1·0316	..	..	Herd milk −0·58 to −0·53
Bruce[2]	*1·0291 to 1·0317	1·0284 to 1·0316	..	..	
Bunce[12]	*1·0325 to 1·0333	1·0330 to 1·0336	−0·550 to −0·580	−0·560 to −0·590	Individual Cows −0·59 to −0·51
Ghosh and Datta Roy[13]	..	1·0280 to 1·040	..	..	(British legal minimum for F.P. of cow milk is −0·530° C.)
Macmahon and Srivastava[23]	..	..	−0·548 to −0·565	−0·555 to −0·575	
Leather[24]	..	..	−0·518 to −0·577	−0·521 to −0·562	
Stewart and Banerjea[24]	1·0295 to 1·0335	..	−0·490 to −0·590 Average −0·549	−0·510 to −0·590 Average −0·554	
Rangappa[16]	1·0289 to 1·0317 (20° C.)	1·0276 to 1·0302 (20° C.)	..	..	
Dastur *et al* (unpublished)	..	..	−0·512 to −0·572 Average −0·550	−0·521 to −0·575 Average −0·549	

* Temperature of determination not mentioned.

Bunce[12] has stated that the freezing point of milk can be relied upon to distinguish the two types of milk. But the observation is hardly justified in view of the marked overlapping of the ranges of freezing points of the two milks. He considers that for the purpose of deducing the percentage

[23] *Analyst*, 1935, **60**, 307.

[24] *Indian J. Med. Res.*, 1930–31, **18**, 57.

[25] Quoted in Winton, "Structure and Composition of Foods" Chapman & Hall, London, 1937.

of added water, it is reasonable to adopt $-0\cdot550°$ C. for cow milk and $-0\cdot560°$ C. for buffalo milk as the freezing point, the relatively small difference of 0·01° C. corresponding to less than 2 per cent. of added water. Macmahon and Srivastava[23] also suggest the same standards. However, Stewart and Banerjea[24] failed to find any difference in the average freezing points of the milks of the two species. Data presented by all the workers show that considerable overlapping occurs in the range of the freezing point of the two milks. On this basis, therefore, it is not possible to classify them.

Another feature for consideration is the range of variation of the freezing point. It will be seen that the extremes differ in both milks from 0·02 to 0·1° C., which theoretically allows 4–20 per cent. of added water. But if the legal standard is accepted as $-0\cdot550°$ C., as suggested by Bunce, about 8 per cent. of added water becomes possible with samples having very low freezing point. Such a standard however needs wider and more systematic investigation before it is fixed.

Moreover, Ghosh and Datta Roy[13] point out that the cryoscopic test is unsuitable inasmuch as the public analyst usually receives for examination samples preserved with formalin or other preservatives which interfere very considerably with the freezing point. If no preservative is added, and if the test is not carried out on fresh milk, the little amount of acid developed between milking and examination will also result in unreliable values. Keister[26] has shown that the average lowering of the freezing point for each 0·01 per cent. of acid calculated as lactic acid varies in different samples from 0·0027 to 0·0044° C., and as such a correction has to be applied to the cryoscopic test. Ghosh and Datta Roy[13] have suggested that the estimation of the ratio of fat and lactose content offers a method of identifying a sample as either cow or buffalo milk. But this obviously

[26] *Ind. Eng. Chem.*, 1917, **9**, 862.

fails as a quick, routine test in a public analyst's laboratory where a large number of samples come in for examination every day.

It will also be noticed from the table that the freezing points of the individual and herd samples of milk of the European cow reach a level slightly higher than that of the Indian cow.

Refractive Index of Milk

The refractive index of nearly 200 samples each of cow and buffalo milk (in contra-distinction to that of milk serum) has been estimated by Rangappa.[27] Further, the relationship between the density (*d*) and refractive index (*n*), expressed as the refractive constant (*K*) has been calculated according to the Lorentz and Lorenz formula,

$$\frac{n^2 - 1}{n^2 + 2} \times \frac{1}{d} = K.$$

Table XI gives the range of these values for samples examined over a year.

TABLE XI

REFRACTIVE INDEX AND REFRACTIVE CONSTANT OF COW AND BUFFALO MILK

	Refractive Index (40° C.)		Refractive Constant	
	Cow	Buffalo	Cow	Buffalo
Maximum ..	1·3481 (<1%)	1·3502 (<1%)	0·2083 (1%)	0·2088 (1%)
Minimum ..	1·3448 (<3%)	1·3466 (<1%)	0·2064 (1%)	0·2072 (1%)
Average ..	1·3461	1·3477	0·2071	0·2079

In practice most of the samples of cow milk have values of *n* (40° C.) between the extremes of 1·3450 and 1·3472, and of buffalo milk between 1·3460 and 1·3490. The range of the refractive constant is still more narrow and distinct, with 0·2065 to 0·2075 for cow, and 0·2076 to 0·2088 for buffalo milk. The latter constant thus provides the simplest method of classifying the type of milk under examination. A feature of great advantage is that the refractive constant,

[27] *Proc. Indian Acad. Sc.*, (B), 1947, **26**, 86.

unlike the refractive index of milk is not a function of the solids-not-fat. Thus, genuine milk with S.N.F. below the legal minimum will still have values of refractive constant within normal limits.

Among the principal constituents of milk, it has been found[28] that, in their natural state, proteins contribute (next to water) the largest fraction, lactose next, and minor constituents (mineral matter and other solutes) the least to the absolute value of the refractive index of milk. Among the proteins, the lactalbumin and globulin contribute, considering their proportion, much more than casein.

Hardly any data are available on the specific heat, electrical conductivity or fluorescence of Indian milks or milk sera. Although no radical departures are to be expected from the physical constants of European samples, it would be interesting to note the slight variations, if any, that exist.

Kothavalla and Sunawala[29] have examined the size, shape and number of fat globules in the milks of various breeds of Indian cows and buffaloes. They conclude, contrary to the observation of Levine and Koo,[30] that the fat globules of buffalo milk are, on an average, distinctly larger in size (5·4 to 5·7 μ) but fewer (712 to 945) in number per c.c. Those of the cows, on the other hand, vary in size from 2·9 to 3·4 μ, and in number from 1,000 to 1,267. Globules of the smallest size usually belong to the draft breeds of cows; globules of medium size reveal the largest uniformity in shape. Among the cows examined were two Ayrshire cows whose milk showed no distinctive character of the fat globules except that their average size (3·7 μ), slightly exceeded the highest Indian average.

Composition and Yield of Goat Milk

This has received little attention in India. While data are available on the composition of Indian donkey's milk,

[28] Rangappa, *Nature*, 1947, **160**, 719.
[29] *Indian J. Vet. Sc. and Anim. Husb.*, 1937, **7**, 8.
[30] *Lingnaam Agric. Review*, 1923, **1**, 13.

no detailed analysis has been done on the milk of Indian goats which forms a very promising source of milk supply for the Indian population. Reliable figures are available only on the average fat content of two breeds of goat milk. They are given in the following table.

TABLE XI

AVERAGE FAT CONTENT OF GOAT MILK

Breed	No. of animals tested	Fat per cent.	Yield per Lactation (lb.)
Beetal[31] ..	..	4·5	356
Jamna Pari[31] ..	..	5·2	540
Jamna Pari[32] .	215	5·2*	..
Jodhpur (local)[33] ..	40	3·5	..
Bar Bari[32] ..	150	4·9*	..
Bar Bari[31] ..	..	..	307

* Fat content has been determined on herd milk every day for a period of six years.

The Kamori, a dual-purpose goat like the Jamna Pari, is said to yield under ordinary conditions of jungle grazing 4 lb. a day, but a yield of 8–12 lb. a day under conditions of intensive feeding has been recorded. This breed is held to have considerable potentialities. The Baluchi and the Cutchi have an average milk yield of 2 and 3 lb. a day respectively.[32] Sen[34] has described a few more breeds of Indian goats with good promise as dairy animals. Under proper management the Osmanabad is said to yield 2–5 lb., Lerri 4 lb., Bari 3–3·5 lb., Thori Bari 3–3·5 lb. and Der Din Panah 5–9 lb. of milk per day.

[31] Kaura, *Indian Farming*, 1943, **4**, 549.
[32] *Rep. Marketing of Milk in India and Burma*, 1943, p. 205.
[33] Mehta, *Proc. 33rd Ind. Sc. Congress*, 1946.
[34] *Indian Farming*, 1944, **5**, 356.

The goat is, in addition, a more economical milch animal than the cow and buffalo. It is able to thrive on a class of fodder which is of little value to others, and can therefore be successfully reared even in areas where fodder resources are limited. The fat content of its milk also indicate that selected breeds of goats rival the cow in yield and richness of milk. The wisdom of developing this source of milk supply is therefore only too obvious.

Chapter III

MAJOR AND MINOR CONSTITUENTS OF MILK

Major Constituents

Protein—Attention has already been drawn (Table VII) to the slight excess of the total nitrogen content of Indian cow milk over that of the European cow. This is in keeping with the generally higher average of fat and solids-not-fat (Tables VIII and IX). According to Davies,[1] the higher proportion of S.N.F. in milk leads to a higher Casein Nitrogen content. In buffalo milk also where the protein content is distinctly higher than in cow milk the Casein makes up the largest part of the total Protein and is correspondingly higher than in cow milk. Kothavalla and co-workers[2] observed that the protein distribution in Indian cow milk is similar to that of western breeds, but that the total protein in buffalo milk (3·60 per cent.) is generally higher than in cow milk[3] (3·36 per cent.). Investigators in different parts of the world have determined the total nitrogen as well as the protein distribution in buffalo milk which are given in Table XIII.

TABLE XIII

DISTRIBUTION OF PROTEINS IN BUFFALO MILK (PER CENT.)

Workers	Country	Total Nitrogen	Proteins	Casein	Albumin + Globulin	Non-Protein Nitrogen
Pappel and Richmond[4]	Egypt	0·64	3·86	3·26	0·60	0·035 (approximately)
Pappel and Hogan[5]	.. Egypt	..	4·16	..	..	..
Levine[6]	.. China	..	6·04	..	..	..
Tartler[7]	.. Europe	..	4·42	3·54	0·52	..
Bruce[8]	.. Ceylon	0·735	4·59	..	..	0·016 (by difference)
Leather[9]	.. S. India	..	4·34	..	..	..

[1] *J. Dairy Res.*, 1932, 4, 144.

[2] *Rep. Imp. Dairy Dept.*, 1939-40, p. 26, Manager of Publications, Delhi.

[3] Anantakrishnan, Dastur and Kothavalla, *Indian J. Vet. Sc. and Anim. Husb.*, 1943, 13, 297.

Except for the Chinese buffalo milk, which is very rich in proteins, as in fat, the others have a uniform average, higher than that of both Indian and European cow milk. Sufficient data are not available, however, to comment on or to compare the proportions of the different constituents making up the total nitrogen. But it may be surmised that the protein content varies, like the S.N.F., with the dry and the rainy season of the year.

Kothavalla and co-workers[2] have studied the nitrogen distribution in certain breeds of Indian cows and buffaloes. The results (which include certain unpublished data from the Indian Dairy Research Institute) are given in Table XIV.

TABLE XIV

NITROGEN DISTRIBUTION IN THE MILK OF INDIAN CATTLE (PER CENT. TOTAL NITROGEN)

Constituent	Murrah Buffalo	Gir cow	Sindhi cow	British cow[10] (averaged)
Protein Nitrogen ..	88·8–95·54	94·8	92·9	94·5
Casein " ..	71·60–83·51	76·2	76·0	77·5
Albumin " ..	8·25–17·15	11·2	11·4	12·3
Globulin " ..	3·90–8·24	4·9	2·9	5·25
Proteose-peptone Nitrogen ..	..	2·5	2·6	..
Non-protein Nitrogen	4·62–11·20	5·2	7·1	5·47
(Total Nitrogen) ..	..	..	..	0·535

There appears to be a remarkable similarity n the distribution of nitrogen among the milks of the cow and the

[4] *J. Chem. Soc.*, 1890, **57**, 754.

[5] *Analyst*, 1916, **41**, 307.

[6] *Phil. J. Sc.*, 1919, **15**, 91.

[7] *Z. Gleisch-Milchyg*, 1918, **28**, 327; cited in "Structure and Composition of Foods," Winton, Chapman & Hall, London, 1937, p. 24.

[8] *Analyst*, 1922, **47**, 291.

[9] *Mem. Dept. Agric. India* (Chem. Ser.), **2**, 199.

[10] Davies, "Chemistry of Milk," 1939, p. 113.

buffalo as well as those of the British and Indian cattle. The only variation to be noted is the globulin factor, which seems to differ with the breed of the animal. It has however been observed that the non-protein nitrogen content is more or less constant both in cow and buffalo milk. But more extensive investigations are needed before useful conclusions can be drawn.

Much work also remains to be done on the elementary analysis and amino acid content of buffalo milk proteins; this would throw interesting light on their phosphorus and sulphur contents as well as on their digestibility in the human system.

Lactose—There seems to be no appreciable difference in the lactose content in the bulk milk of Indian and European cows. Although this constituent is slightly richer in buffalo milk, contributing to the higher S.N.F. content, the difference is not so marked as in the case of fat and nitrogenous constituents. It has also been pointed out that the rough inverse ratio between lactose and fat, which exists in cow milk, also holds for buffalo milk.

Pappel and Richmond[4] observed different powers of hydrolysis and polarisation of light for buffalo milk sugar from those exhibited by the lactose of cow milk. But this has been contradicted by Porcher[11] who examined the milk sugar of Italian and Annam buffaloes and found them to differ in no way from cow milk sugar.

Fat—Fat is the most variable of all the constituents present in milk. A detailed study of butterfat is made in Part III of this volume from the Chemical, Technological and Commercial points of view. It may, however be mentioned here as a distinguishing feature of buffalo milk-fat, that it contains Phosphorus and a larger proportion of Sulphur than does cow milk-fat—Sulphur 0·05 per cent. and Phosphorus 0·01 per cent.

[11] *Bull. Soc. Chim.*, 1903, **29**, 828.

Minor Constituents

Ash—The content of mineral matter (Tables VI and VII) in Indian buffalo milk is generally slightly higher than in the milk of the cow, European or Indian. And mention has already been made (opp. p. 15) that the ash content of Indian cow milk is frequently (particularly during periods of drought) below the European standard of 0.7 per cent. The composition of the ash also varies to a certain extent. Bruce[8] gives the following figures for the ash of Indian cow milk, averaged from a number of samples. Similar figures not being available for the milk of the Indian buffalo, Tartler's[7] values have been used for comparison.

TABLE XV

AVERAGE COMPOSITION OF MILK ASH OF COW AND BUFFALO (PER CENT.)

Constituent	Indian cow[8]	Buffalo[7]	European cow[8]
Iron ..	Trace	..	0·53
Lime ..	22·24	33·51	22·00
Magnesia ..	3·39	3·75	3·05
Potassium oxide ..	14·67	13·33	24·67
Sodium oxide ..	13·52	8·17	9·70
Phosphoric acid ..	38·72	33·50	28·45
Sulphuric anhydride ..	Trace	..	0·30
Chlorine ..	9·85	9·19	14·28

The phosphoric acid and sodium oxide are higher and the potassium oxide and chlorine are lower in the Indian cow milk than in the English cow milk. The lime content of buffalo milk is higher, nearly by 30 per cent. than that of either cow. This is in keeping with the finding of Anantakrishnan *et al.*[12] who also report a slightly higher phosphorus content in buffalo milk. The figure given by De[13] for calcium in buffalo milk, however, appears to be a little too

[12] Anantakrishnan, Dastur and Kothavalla, *Indian J. Vet. Sc. and Anim. Husb.*, 1943, **13**, 297.

[13] *Indian J. Med. Res.*, 1934–35, **22**, 499.

low. Except for sodium oxide, which is rather low, the mineral constituents of this milk resemble very closely those of the Indian cow milk.

Basu and Mukherjee[14] have estimated the phosphorus content of milk of a number of mammals.

TABLE XVI

PHOSPHORUS CONTENT OF MAMMALIAN MILK (mg./100 c.c. MILK)

Animal	Cow	Sheep	Goat	Buffalo	Human
Total Phosphorus ..	97·6	99·5	105·0	125·3	35·25

Of the total phosphorus content more than 50 per cent. is inorganic, except in human milk in which the ester phosphorus predominates. The authors report that in these milks hardly any phosphorus exists in the form of adenyl pyrophosphate or as hexose di- or mono-phosphate. In a feeding experiment on rats the same workers[15] found that nearly 80 per cent. of the phosphorus in cow, buffalo and goat milk is available to the system. Therefore, in view of the higher content of this element buffalo milk is superior in this respect.

Acharya and Devadatta[16] have estimated and classified the phosphorus (organic, inorganic, protein, lipoid, dialysable, undialysable, etc.), magnesium, and calcium (acid-soluble and insoluble, dialysable and non-dialysable) in buffalo milk. Table XVII, however, gives the absolute contents of these

TABLE XVII

CALCIUM, MAGNESIUM AND PHOSPHORUS CONTENT OF BUFFALO MILK (mg. PER 100 c.c. OF MILK)

Type	Calcium	Phosphorus	Magnesium
Acid-soluble ..	99·30	96·57	16·59
Acid-insoluble ..	49·32	25·68	1·21
Total ..	149·0	122·95	18·0

[14] *J. Vet. Sc. and Anim. Husb., India*, 1943, **13**, 231.

[15] *Ibid.*, p. 236.

[16] *Proc. Ind. Acad. Sc.*, 1940, **10**, 221.

elements in acid-soluble and acid-insoluble portions of mineral matter in milk.

From these figures it is evident that the greater part of these elements exist in the inorganic and organic form, uncombined with protein and fat. Of the total calcium, Singh, Verma and Anantakrishnan[17] found that the major portion (67–77 per cent.) exists in the colloidal form.

Desai and Mathur[18] have made the interesting observation that the lowest phosphorus and calcium content of milk falls in with the period of peak yield.

Pal[19] has estimated the total and ionisable iron in Indian cow milk, and has found that very nearly all of it exists in the ionisable (available) form.

No. of samples	Total iron mg./100 c.c. milk	Non-Ionisable iron mg./100 c.c. milk	Ionisable iron per cent. of total iron
10	0·62—0·71	0·6 –0·7	89·9—98·5

The manganese content of cow milk, however, has been found to be as low as 0.006 mg. in 100 c.c. of milk.[20]

Vitamins and Other Constituents—Milk contains, although in minute quantities, nearly all the vitamins so far known. They have been enumerated in Chart I. Quantitative figures, wherever known, for Indian milks are given in Table XVIII.

De[13] has reported that the spectrographic examination of milks reveals similar proportions (50 to 100 γ) of vitamin A in both cow and buffalo milk. This finding is supported by recent workers.[21]

[17] *Indian J. Vet. Sc. and Anim. Husb.*, 1946, **16**, 177.

[18] *Ibid.*, 1945, **15**, 198.

[19] *Indian Med. Gaz.*, 1939, **74**, 471.

[20] Ray, Chand and Rau, *J. Dairy Res.*, 1941, **12**, 109.

[21] Dharmani and Chopra, *Indian J. Vet. Sc. and Anim. Husb.*, 1946, **16**, 158.

TABLE XVIII

VITAMIN CONTENT OF INDIAN MILKS

Animal	Carotene[20]*	Vitamin[20]* A	Nicotinic acid‡[22]	Vitamin ‖[20] C
Cow ..	71 ±32·03	115·6 ±35·46	0·1	1·94 ±0·35
Buffalo ..	..	†23–27[21]	0·1	6·72[23]

* Moore's Yellow and Blue Lovibond units respectively per 100 c.c. of milk.
† Moore's Blue Units per gram of butterfat.
‡ mg. per 100 gm. of milk.
‖ mg. per 100 c.c. of milk.

Among the milks of domestic animals only cow milk-fat is pigmented. Fresh buffalo milk-fat has no carotinoid pigments in it.[24] This accounts for the whiteness of buffalo milk, and of butter and ghee made from its milk or cream, although Levine and Koo[25] attribute the difference to the smaller size of the fat globules in the milk of this animal. Vitamin A, however, is present in buffalo milk-fat, although perhaps to a slightly lesser degree than in cow milk-fat. When compared with the western breeds of cows, Ray and his co-workers[20] have found that Indian cow milk contains more of carotene and less of vitamin A, both of which are, however, subject to fluctuations due to the feed and breed of the animal. The riboflavin (B_2) content of colostrum has been found to be three times as high as that of normal milk; approximately 30 per cent. of the initial concentration in colostrum is lost within 24 hours after parturition.[26] In addition, colostrum is also richer than milk in thiamine and pantothenic acid, all the three vitamins being reduced to normal in 7 to 10 days after calving.[27] Buffalo milk

[22] Swaminathan, *Indian J. Med. Res.*, 1944, **32**, 43.
[23] *Dairy Sc. Abstracts*, 1939-40, **1**, 187.
[24] Davies, "Chemistry of Milk," 1939, p. 215.
[25] *Lingnaam Agric. Rev.*, 1923, **1**, 15.
[26] Theophilus and Stamberg, *J. Dairy Sc.*, 1945, **28**, 259, 269.

appears to be much richer in vitamin C than the milk of the Indian or European cow, both the latter containing very nearly the same amount of this Vitamin. The nicotinic acid content of Indian cow milk agrees well with that of Western breeds.[28]

Little work has been done on the enzymes present in Indian milk; but it is a fair inference that they are present in Indian cow and buffalo milk also.

[27] Pearson and Darnell, *J. Nutrit.*, 1946, **31**, 51.
[28] *J. Nutrit.*, 1945, **29**, 137.

Chapter IV

VARIATIONS IN COMPOSITION OF MILK

It may be stated as a matter of generality that Indian milks, especially cow milk, are subject to the same variations as those of Europe and America with regard to feed, breed, season, time of milking, stage of lactation, etc. In certain aspects, however, buffalo milk differs by its characteristic constancy. The factors affecting the variation of composition and other characteristics of milk will be dealt with in order.

Breed of Animal

The largest variations in the composition of milk are due to differences in breed of cattle. Data are only available with regard to the fat and total solids content of the milks of the cow and buffalo. In almost all cases, the total solids have been calculated on the basis of the specific gravity and fat content of milk according to the Richmond formula. The S.N.F. is deduced by difference. Table XIX (p. 37) illustrates the variations due to breed.[1]

Figures in this table indicate that, although all breeds exhibit a high average of fat and S.N.F., the differences in the quality of milk between the various breeds are fairly marked. The differences in the averages for the same breed, analysed by different authorities, can perhaps be explained by the presence or otherwise of selected dairy animals in the herd of the farm. Moreover, where the calf is allowed to suck the fore milk, as in the Poona samples, the fat content tends to be higher.

[1] *Rep. Marketing of Milk in India and Burma*, **1943, p. 203.**

TABLE XIX

VARIATION OF COMPOSITION OF MILK DUE TO BREED OF COW

Breed	No. of Samples tested	Sp. Gr. at 60° F.	Per cent. Fat	S.N.F.	Authority
Amritmahal	58	1·027	4·58	..	S. Rau, Bangalore (1913)
Ongole ..	48	..	5·05	..	Imp. Dairy Inst., Bangalore (1927)
Sahiwal ..	134	1·032	4·65	9·20	Military Dairy, Peshawar (1916)
Sindhi ..	929	..	6·0	9·42	H. H. Mann, Poona (1907)
Sindhi* ..	..	1·030	4·84	8·42	Agric. Inst., Allahabad (1938)
Sindhi ..	2 500	..	4·65	8·69	Imperial Dairy Inst., Bangalore (1940)
Gir ..	359	..	5·76	8·92	H. H. Mann, Poona (1907)
Gir ..	730	..	4·54	9·15	Imperial Dairy Inst., Bangalore (1940)
Tharparkar*	50	1·031	4·60	9·0	Rep. Imp. Dairy Expert (1934–35), p. 177
Hariana* ..	40	1·031	4·60	9·0	
Montgomery	25	1·029	4·60	8·44	Leather and Dobbs[2]

* Averaged from daily analyses over a year.

Similar figures are available (except for Sp. Gr.) for the buffalo milk of three breeds—the Murrah, Jaffarbadi and Surti animals. They are given in Table XX.[3]

TABLE XX

VARIATION OF COMPOSITION OF MILK DUE TO BREED OF BUFFALO [3]

Breed	No. of Samples tested	Per cent. Fat	S. N. F.	Authority
Murrah ..	Average of 75 animals	6·6	9·6	Military Dairy, Peshawar (1916)
do ..	Annual average of daily herd tests	7·3	9·25	Agric. Inst., Allahabad (1938)
do ..	2,200 herd samples	6·75	9·50	Imperial Dairy Inst., Bangalore (1940)
do ..	50	6·8	10·1	H. H. Mann, Poona (1908)
Jaffarbadi ..	82	7·3	10·1	
Surti ..	932	8·4	10·3	

[2] *Mem. Dept. Agric. India* (Chem. Ser.), 3, 148.

[3] *Rep. Marketing of Milk in India and Burma*, 1943, p. 205.

The Surti breed of buffalo obviously yields the richest milk among the breeds examined. The majority of workers give a fairly constant average of fat content for the Murrah breed. The higher figure of 7·3, however, indicates that there is still scope for improving the richness of the milk of this breed by a rigorous process of selective breeding and scientific management. It may also be worth considering the feasibility of further improvement of the dairy qualities of the Indian buffalo by breeding it with selected Cantonese animals whose milk has a high average of over 10 per cent. of fat.[4]

Individuality of Animal

Great variations are exhibited in the composition of milk from animal to animal of the same breed. Meggitt and Mann[5] have found such variations to be greater among buffaloes than among cows. The departure from the average composition naturally decreases with the bulking of milk of increasing numbers of animals. Table XXI gives the composition of

TABLE XXI

VARIATIONS IN COMPOSITION OF MILK DUE TO INDIVIDUALITY OF ANIMAL[6]

Animal	Sp. Gr. at 60° F.	Per cent. Fat	Per cent. S. N. F.
Sindhi Cow—			
1	1·0322	5·69	9·03
2	1·0324	4·81	8·46
3	1·0293	4·66	8·16
Murrah Buffalo—			
1	1·0334	7·05	9·49
2	1·0334	7·38	9·64
3	1·0340	6·57	9·41

[4] *Phil. J. Sc.*, 1919, **15**, 91.

[5] *Mem. Dept. Agric. India* (Chem. Ser.), **2**, 232.

milk of individual cows and buffaloes, each belonging to the same breed.[6]

Although animals of each group were maintained on the same feed, were of about the same age and weight, and were in the same stage of lactation, the differences in composition are very marked. Meggitt and Mann[7] state that large variations are to be expected among the individuals of a breed of cows which have undergone no selection for many generations.

Time of Milking

A definite variation is observed in the fat content of both the individual and herd milks between the morning and evening samples. Table XXII gives the figures for bulk samples of cow and buffalo milk.

TABLE XXII

VARIATION IN FAT CONTENT OF MILK DUE TO TIME OF MILKING[8]

Time of Milking	Sindhi Cow	Gir Cow	Buffalo
Morning ..	6·0	5·2	7·1
Evening ..	6·3	6·2	7·9

This difference varies from 0·3 to 1·5 per cent. according to the season, breed and individuality of the animal. The fat content of the bulk samples of the Surti buffaloes and Sindhi cows, for instance, differ hardly by 0·2–0·3 per cent., but those of the Gir cow and the Delhi buffalo herd vary from 0·9–1·2 per cent. between the two milkings. Such a variation is caused by the difference in the intervals between milkings. For, the longer the interval, the lower the fat content and the higher the yield of milk[9]; and the more frequent the milkings, the greater the fat variation.[10]

[6] *Rep. Marketing of Milk in India and Burma*, 1943, p. 206.
[7] *Mem. Dept. Agric. India* (Chem. Ser.), 2, 1.
[8] *Ibid.*, 2, 36, 231.
[9] *Rep. Marketing of Milk in India and Burma*, 1943, p. 208.
[10] "Fundamentals of Dairy Science," 1928, A.C.S. 41, p 18.

If the interval between the two milkings were made the same both in the night and day, Leather and Dobbs[2] found that the morning milk tends to be richer in fat than the evening milk. Figures are given in Table XXIII for Montgomery cows.

TABLE XXIII

EFFECT OF EQUALISING THE TIME INTERVAL BETWEEN MILKINGS ON FAT CONTENT OF MILK (PER CENT.)

Cow	Morning Fat	Evening Fat	Difference
1	4·03	3·72	0·31
2	3·85	2·95	0·90
3	5·76	3·99	1·77

The solids-not-fat of milk, however, remain very nearly the same, irrespective of the time and frequency of milking. Table XXIV illustrates this fact clearly.

TABLE XXIV

FAT AND SOLIDS-NOT-FAT CONTENT OF MORNING AND EVENING MILKS[11] (PER CENT.)

Breed	Fat		S. N. F.		
	Morning	Evening	Morning	Evening	
Tharparkar 50 Cows	4·4	4·8	9·14	8·92	The cows were milked at
Hariana 40 Cows ..	4·4	4·8	9·20	8·96	4 A.M. and 2 P.M.

The findings of various other workers, Indian and Western, confirm the constancy of the S.N.F. of milk drawn at different intervals.

Such differences in fat content vary a good deal from animal to animal and from season to season. Generally the difference lies between 0·75 and 1·50 per cent. The mean difference, after weighting for differences of period, for all the tested cows of the Pusa herd was 0·91 per cent. fat. In an English herd also, the morning milk showed a small

[11] *Rep. Imp. Dairy Expert*, 1934-35.

excess of fat over the evening milk, when the interval between the milkings was exactly 12 hours.[12] Dave and co-workers have come to the conclusion that the yield and fat content of milk remains very nearly the same (with a very slight excess of fat in the evening) when the interval between the two milkings is made the same. In general, with equal intervals between milkings, the lower fat content in the evening is believed to be due to the greater activity of the animal in daytime.

Stage of Lactation

The composition of milk from the birth of the young one to the end of the period of lactation undergoes characteristic changes. The milk secreted immediately after the birth of the calf and for a few days after is appreciably different from normal milk in composition as well as in physiological function. Its laxative effect is supposed to facilitate the early expulsion of the meconium from the intestinal tract of the infant animal. The extra-ordinary richness of the globulin fraction during the first twenty-four hours after calving indicates the prompt transference of a corresponding proportion of immunising bodies from the mother to the new-born. The change from colostrum to normal milk takes about three days in the buffalo, and four to five days in the cow.

In this connection it is interesting to observe the transitional changes from prepartum milk through colostrum to normal milk. Mukherjee *et al.*[13] have reported that the mammary secretion before parturition is extraordinarily rich in proteins (most of it being made up of globulin) and comparitively poor in lactose and fat.

Shah[14] and Sivasubramanian and Dover[15] have analysed the colostrum of certain breeds of Indian cows and buffaloes.

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[12] *Proc. Univ. Durham, Phil. Soc.*, 1911, IV, Part I; (cited in 2).

[13] Mukherjee, Swaminathan and Viswanath, *Indian J. Vet. Sc. and Anim. Husb.*, 1944, **14**, 232.

[14] *Ibid.*, 1936, **6**, 234, 241.

[15] *Ibid.*, 1938, **8**, 29.

The results given in Table XXV relate to the composition of milk immediately after the birth of the calf.

TABLE XXV

COMPOSITION OF THE COLOSTRUM OF THE COW AND BUFFALO[15] (PER CENT.)

Composition	Sindhi Cow	Murrah Baffalo	Montgomery Cow (average)
Sp. Gr. (20° C.) ..	1·063	1·074	1·058 (15° C.)
Acidity (c. c. N. alkali neutralising 10 c.c. of sample)	0·41	0·35	0·368 (% acid)
Chloride (NaCl) ..	0·46	0·61	..
Water ..	81·8	71·1	77·5
Total Solids ..	18·2	28·9	22·55
Fat .	2·0	3·9	4·6
Total Protein (N × 6·38)	12·8	21·4	13·21
Casein (N × 6·38) ..	4·0	6·8	4·58
Albumin ..	2·6	3·8	8·70 (Albumin and Globulin)
Globulin ..	6·2	10·8	
Lactose ..	2·2	2·2	3·30
Ash ..	1·0	1·1	1·20

While there are variations in composition due to the species and breed (as also to a great extent to individuality) of animal, the colostrum of all animals are characterised by a high specific gravity and high average content of free acidity, chlorides, total solids, total proteins (the excess being mostly made up of globulin) and ash. The low average content of fat and lactose is equally remarkable. In each of these constituents, except lactose in which it is generally inferior, buffalo colostrum is consistently richer than cow's. With the change into normal milk the fat and lactose increase and the rest of the factors decrease steadily. The rate of change, however, differs with the constituent as well as with the type of animal, the first to fall into place being the globulin fraction. All the observations agree with those made with western breeds of animals.

Prepartum milk and colostrum have the property in common, based on their high globulin content, of being coagulated by heat.

Among the physical properties, parturition is accompanied by a high value of the density, refractive index and refractive constant[16] of the colostrum owing, evidently, to its abnormal richness in the total solid and S.N.F. content. The freezing point, because of its higher content of crystalloids, is also lower than that of normal milk. All these values, however, reach normality in 3–7 days after calving.

As the lactation progresses, the fat rises to a constant level within a few weeks and maintains itself at that level for some months after which it further rises to the end of the period.[17] Meggit and Mann[8] found such an increase more pronounced among the Sindhi than the Gir cows, and least among the Surti buffaloes, which yielded richer milk only at the very end of the lactation period. For the Montgomery herd at Pusa, Henderson[18] observed a rise in the fat content until the beginning of the second month after which it remained constant up to the sixth month and rose progressively to the end of the lactation. The fat level of the cross-bred herd, however, rose to constancy and commenced this rise sooner than did the pedigree herd.

The milk yield of the animal also exhibits a relationship with the stage of lactation. Rising to a maximum in 3 to 5 weeks after calving, it declines steadily to the end of lactation. Kartha[19] has found that the rate of decline both for cows and buffaloes can be expressed as an exponential equation that has been formulated by Brody. This decline also varies with the type, breed and individuality of animal.

[16] Rangappa, *Proc. Indian Acad. Sc.*, B., 1947, **26**, 125.
[17] *Sci. Rep. Agric. Res. Inst., Pusa*, 1929-30 to 31-32, 41.
[18] *J. Cent. Bur. Anim. Husb. and Dairying, India*, 1928-29, **2**, 29.
[19] *Indian J. Vet. Sc. and Anim. Husb.*, 1934, **4**, 36.

Meggitt and Mann[8] have remarked little relationship, both among the cows and buffaloes, between the yield and composition of milk of individual animals. When the animal is rapidly drying off, however, the milk tends to become slightly richer in fat and chloride content.

Different Quarters of the Udder and Different Portions of a Milking

The changes in composition of milk due to these factors are of importance in India inasmuch as the calf is made to suck portions of milk from different quarters of the udder during milking. This fact may have therefore to be taken into consideration while judging the quality of a given sample of market milk.

Leather and Dobbs[2] have noted that while the quality of milk from the four different quarters of the animal exhibits characteristic differences, very little variation exists from one side of the udder to the other. Table XXVI illustrates these facts.

TABLE XXVI

VARIATION IN QUALITY OF MILK DUE TO DIFFERENT PARTS OF UDDER (FAT PER CENT.)

Animal	L. Side	R. Side	L. Fore	R. Fore	L. Hind	R. Hind
1	4·39	4·29	4·36	4·33	4·42	4·20
2	4·37	4·52	4·42	4·68	4·31	4·38
3	4·28	4·19	4·19	4·12	4·32	4·27

It is well known that different portions of milk during a milking possess different composition, the fore-milk being always poorer in fat than the strippings. Working on a few Indian animals Kothavalla and his co-workers[20] have found that the first portion of the milk drawn is richest in solids-not-fat but poorest in fat and *vice versa*. In other words the fat content increases and the S.N.F. decreases with the

[20] *J. Cent. Bur. Anim. Husb. and Dairying, India*, 1929-30, 3, 122.

progress of milking. They have observed however that the rate of decrease of S.N.F. is not so marked as the rate of increase of fat. Table XXVII gives the fat and S.N.F. contents of different portions of the milk at a milking.

TABLE XXVII

COMPOSITION OF DIFFERENT PORTIONS OF MILK DRAWN AT ONE MILKING (PER CENT.)

Portions	Fat	S. N. F.
1	1·04	8·64
2	1·92	8·63
3	3·02	8·51
4	4·40	8·37
5	5·32	8·15
6	7·63	7·77

These figures vary slightly with the method of drawing the portions of milk; but the general trend remains the same.

Feed

The effect of variation of feed on the composition of milk is a very wide field of study in which a good deal of work has been done in western countries by animal nutrition workers and others. Some work has also been done here on the effect of feeding indigenous oil cakes, grasses and silages on the composition and yield of milk.

Working on the effect of high and low protein feeds, Warth[21] has found that the former leads to a slightly higher fat content in milk, probably due to the ingestion of an excess of fat along with the cake. There was otherwise no difference in the yields of milk. He observed however, that with a higher starch equivalent in the feed of the animal there was an increase in the yield of milk.[22] Cows fed on

[21] *Sc. Rep. Agric. Inst., Pusa,* 1934-35, 180.
[22] *Ibid.,* 1933-34, 125.

different types of oil cakes showed that linseed cake produced a slightly higher yield of milk but slightly poorer in quality, while coconut cake led to a higher percentage of fat, and cottonseed meal to a higher content of nitrogen in the milk.[23,24] Davis and Harland[25] report that replacement of a part of the concentrates in the ration with cotton seed only influences the fat content of milk by a slight rise without affecting the milk yield. The substitution of molasses, by Warth,[22] for a portion of the concentrates, on the other hand, led to a reduction in milk yield. But he observed no such reduction in yield of milk or fat when a portion of the silage was made up by roughages like ragi straw in the feed. This is encouraging in view of the high cost and difficulty of preparation of silage. He also states that fodder fed in a chaffed state leads to more economic food consumption and milk production.[26] The composition and yield of milk remained practically the same when Das Gupta,[27] and Ray Sarkar and Sen[28] fed heavy rations of berseem hay in place of the concentrate mixture. The carotene and vitamin A contents, however, increased up to a limit with the replacement of dry rations by green feeding, beyond which further green feeding made no change. The importance of an adequate quantity of roughages in the feed of the animal has been brought out by Loosli and his co-workers,[29] the lack of which results in a reduction of the butter-fat and milk yield. A finding of considerable importance in nutrition is that shortage of energy (starch equivalent) in the animals diet is more serious in its effects in diminishing the milk protein than is shortage of protein, provided the diet

[23] *J. Cent. Bur. Anim. Husb. and Dairying, India*, 1929, **3**, 77.

[24] *Ibid.*, 1930, **4**, 7.

[25] *J. Dairy Sc.*, 1946, **39**, 839.

[26] *Sc. Rep. Agric. Inst., Pusa*, 1930-31, 130; *Agric. and Live Stock*, 1932, **2**, 13.

[27] *Indian J. Vet. Sc. and Anim. Husb.*, 1943, **13**, 196, 275; 1944, **14**, 279.

[28] *Ibid*, p. 219.

[29] *J. Dairy Sc.*, 1945, **28**, 147.

contains sufficient energy.[30] The practice of feeding cod-liver oil to cows appears to influence the fat content of milk. When the oil is fed all at once the fat per cent. suffers a fall. But the fall is negligible if it is distributed over a large number of feeds.[31] It is perhaps possible that the sudden influx of an unusual amount of a metabolic accelerator like cod-liver oil helps a portion of the fat which would otherwise have entered the milk to be utilised in the animal system itself.

Recent findings[32, 33, 34] indicate that the level of true Vitamin A in milk may be increased by feeding large amounts of the Vitamin (700,000 I. U./day) in the form of fish liver oil concentrates. But this rise is partially offset by a decrease in carotene content. Supplementation of the diet of the cow with concentrates of natural, mixed tocopherols has little effect on the Vitamin A content of milk, but it causes a rise in the milk fat and total milk production.[35] However, the increased production of butterfat which follows administration of Vitamin E does not occur if both Vitamins A and E are supplied, and the fall in carotene content of milk is also less marked than when Vitamin A is fed alone.

A condition of frequent occurrence in India, starvation and under-feeding of the animal, is characterised by a considerable drop in the yield of milk as well as in the fat content (about 40 per cent. in 12 days) of the secretion. Even after the animal is put on a balanced feed the composition and yield fail to come back to normal for a number of weeks.[36] It is clear, therefore, that the nutritional status of the milch animal must be maintained at the normal level under all circumstances.

[30] Kay, *Nature*, 1947, **160**, 307.
[31] Moore, Hoffmann and Berry, *J. Dairy Sc.*, 1945, **28**, 161.
[32] *Nutrit. Rows.*, 1943, **1**, 145.
[33] Deuel *et al.*, *J. Nutrit.*, 1941, **22**, 303.
[34] *Ibid.*, 1942, **23**, 567.
[35] Harris, Swanson and Hickman, *J. Nutrit.*, 1947, **33**, 411.
[36] Smith and Dastur, *Biochem. J.*, 1938, **32**, 1868.

Chapter V

BACTERIOLOGY AND HEAT TREATMENT

Dairy Practice in India

Among the civilised nations of the world it is unfortunate that India ranks very low in its standards of dairy sanitation, hygiene and farming. The cattle are not allotted in the farmer's house separate byres and sheds according to sanitary laws, but are huddled together in a part of the house, almost jostling with the human inmates. The floor being earthy and uneven, they wallow in their own dirt as long as they are tied up in the house, usually from dusk to dawn. The animals are never brushed and rarely washed. During milking the udder may or may not be washed with water; and the milk is drawn in a metallic (commonly brass) vessel after allowing the calf to suck a portion of the milk from the animal's udder.

The cattle are let out into the fields after the morning milking, ostensibly for grazing. But there is no practice in India, unlike in other dairy countries of the west, of allotting lands and cultivating pasture for cattle-grazing by individual farmers. In large villages, an indiscriminate piece of dry land is earmarked by Government for grazing cattle. Even here no attempt is made to grow any pasture, and the animals seem to visit the field only for gathering more dirt. In summer, the buffaloes wallow in dirty ponds throughout the day and come back home in the evening richly coated with mud. Once in a way, they are washed in the same ponds in which they cool themselves.

In cities where milk is generally retailed by milkmen who own a few animals each, the conditions are not much different. Either the milk is drawn in the milkman's house, watered according to his conscience and the price he sets and carried to the consumer's in open cans or other metallic containers, or the animal itself is taken to the buyer's house and milked in the open at his door.

Under these conditions of dairy farming, it is easy to imagine the poor bacterial quality of village and market milks in India. The Report on the Marketing of Milk in India and Burma (*loc. cit.*) sums up the situation thus: "The universal Indian practice of allowing the calf to suck a little milk before each milking, the practice of milking the animals on the streets for door-to-door delivery, the dirty udder and unwashed flanks of the animals, particularly the buffaloes which wallow in the mud, the unwashed and contaminated hands of the milker, his dirty clothes and insanitary habits, *e.g.*, coughing, sneezing and blowing the nose with fingers, etc., the diseases, if any, of the milker and others handling the product, the filthy condition of the cattle stable which exposes milk to manurial contamination, the unclean and defective milk vessels, the polluted water supply in the villages, the defective transport arrangements, the practice of putting leaves, paper and straw in milk to prevent spilling from open cans, the warm climate of the country so favourable for rapid growth of bacteria, the ignorance and indifference of persons generally engaged in the milk trade even towards the elementary principles of cleanliness, the insanitary conditions of the open milk shops in the streets, the lack of suitable technical and educational facilities to create better understanding in the people concerned, the addition of dirty water to milk, the lack of adequate control by the health authorities, etc., are only a few of the many sources and causes which unfortunately pollute the supply of market milk in India and help to keep its standard low compared to other countries."

The report also makes recommendations for the improvement of bacterial quality of milk by the promulgation and enforcement of laws and standards on the same lines as in other countries. In England the bacterial content of "certified" milk does not exceed 30,000 per c.c. It has been abundantly shown that this standard is easily attained in India if proper precautions are observed.

Bacterial Quality of Market Milk

The last mentioned report rightly complains that "in India although the defective, filthy and often dangerous methods employed in the production, handling and distribution of milk are generally wellknown to all including the Public Health authorities, little or no biological work has been done so far to bring to light the real state of affairs or their effects on public health. Figures of infantile mortality in India, said to be highest among all countries of the world, are often quoted in the various reports, but no study has been made to show how far the unhygienic conditions prevailing in the production and distribution of the most important child's food—milk—are responsible for the heavy toll of death and disease."

Although milk within the healthy udder is practically free from bacteria, it gets contaminated soon after it is drawn. The amount of contamination varies with the conditions enumerated before, the temperature of the day, the season of the year and the time lapse between milking and distribution.

Joshi[1] has analysed a number of samples of market milk in Bombay, which give an idea of the average bacterial content of such milks. Table XXVIII gives these figures.

TABLE XXVIII

BACTERIAL QUALITY OF BOMBAY MILK

Time of Collection		No. of Samples	Average per c. c.	Average of 68 Samples
June	1913	8	8,981,000	
September	,,	12	16,195,000	
December	,,	12	18,833,000	
January	1914	6	10,416,000	17,103,000
April	,,	9	19,000,000	
May	,,	21	19,767,000	

[1] Joshi, L. L., "The Milk Problem in Indian Cities," 1916, p. 54; Taraporevala & Sons, Bombay.

When samples were collected from milk shops, railway stations, street vendors, etc., the average of 240 samples rose further to 36,385,000 organisms per c.c.[2]

Although the bacterial quality of the milk sold is deplorably low, it is encouraging that the samples were free from pathogenic organisms like *B. Typhus*, tuberculosis, diphtheria or cholera.[3] But almost every sample showed the presence of Coli organisms, and nearly all the bacteria of the milk were found to ferment lactose.

In contrast to this, when Joshi[4] collected fresh milk from healthy animals of Bombay under strictly aseptic conditions, he found the bacterial count to be as low as 292 per c.c. That it is possible to produce and distribute low count milk in India has been amply proved by the workers at the Agricultural Research Institute, Pusa,[5] and the Imperial Dairy Research Institute, Bangalore. Bacterial counts of the milk of the Pusa Dairy Farm are given in Table XXIX.[6,7]

TABLE XXIX

BACTERIAL COUNT OF DAIRY FARM MILK

Month		Average Count per c.c.		Remarks
		1925	1931-32	
January	..	20,800	8,845	Open pails were used in 1925; 'Covered' pails in 1931-32
February	..	8,300	4,000	
March	..	11,500	9.770	
April	..	..	8,617	
May	..	22,000	5,871	
June	..	28,500	7.192	
July	..	..	3,833	
August	..	..	4,092	
September	..	..	4 753	
October	..	..	2,333	do
November	..	..	3,470	
December	..	..	3,400	

[2] *Ibid.*, p. 56.
[3] *Ibid.*, p. 69, 76.
[4] *Ibid.*, p. 52.
[5] *Sc. Rep. Agric. Res. Inst., Pusa*, 1924-1932.
[6] *Ibid.*, 1925-26, p. 51.
[7] *Ibid.*, 1931-32, p. 149.

It may be noticed that the use of a 'covered' (partially covered) pail for the collection of milk appreciably brings down the bacterial count of milk.

Verma and co-workers[8] have studied the bacterial quality of 1,340 samples of morning milk produced and handled under different conditions in Bangalore by the plate count and the methylene blue reduction time. These included individual and herd samples from private-owned and dairy animals, from villages and town stables, from hawkers and from animals milked from door-to-door in the city. The results are given in Table XXX.

TABLE XXX

BACTERIAL QUALITY OF MARKET MILK IN BANGALORE

Sample	Plate Count per C.C.	Methylene blue reduction time (hours)
Individual (Imp. Dairy Inst.)	<50,000	>5·5
Herd (Imp. Dairy Inst. and private-owned cows)	<100,000	4—5·5
Dairy, and from animals milked from door-to-door	<500,000	>4
Town stables ..	200,000–5 millions	2–5·5
Village ..	500,000–10 millions	1–4
Milk hawkers ..	500,000–5 millions	<3
Town dairies ..	>1 million (maximum recorded >90 millions)	<2

These figures clearly indicate, not merely the insanitary conditions of collection and supply of milk in villages and town stables and the urgent need for measures of control, but the possibility of producing low-count, high-grade milk.

[8] Verma, Kothavalla and Seshacharyulu, *Indian J. Vet. Sc. and Anim. Husb.*, 1944, **14**, 223.

Variations in Bacterial Count of Milk

Several factors influence the bacterial quality of milk. The deterioration caused by the unhygienic methods of maintenance of cattle, conditions of collection and distribution of milk have already been pointed out. The changes due to season, time lapse between milking and distribution, temperature of storage of milk, etc., will now be considered.

Season

The results obtained at the Pusa Agricultural Institute (*loc. cit.*) show an unfailing correlation between the season of the year and the bacterial count of milk. Beginning with a low average count in the cold months of the year (October to January), there is a gradual rise till the break of rains in June, when it suddenly shoots up to a high figure and then slowly falls off (July to September) to the winter level again. The reason for the abrupt rise in the bacterial count with the commencement of the monsoons is that cattle gather quantities of mud and dirt in the fields and shed them into the milk during milking.

Time of Storage

Under tropical conditions which favour a quick multiplication of bacteria in the milk, the time lapse between milking and distribution or processing is a major cause of high bacterial counts.[9] Joshi[10] observed the following rate of rise with storage of milk collected under strict aseptic conditions in Bombay.

Up to a limit, the bacteria continue to multiply with the time of storage of the milk. After passing through a phase of logarithmic rise the count sharply attains a steady level. This is clearly seen in Table XXXI.

[9] *Sc. Rep. Agric. Res. Inst., Pusa*, 1929–30, p. 53; 1930–31, p. 66.
[10] Joshi, L. L., "The Milk Problem in Indian Cities," 1916, p. 62.

TABLE XXXI

RISE IN BACTERIAL COUNT OF MILK WITH STORAGE

Time of Storage (Hours)		Bacteria per c.c.
Soon after milking		198
1	..	304
2	..	624
3	..	1,035
4	..	7,200
5	..	19,400

Temperature of Storage

This is another important fatctor that determines the bacterial count of milk under storage. The rate of multiplication of micro-organisms increases up to a limit of about 40° C., the optimum being 25°–35° C. This range of temperature commonly prevails in this country, and is therefore most favourable for bacterial growth. Table XXXII gives the *relative* increase of bacteria in milk at two different temperatures of storage.[11]

TABLE XXXII

EFFECT OF TEMPERATURE OF STORAGE ON BACTERIAL COUNT OF MILK

Temperature of Storage (°C.)	Relative Number of Bacteria at the end of Hours 0	6	12	24	48
20	1	1·7	24·2	6,128·0	357,499·0
10	1	1·2	1·5	4·1	6·2

The enormous increase in the multiplication of bacteria at the higher temperature is correspondingly reflected in the lower keeping quality of milk. At Pusa,[12] milk stored at 10° C. kept for 6–9 days during the cold months, and for 4–6 days during the warmer months.

[11] *U.S. Dept. Agric. Bull.*, 348, p. 11.

[12] *Sc. Rep. Agric. Res. Inst., Pusa*, 1928–29, p. 41.

The difference in the keeping qualities of milk between the two seasons at the same storage temperature is accounted for by the difference in the initial counts of milk, a higher count in summer resulting in lower keeping quality.

The bacterial count of milk also varies from animal to animal; and in each animal different portions of milk at a milking show different bacterial counts. The fore milk is the richest in bacterial content, and is five or six times that of middle milk.[13] Again, certain cows are found to yield consistently low count milk; and it is not known whether this is a mere result of healthiness, or a herditary character.

Heat Treatment of Milk

The fact that the raw milk ordinarily produced in the country is of very low bacterial quality is not so very discouraging as it appears at first. For, the situation is redeemed by the almost ubiquitous practice of boiling milk before consumption. And it is to this practice that the absence of milk-borne diseases in India is to be mainly attributed.[14] Unlike in Western countries, there exists neither legislation for, nor the common practice of, heat treating or refrigerating milk soon after it is collected by the producer. But boiled milk is sometimes sold in cities where the milk retailer has to preserve it for the longest possible period.[15]

Reduction in Bacterial Count due to Processing

The multiplication of bacteria in milk leads to the progressive development of acidity and reduction of *p*H, culminating in the curdling of milk. To lengthen the life of the milk, therefore, the bacterial growth should be checked by cooling the milk to below 10° C., or the viable organisms should be destroyed by suitable preliminary heat treatment.

[13] *Ibid.*, 1927–28, p. 50–52.

[14] Wright, N. C., "Report on the Development of the Cattle and Dairy Industries of India," 1937, p. 21, Manager, Govt. Pub., Delhi.

[15] *Ibid.*, p. 22.

In Western countries pasteurisation (flash or holding) and immediate refrigeration is the legal standard of preliminary treatment of milk. This is the practice adopted in the military and civil dairy farms in India. Data are available on the changes in bacterial count of dairy farm milk in India before and after such treatment.[16]

TABLE XXXIII

BACTERIAL COUNT OF MILK BEFORE AND AFTER PASTEURISATION

No. of Hours after milking	Stage	Bacteria per c.c.
..	Milking pail	6,300
¼	Weighing pail	27,300
2	Bulked milk before pasteurisation	225,000
2½	After pasteurisation	9,400
3	After bottling	15,000
18	Milk returned from delivery	121,000

It is easily seen that in spite of the elaborate process of pasteurisation and subsequent cooling of the milk, there are more viable bacteria left in it than are found in the fresh sample in the milking pail. The quick multiplication of these under ordinary conditions of preservation shorten the life of the milk. That this method of treatment and preservation in cold storage is not commendable to the Indian farmer, not only from the point of view of economy but also from its unsuitability under tropical conditions, has been emphasised by Wright[17] in his report on the development of the Indian dairy industry. The expensive and elaborate nature of the equipment are the obvious drawbacks, while its sterilising efficiency is questionable.

[16] *Rep. Marketing of Milk in India and Burma*, 1943, p. 213.

[17] Wright, N. C., *Rep. Development of Cattle and Dairy Ind., India*, 1937, p. 25–26.

In contrast to this method of processing the simple method of boiling, or evaporating milk at the boiling point, appears to be best suited for Indian rural conditions. Srinivasan and Banerjee[18] have investigated the bacterial destruction after so processing the milk, and have compared it with the method of steaming for 1 hour in an autoclave at atmospheric pressure. The results are given in Table XXXIV.

TABLE XXXIV

BACTERIAL COUNT OF MILK AFTER PROCESSING

Bacteria per c.c. (Raw milk)	Method of processing	Bacteria per c.c. (processed milk)
120,000	Steamed for 1 hour	100
150,000	Boiled for 5 minutes	5,000
150,000	Boiled for 10 minutes	30
150,000	Boiled to reduce volume by 5%	23
150 000	Boiled to reduce volume by 10%	23

Rangappa[19] has also estimated the standard agar count of milk so processed after different intervals of storage at room temperature (17°–25° C.). Table XXXV illustrates the slow

TABLE XXXV

BACTERIAL COUNT OF MILK STORED AT 17°–25°C. AFTER BOILING FOR 10 MINUTES

Hours after milking	Sample	Standard Agar count per c.c.
2	Fresh Milk	566,000
2½	Milk boiled for 10 minutes and cooled	180
4½	Cooled in a closed flask and stored	1,800
8½	Do	3,200

[18] *Indian J. Vet. Sc. and Anim. Husb.*, 1946, 16, 72.
[19] *Indian Med. Gaz.*, 1947, 47, 320.

multiplication of bacteria in such milk as compared to pasteurised milk (*cf.* Table XXXIII).

The method of boiling milk for 10 minutes, or of reducing its volume by 5 per cent. is, as can be seen from the above table, evidently more efficient than pasteurisation or steaming for one hour at atmospheric pressure. It has also been found that fresh milk so processed keeps for more than 24 hours at room temperature when cooled in a closed vessel and then stored. The method has the great advantage of being perfectly suitable to the petty farmer with a few pounds of milk, as it needs no special equipment and demands little skill from the villager.

The practice of simmering the morning milk over the remains of the kitchen fire till evening and then adding to it the evening lot is also common. But the bacterial quality of milks resulting from this and other indigenous methods of processing are yet to be investigated. Anantakrishnan and coworkers[20] have, however, found that milk maintained at 55° C. keeps sweet for 48 hours, and they recommend this method for transportation under tropical conditions prevailing in India. That this method requires further investigation is clear from the findings of Verma and Lakshminarayana[21] who have isolated four distinct types of aerobic spore-formers that survive heat-treatment at 55° C., and some of which, in fact, have a growth range extending from 50°–80° C.

Chemical Changes due to Processing

Variations take place in the composition of milk, the physical nature of its constituents and its digestibility with different types of heat-treatment. Such changes are chiefly determined by the time-temperature relationship of the treatment—the higher the temperature and the longer the processing, the more marked the changes.

A great deal of work has been done on the chemical changes in milk constituents resulting from the holding

[20] Anantakrishnan, Dastur and Kothavalla, *Curr. Sci.*, 1946, **15**, 354.
[21] *Curr. Sci.*, 1947, **16**, 228.

method (140°–145° F. for 30 minutes) of pasteurisation. Denaturation of the protein molecule, as well as its flocculation due to the neutralisation of the electric charge on it occurs to some extent. The albumin fraction suffers a reduction of about 5 per cent. while the casein content remains practically the same. The coagulation of the albumin progressively increases with temperature of treatment—82 per cent. of it being precipitated when held at 115° F. for half-an-hour.[22]

A partial precipitation of calcium salts[23] and phosphates[24] also occur during pasteurisation, the diffusible calcium being reduced from 26 per cent. to 20 per cent. of the calcium in milk.[25]

Boiling milk and slowly cooling it to room temperature without stirring causes a skin to be formed at the liquid-air interface, and a layer of residue at the bottom of the vessel. While the residue consists mostly of denatured casein and precipitated mineral matter, the composition of the skin is variable, depending on the amount of fat rising to the surface, ash (mainly tricalcium phosphate) and denatured protein. The fat content of the skin has been found to vary from 20 to 60 per cent. of the dry matter.[26]

Anantakrishnan and his co-workers[27] have recorded a fall in the content of dry matter in boiled milk, from which the skin and the residue had been removed. With a loss of 6 per cent. in the volume of milk the total solids were reduced by about 5 per cent. The skin and residue together contained, in the dry matter, 49.3 per cent. of fat, 28.1 per cent. of proteins and 4.4 per cent. of ash rich in calcium and phosphorus. But it must be pointed out that the contents of the skin and residue of milk cannot be reckoned as a loss

[22] Kieferle and Gloetzl, *Milschw. Forsch.*, 1930, **11**, 62.
[23] Soldner, *Landw. Versuch,—stat.*, 1888, **35**, 351.
[24] Diffloth, *Bull. Sc. Pharmacol.*, 1904, **10**, 273.
[25] Magee and Harvey, *Biochem. J.*, 1926., **20**, 873.
[26] Davies, W. L., "The Chemistry of Milk," 1939.
[27] *Indian J. Vet Sc. and Anim. Husb.*, 1943, **13**, 297

inasmuch as they are entirely utilised either for consumption or for the production of curd or butter.

The formation of the skin and the residue in boiled milk is, on the other hand, totally prevented by slow but continuous agitation during boiling and cooling of the milk to room temperature. Rangappa[19] has analysed the milk boiled in this way for 10 minutes. While the volume suffered a loss of about 10 per cent., the total solids rose by about 2·3 per cent. and fat by 1·15 per cent. The acidity and ash also showed a very slight increase. But when the boiled milk was made up to original volume the loss in total solids, fat and lactose were almost insignificant. A slight loss in lactose was noticed by Kieferle and Gloetzl[22] also when milk was boiled for 30 minutes at 100° C. When correction was however made for the reduction in volume, the total solids showed a loss of only about 0·05 per cent. with regard to proteins. Gould[28] has found that rigorous processing (2·5 hours at 116° C. or 8 hours at 100° C.) destroys 25–30 per cent. of the lactose content. Wright[29] has observed no change in the casein content or molecule on heating to 120° C. for 30 minutes although its coagulability with acid and rennet was affected by the treatment. The soluble calcium and phosphorous salts, however, suffer a slightly greater loss by boiling than by pasteurisation.

Among the vitamins in milk, A is the most resistant and C. the most vulnerable to heat treatment. While hardly any vitamin A is destroyed by boiling, Kothavalla and his co-workers[30] have found about 22 per cent. of vitamin C is lost at the " First Boil, " 66 per cent. after 10 minutes and 100 per cent. at the end of 30 minutes. The loss of vitamin C is dependent both on the time of treatment and of exposure to light. Most of it can, therefore, be conserved by processing for the shortest interval in a closed system.[31]

[28] *J. Dairy Sc.*, 1945, **28**, 367.
[29] *Biochem., J.*, 1924, **18**, 245.
[30] *Indian J. Vet. Sc. and Anim. Husb.*, 1943, **13**, 35.
[31] Woessner, Weckel and Schuette, *Dairy Sc. Abs.*, 1940, **2**, 325.

A slight reduction in the thiamine (B_1) content of milk occurs due to the simple cleavage of the molecule into pyrimidine and thiazcle.[32] Theophilus and Stamberg[33] have reported that riboflavin (B_2) is very little affected by the type of processing or preservation of milk although photolysis of the vitamin readily takes place when milk is exposed to direct or diffused sunlight.

All the enzymes of milk are destroyed both during pasteurisation and boiling; and the digestibility of milk increases in the order, raw, pasteurised and boiled.[34,35,36]

Escudero, Herraiz and Herrero[37] also report that the biological value of milk for rats, and the availability of calcium and the vitamins (except vitamin C) is little affected by boiling. Consideration of all factors involved in the processing of milk, therefore, leads one to conclude that boiling for 10 minutes in a closed vessel is the most suitable for the dairy conditions prevailing in India.

[32] Knott, *Ibid.*, 1942–43, **4**, 195.
[33] *J. Dairy Sc.*, 1945, **28**, 259, 269.
[34] Leary and Shieb, *J. Biol. Chem.*, 1917, **28**, 393.
[35] Doan and Dizikes, *Dairy Sc. Abs.*, 1942–43, **4**, 176.
[36] Turner, *Food Res.*, 1945, **10**, 52.
[37] *Nutrit. Abs.*, 1945, **15**, 30.

Chapter VI

UNFERMENTED MILK PRODUCTS

The importance and completeness of milk as human food, when once realised, will naturally lead to attempts and devices to retain its wholesomeness for the longest possible period. But the prized perfection of the product is itself against its preservation for an indefinite length of time, since it offers an equally perfect medium for the growth of micro-organisms which thrive on the sugar, fat and proteins of milk. The multiplication of these organisms leads first to the smell and taste of acidity, and soon after to the clotting of milk on warming. The aim has always been, therefore, to conserve either all or as many as possible of the constituents of milk in a form most akin to that in the original milk. Concentration and partial desiccation of milk, and the addition of preservatives like sugar and fillers like flour to resulting concentrates have given rise to various unfermented milk products. While some of these are peculiar to India, many have their counterparts among the whole-milk products of the West.

India is reported[1] to produce annually 66·2 crores of rupees worth of unfermented milk products, forming little more than 10 per cent. of the total milk production, and 15 per cent. of all the milk products (Table V). The chief products are Khoa, Rabbri, Kheer, Mallai, Khurchan, Cream, Ice-cream and Casein. Except for Casein, which is manufactured to the extent of about 8,000 cwts. per year, mostly for export to industrial countries,[2] the others are prepared on a very small scale either in households, or in hotels and confectionery shops for limited local consumption. No attempt has so far been made to devise manufacturing

[1] Wright, N. C., *Rep. Development of Cattle and Dairy Ind. India*, 1937, p. 160.

[2] Wright, N. C., (*loc. cit.*), p. 49.

processes to exploit them on large factory scales, as is the case with similar products in the west. There is, however, an appreciable import trade in western milk products most of which could be replaced by the manufacture of similar products in India with the sympathetic support of Government.[3] The following table illustrates the import trade in these products[4] before the war.

TABLE XXXVI

IMPORTS OF UNFERMENTED MILK PRODUCTS INTO INDIA[4]

Product		Approximate quantity Imported (Cwt.)
Unsweetened Condensed Milk, whole	..	25,000
Sweetened Condensed Milk, whole	..	25,000
do do (skimmed)	..	150,000
Dried Milk Powder, whole	..	25
do (skimmed)	..	250
Preserved Cream	..	50
Sterilised Milk	..	150
Total	..	200,300

The milk equivalent of these products amounts to 694,200 maunds.

These figures remained fairly constant till 1939, after which, as was to be expected, the imports considerably increased owing to the exigencies of War. But figures are lacking for such imports. As they have been mostly for Military consumption, the excess imports might vanish to some extent when conditions return to normal. But with the inordinate depletion of the milch cattle during the war and the rising income levels of the working classes, a sustained importation of large volumes of milk products like whole and skim milk powders is likely to prevail until the

[3] Wright, N. C. (*loc. cit.*), p. 50–51.
[4] *Ibid.*, p. 167.

milk production in the country can satisfy the indigenous demand.

Khoa—This is the most important of the unfermented milk products, making up the largest proportion of them. The keeping quality of the milk is preserved by its conversion into semi-solid form, the heat and the concentration of milk constituents both tending to eliminate bacterial growth.

In the preparation of Khoa, small quantities of milk (usually about 5 lbs.) are evaporated in a round-bottomed shallow iron pan, (*Karhai* or *Khola*) over a fairly hot and steady fire. To prevent scorching, the milk is continuously stirred with a special scraper (*Khunti*). With the diminution in volume the rate of stirring is increased, and the operator has to exercise skill in maintaining a uniform consistency in the mass. After about 15 minutes, the mass assumes a pasty consistency, when the pan is removed from over the fire, and the product well worked with the flattened end of the scraper by alternately spreading into thin layers and collecting it repeatedly until it can retain its own shape. At first the mass has the consistency of butter, but after cooling it becomes more solid, like dough. It is now shaped into large circular pats and allowed to cool on the floor. It tastes sweet, and has a wholesome, lightly cooked flavour.

Khoa is mostly used in the preparation of sweetmeats. By itself it keeps well for 4–5 days in cold weather and for 2–3 days in hot weather. But the addition of sugar prolongs its life to 3 or 4 months.

The main reaction in the preparation of Khoa is the heat denaturation and coagulation of the milk proteins. Most of the albumin and globulin are rapidly denatured, the protective properties of the other colloids are destroyed early in the boiling process, and the process is accelerated by the incorporation of air and the frothing during the stirring. The total heat coagulation of the proteins occurs when the boiling mixture thickens to a buttery consistency in the pan. The vigorous agitation of hot milk has an appreciable homogenising action so that when the stage of coagulation is reached all the fat globules are entrained in the coagulum. The water is similarly dispersed in the final product, and the Khoa pats do not therefore appear to be wet.

Davies[5] states that the lactose in Khoa is present mostly as a super-saturated solution, since the available water is only sufficient to make roughly a 50 per cent. solution, while the solubility of *l*-lactose hydrate at atmospheric temperature is only 16 per cent. In the hot Khoa the lactose would be anhydrous, but it is doubtful if any of this would crystallise out since the drops of water are too small in size. In no case, however, are large crystals of lactose found in the ordinary Khoa. The viscosity of the product may come in the way of their formation. The composition of Khoa is given in Table XXXVII.

With sufficient care in preservation, it is easy to keep Khoa for long periods, for external contamination with moulds and bacteria is the chief cause of deterioration resulting in proteolysis and acidity development. Discolouration and the presence of cob-web-like threads on breaking are the criteria of spoilage.

Kheer—Like Khoa, this is also a partially desiccated product, but Kheer is much more liquid than Khoa. While in Khoa the "ratio of concentration" is roughly 6, that of Kheer is only 3 to 4. It is usually sweetened before consumption, and resembles sweetened condensed milk. This product is not usually meant to be preserved for a long time, and is consumed soon after preparation. Although there is some evidence of lactose crystal formation, the length of storage is not enough for the sugar crystals to grow appreciably in size. The composition of Kheer is given in Table XXXVII.

Rabbri—This product is a great delicacy. During its preparation, milk is not stirred; and the solids are separated from the milk by removing successive thin films of coagulated

[5] Davies, W. L., "Indian Indigenous Milk Products" (Thacker, Spink & Co., Calcutta, 1940), pp. 19–21.

material from the surface by means of bamboo splints. These skins are laid aside on the cooler parts of the surface of the pan. When the milk has been reduced to about one-fifth to one-eighth of its initial volume the pan is removed from the fire and the whole mass is gently mixed without injuring the flakes. Sugar is then added and the product allowed to cool.

Khurchan is a similar product to which arrow-root has been added during preparation.

Mallai—This is usually made by simmering large quantities of milk (about 20 lbs.) until a thick layer of milk fat and coagulated proteins forms on the surface. This is skimmed off with a flat ladle and laid aside to cool. The process is repeated twice, when most of the fat has been removed. The product is smooth and white in appearance and tastes like clotted cream. Mallai is sold and consumed without any addition of sugar.

A number of unfermented products of India have their near counterparts in the west, and they are given below.

Indigenous poduct		Western product
Mallai ; Sar	..	Clotted cream
Khoa	..	Dried or evaporated milk
Rabbri	..	Sweetened condensed milk
Channa (Chhana)	..	Cheese

The composition of the Indian products are given in the following table.

TABLE XXXVII

COMPOSITION OF INDIAN UNFERMENTED MILK PRODUCTS[6] (PER CENT.)

Product		Water	T.S.	Fat	Lactose	Protein	Ash	Added Sugar
Khoa	..	30	70	36	30	18	5·0	..
Kheer	..	50	50	26	20	13	3·3	..
Rabbri	..	30	70	20	17	10	3·0	25 to 30
Kheer (sweetened)	..	45	55	20	16	10	2·6	15 to 25
Kheer (skimmed milk)	..	45	55	5	18	11	2·7	15 to 25

[6] Davies, W. L., "Indian Indigenous Milk Products," 1940, p. 23.

The nutritive value and richness of the foods mentioned above are self-evident. But the fact remains that the processes have not yet been worked out on a large manufacturing scale, and the methods of preservation have not been standardised. There has been no attempt either to produce and supply the Indian market with dried milk powders or condensed milks or infant milk foods in place of the imported products from other countries. Wright[7] has stated that the possibility of establishing condensing and drying plants in India with the available milk and skimmed milk in the country justifies serious consideration. It must, however, be pointed out that Indian enterprise cannot successfully compete with foreign products until the dairy industry here begins to produce large quantities of surplus milk at competitive prices. On the other hand, the duty of 20 to 30 per cent. on products imported into India would give the Indian manufacturer a great advantage in the home market. The Indian Council of Agricultural Research has now proposed to give a start to this industry by trying out a pilot plant in one of the " milk pockets " in the country.

Indian Cheese—Cheese is neither a common nor a very popular product in India. It is prepared on a very small scale, and only in certain confined areas. The reason for this lies both in the unfavourable economic and climatic conditions. The small producer cannot afford to tie up capital or nutriment in cheese for the long periods required for ripening of the product. Besides, cheese requires a high humidity and low temperatures of ripening (12° to 15° C.), which are hardly obtainable in India. The prevailing temperature, on the other hand, is conducive to putrefaction and excessive desiccation.

The imported cheese, about 10,000 cwt., is consumed by the European population or by Indians with western habits of diet.

[7] Wright, N. C., *Rep. Development of Cattle and Dairy Ind. India*, 1937, p. 51.

Davies[8] has described that three types of country cheese are made in this country. The following descriptions are taken from him and the Wright's Report.[7]

Dacca and Surti cheese are both made from whole milk. The milk is clotted with rennet, and the curd filled into wicker baskets. A weighted board is placed on top to help drain the whey. The Dacca variety is removed after 10 to 14 days, when it has formed a dry cheesy coat, and smoked with wood or dry cowdung cake. The smoky flavour is objectionable to some palates.

The Surti cheese is immersed, as soon as it is dry, in acid whey containing salt. This makes it firm and lightly salty at the same time. It is then drained and dried. This unsmoked variety keeps for a shorter period (10 to 15 days) than the Dacca cheese, and tastes of acid curd.

Bandal cheese is a soft type prepared from cream. Its preparation differs from the Surti in that it is finally smoked. The flavour is more mellow and softer than that of whole-milk cheese.

Davies[6] gives the following figures for the composition of Indian cheese.

TABLE XXXVIII

COMPOSITION OF INDIAN CHEESE

Variety	Moisture	Fat	Protein	Mineral matter	Lactose
Dacca or Surti	38 to 42	35 to 45	20 to 25	About 50 per cent. of	..
Bandal ..	40	50 to 65	10 to 15	the content in milk	..
Channa ..	35 to 50	25 to 35	15 to 20	0·3 to 0·4	2·0 to 2·5

Channa (*Chhana*) is also a counterpart of western cheese; but, unlike the latter, it contains high proportions of fat. It is precipitated from boiling milk by the addition of lemon juice, or acid whey from other batches of preparation. Citric

[8] Davies, W. L., "Indian Indigenous Milk Products," Calcutta, 1940, p. 67.

acid may also be used for the purpose when preparing it on a small scale.

Lumps of casein with entrained milk-fat are formed as soon as the acid is added to boiling milk. After filtering through a cloth, much of the remaining whey is pressed out by squeezing the bag containing the precipitate between boards for a few hours. The yield or condition of drainage of whey is best when the correct amount of lime juice has been added. Too little juice will give a milky whey and a soggy product, and too much will give sour channa. The product is used for cooking and for making confectionery.

The approximate composition of channa is given in Table XXXVIII.

An appreciable difference between the cheese prepared in India and that imported from the west is the absence of any ripening in the Indian product.

PART II

FERMENTED MILK PRODUCTS

K. S. RANGAPPA

Chapter VII

FERMENTATION IN INDIAN DAIRY INDUSTRY

Introduction

The necessity for controlled fermentation of milk in a tropical climate. is fairly obvious. The prevailing temperature of 20° to 35° C. is most favourable for the growth of micro-organisms which bring about spoilage of milk hardly within a few hours of milking. The rate of multiplication of these organisms and the consequent development of acidity and curdling of milk is disproportionately high with the rise of the temperature of storage. It has been found that milk which keeps for 24 hours at 60° F. keeps only for 6 hours at 100° F.[1]

In the Indian household the life of the milk is partially extended for a period from 12 to 24 hours by simple heat treatment which destroys a great part of the microflora. It is, in fact, possible to keep milk indefinitely by sterilising it and maintaining its sterility. While this process was obviously impossible in ancient days, it is rendered impracticable even in modern times by the elaborateness of the equipment needed for it. The simplest way of preserving milk for human consumption in a tropical country is to allow it to sour, since this checks putrefactive changes while giving to the milk an acid taste which is particularly refreshing in a hot climate.[2] The Aryans therefore made a virtue of necessity, and turned to benefit the irrepressible phenomenon of fermentation. Thus the life and utility of milk nutrients were extended and, incidentally, the separation and isolation of the proximate principles, like fat and casein, were also facilitated.

[1] Wright, N. C., *Rep. Development of Cattle and Dairy Ind. in India*, 1937, p. 22.

[2] Wiser, *U.P. Bull.* 2, 1936, Allahabad.

The Mechanism of Fermentation

Fermentation in milk consists essentially in the gradual conversion of lactose into acids by lactose-fermenting organisms. The lactic acid causes at first an acid flavour in milk. A portion of the acid combines with the calcium of casein to form calcium lactate, thus setting free the casein and coagulating it when its iso-electric point (pH 4·6) is reached. The calcium combined as $CaHPO_4$ in milk goes completely into solution more rapidly than calcium combined as caseinate during souring. The albumin-nitrogen increases in the serum with the rise in acidity, and the citric acid completely disappears, while the insoluble inorganic constituents of fresh milk are rendered soluble by the lactic acid.[3] Chitre and Patwardhan[4] have made an interesting observation that fermentation of milk by lactic acid organisms considerably increases the riboflavin content, to a lesser extent the thiamine, but reduces markedly the content of nicotinic acid. This is clearly caused by the metabolic activity of the fermenting organisms. But the recent finding of Roy and Bhatnagar[5] that the nitrogen content of milk, as determined by the Kjeldahl method, rises on fermentation remains to be accounted for.

During the process, the electrical charges on the fat particles are also neutralised, causing the globules to coalesce and rise to the top. Rangappa and Banerjee[6] have shown that the isoelectric point (pH 4·5–4·6) of fat globules in milk is slightly higher than that (pH 4·3) of washed globules from cream.[7] This remains constant irrespective of the nature of the starter and temperature of incubation. But, with respect to acidity, a pure culture is able to neutralise the charge on the fat globule at a lower level (1·0 per cent.) of acidity than a mixed starter (1·3–1·5 per cent.). Viewed

[3] Vanslyke and Bosworth, *New York Agric. Expt. Sta. Bull.*, 1916, **48**, 3.
[4] *Curr. Sci.*, 1945, **14**, 320.
[5] *Ibid.*, 1948, **17**.
[6] *Proc. 34th Indian Sc. Cong.*, 1947, Sec. 10, p. 2.
[7] Mohr and Brockman, *Milch. Witschaft. Forsch.*, 1930, **11**, 21.

through the microscope, the discrete globules, though retaining their shape throughout souring, increasingly cluster together with the rise in acidity.

Various organisms are capable of fermenting milk, and each produces chemical and physical changes characteristic of it. The common types are *Streptococcus lactis*, *Lactobacillus acidophilus* and *Bulgaricus*. Each of these produces different levels of acidity; and while *S. lactis* is the easiest to maintain and the most predominant in the common fermented milk products, the other two strains demand stricter conditions of propagation. In addition to these, aroma-producing organisms like *S. citrovorus* and *paracitrovorus*, as well as several types of moulds and yeasts are usually present in soured milk and its derivatives.

Enumeration and Statistics

Dahi or curd, *lassi* or buttermilk, and butter constitute the main fermented milk products of India. Some of these are used directly for consumption while others form an intermediate stage in the preparation of a final commodity like *ghee* (clarified butterfat) or certain types of sweetmeats.

The manufacture of dahi and butter[8] account for 43·5 million maunds of milk, or 6·9 per cent. of the total milk production, and 9·6 per cent. of the milk products produced in India. Lassi is also a by-product in the production of ghee. It has been estimated that 597 million maunds of buttermilk are thus produced annually. Some of this is consumed by the villager's family and forms almost the only dairy product that falls to the share of the agriculturist. As it has no market value in rural parts, the surplus is either given away or fed to cattle or run to waste. The quantity of country cheese manufactured in India appears to be negligible; even in parts of the country where creameries have been started, the small amount of skim milk is not

[8] *Rep. Marketing of Milk in India and Burma*, 1943, p. 66.

usually converted into cheese, but made into dahi either for marketing or consumption.

Conflicting opinions are held regarding the improved nutritive value of fermented milk. Milk is itself the most nutritious of foods; and there is no increase of the fat or protein content of milk on fermentation. But the increased digestibility of soured milk is more or less admitted. There is also a greater amount of phosphorus and calcium made available to the system by their precipitation in the lower intestines due to the acid condition induced by *L. acidophilus*; and the consumption of sour milk also results in increased efficiency of the body to cope with a sudden influx of lactic acid in the system.

Fermented Milks in Other Parts of the World

Soured milk also appears to be a very popular beverage in central Europe, Russia, Rumania and the middle East. Milks other than those of the cow or buffalo are also used for the purpose. While the *yoghurt* of Turkey, the *leben* of Egypt, the *mazum* of Armenia and the *gioddu* of Sardinia are products of lactic fermentation like dahi, *kumiss*, prepared from mare's milk, the *kefir*, from goat's and ewe's milk are soured with yeasts, and contain considerable quantities of alcohol.[9] All these products, it may be noted, had their origin far back in history, and were all necessitated by the ubiquitous property of milk to turn sour on keeping. Fortunately however, man has eminently succeeded in circumventing this difficulty by acquiring a taste for soured milk.

[9] Rogers, *U.S. Dept. Agric.*, 1916, *Bull.* 319.

Chapter VIII

PREPARATION, QUALITY AND COMPOSITION OF DAHI AND LASSI

Dahi is the curd resulting from the lactic fermentation of milk. The method of preparation and the quality aimed at depends on whether it is to be consumed as such or is to be an intermediate product in the manufacture of butter and ghee.

Good dahi is a weak gel, like junket. It has a creamy layer on top, the rest being made up of a homogeneous body of curd. The surface is smooth and glossy, while the cut surface is trim and free from cracks and gas bubbles. A pleasant sweetish aroma and a mild taste are looked for in the product. It is eaten with rice in the south, and with wheat preparations in the north. Beaten up with salt or sugar, it is also taken as a drink. Ordinarily in middle-class families the cream is removed for butter-making before consuming the curd either as such or after dilution with water.

Dahi for Consumption

More care is usually taken in the preparation of curd when it is to be eaten directly than when it is to be used for making butter. Milk is brought to boil in order to destroy the viable organisms, cooled to body temperature, seeded with a small quantity of a lactic culture from the previous run and put away for 12 to 24 hours. The seed is never pure, but a mixed culture in which lactic acid organisms predominate. The quantity of seed depends on the season of the year and the severity of the climate. About 5 to 10 per cent. by volume is added in very cold weather, and the vessel is sometimes wrapped with rags or hay, or placed in a straw box to prevent loss of heat by radiation. In summer, on the other hand, hardly 1 to 2 per cent. of the seed is added, and the vessel is sometimes kept cool by covering it with a wet cloth.

A vigorous culture ordinarily develops 0·9 to 1·0 per cent. of acidity calculated as lactic acid after 10 to 12 hours of incubation. But there is more than one factor that determines the level of acidity reached in the curd. The temperature and time of incubation, the bacterial quality of the milk and the type of starter play an important part.

Time and Temperature of Incubation

Srinivasan and Banerjee[1] report that with a good mixed starter, free from yeasts, moulds and gas-forming organisms, the acidity rises with time up to a limit, beyond which it remains steady. The upper limit of acidity reached also increases steadily with the temperature of incubation, and attains about 1·6 per cent. at 45° C. This is illustrated in Fig. 1. In the same study they have also noted that the pH

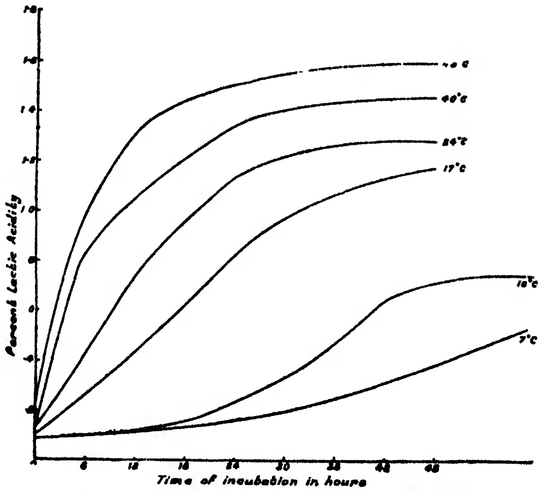

FIG. 1. Diagram of Acidity against Time

falls from about 6·8 to 4·4 irrespective of the temperature of incubation. The rate of fall of pH however rises with the temperature of incubation. Ranganathan and Narasimhamurthy,[2] as well as Knudsen and his co-workers[3]

[1] *Indian J. Vet. Sc. and Anim. Husb.*, 1946, 16, 72.
[2] *Agric. and Livestock in India*, 1938, 8, 421.
[3] *Dairy Sc. Abstracts*, 1940, 1, 61.

arrive at the same conclusion regarding the final pH of starter cultures.

In the experiments conducted by Srinivasan and Banerjee (*loc. cit.*) the dahi had a homogeneous texture at all temperatures of incubation, and a sweet aroma and taste as long as the acidity remained below the 0·8 to 1·0 per cent. level (as lactic acid), the figure depending on the lactose content of the original milk, the higher limit being tolerated with a higher lactose content.

Type of Starter

There is great paucity of published data on the types of organisms present in dahi in different parts of the country in the various seasons of the year. The types that are known will be discussed later. It is however well known that a defective seed with an over-predominance of yeasts, moulds and gas-forming organisms fails to yield a product with a homogeneous texture or a good aroma. All the same the importance of a good seed of the straight lactic type is yet to be appreciated. Davies[4] recommends therefore the establishment of stations wherefrom good starters can be obtained by manufacturers when their stock gets contaminated.

The Lactose-Lactic Acid Ratio

A good starter culture for the preparation of dahi should have a firm and uniform texture, a glossy surface free from moulds, and a sweet aroma. Srinivasan and Banerjee[1] recommend for the seed culture an acidity (as lactic) of 1·0 to 1·2 per cent. and a volume of 2 to 2½ per cent. of the milk to be soured. They have also shown that, chemically, the suitability of the starter culture can be judged by the lactose-lactic acid ratio of the curd. Each molecule of lactose fermented by a pure strain of lactic acid organisms yields, theoretically, four molecules of lactic acid. The molecular weight of lactose being four times that of lactic acid, the lactose disappearance will be equal, weight for weight, to

[4] Davies, W. L., "Indian Indignous Milk Products," Calcutta, 1940, p. 34.

the lactic acid produced. Since the end products of lactose fermentation, however, include small amounts of butyric and acetic acids and alcohol, little more than 90 per cent. of the curd acidity will actually be made up by lactic acid.[5] A starter can therefore be said to be predominantly a lactic culture when the ratio of the lactose it ferments to the acidity (as lactic acid) it develops is very nearly 1 : 0·9. Fig. 2 illustrates the variation of this ratio in different types of seed cultures selected at random. Fig. 3 represents the variation of this ratio in a predominantly lactic culture at different temperatures of incubation.

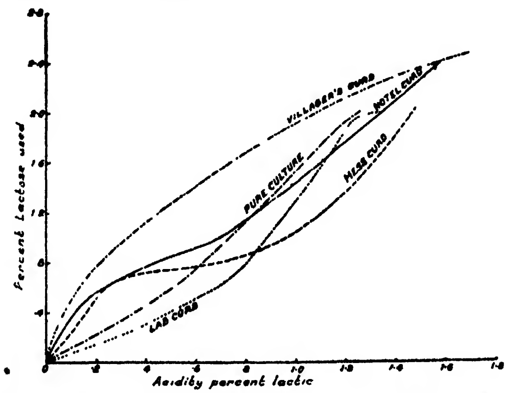

FIG. 2. Diagram of Lactose—Acidity in Differing Types of (Seed)

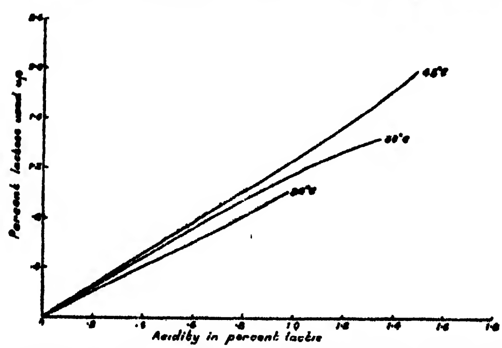

FIG. 3. Diagram of Lactose used—Acidity developed at temperatures of 24° C., 30° C. and 45° C. with mixed flora (Seed curd)

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[5] Vanslyke and Bosworth *New York Agric. Expt. Sta. Bull.*, 1916, 48, 3.

It can be seen from the preceding figures that the production of acidity almost equals the lactose consumption at 35° and 45° C. of incubation, this range of temperature being the most favourable for the growth of lactic organisms.

Dahi for Making Butter

The technique employed in the country method of making dahi for butter is very primitive and needs to be greatly modified if the industry is to have a long lease of life. The most prevalent practice in villages is to utilise raw milk for the purpose. It is either allowed to undergo natural ripening in earthen pots by atmospheric contamination of bacteria, as in Baroda, Kathiawar and other areas, or a small quantity of butter-milk from the last batch of raw-milk curd is added to it as in Mandya and other areas in the south. The result is a ropy dahi with gas bubbles and a mouldy flavour, separating into two distinct layers—serum at the bottom and a cake of curd on top. Although the acidity developed is not as high as in boiled-milk curd, owing to the lower buffering capacity of unboiled milk and the growth of certain yeasts at the expense of the acid developed, its texture, flavour and keeping quality are very poor. The practice of seeding raw milk for butter-making owes its existene to the fact that butter is only an intermediate product in the manufacture of ghee, and the by-product, butter-milk, has no market value in the villages.

Effect of Processing of Milk on Dahi

The salutary effect of boiling and other processes of heat treatment of milk on the bacterial population has already been discussed. In the preparation of good dahi efficient heat treatment of milk is essential. Srinivasan and Banerjee[1] have found that boiling milk for 10 minutes, or evaporating it at the boiling point to a reduction in volume by 5 per cent. yields a curd of very firm texture and pleasant aroma, and eliminates the numerous defects in the curd that result from improper or lack of processing. They also point out that either of the methods of pre-treatment of milk is easily adaptable to village conditions inasmuch as the boiling point can be judged by ebullition, the time lapse by counting, and the extent of evaporation by visible reduction in volume.

Composition of Dahi

The range of composition of this product under differing conditions of manufacture, season, locality, etc., are yet to be worked out. Broadly, however, it may be stated that except for the appearance of lactic acid in place of lactose, and a small loss of water by evaporation, there is no appreciable loss of other constituents in the fermentation process. When a level of pH 4·6 or lower is reached, all the calcium is in the ionic form, and all the phosphorus, except that conjugated to casein, exists as ionic phosphate.[6] The general composition of dahi prepared from whole and skimmed milk is given in Table XXXIX.

TABLE XXXIX

COMPOSITION OF DAHI[6] (PER CENT.)

Product	Water	Fat	Protein	Lactose	Lactic acid	Ash	Calcium	Phosphorus
Dahi (whole milk)	85 to 88	5 to 8	3·2 to 3·4	4·6 to 5·2	0·5 to 1·1	0·70 to 0·75	0·12 to 0·14	0·09 to 0·11
Dahi (skimmed milk)	90 to 91	0·05 to 0·10	3·3 to 3·5	4·70 to 5·3	0·5 to 1·1	0·70 to 0·75	0·12 to 0·14	0·09 to 0·11

It may be observed that almost the only difference in composition in the curds prepared from whole and skimmed milk is that the latter contains very little fat and a slightly higher water and lactose content.

If dahi is meant for home consumption, the creamy top portion is quite often kept by for butter-making, and the rest is used as curd. Anantakrishnan and Kothavalla[7]

[6] Davies, W. L., "Indigenous Indian Milk Products," Calcutta, 1940, pp. 31–32.

[7] *Indian J. Vet. Sc. and Anim. Husb.*, 1946, 16, p. 56.

have estimated the fat distribution in dahi under various conditions. In a 9 cm. column of well-set dahi the milk-fat is roughly distributed as follows.

DISTRIBUTION OF FAT IN DAHI (PER CENT.)

Top layer	Second layer	Third layer	Bottom layer
49·0	23·5	19·9	7·6

The percentage distribution of fat is unaffected by the surface area, and mainly depends on the depth of dahi, nearly 90 per cent. of the fat rising to the top when the height of the column is reduced to 3 cm. However, the implication in their report, that the rise of milk-fat in dahi is caused by fermentation, is not quite tenable, since the process is entirely physical which occurs in milk even without fermentation.

Butter-milk

Lassi or butter-milk is a by-product in the preparation of butter from dahi by the indigenous process. Dahi is churned, with frequent additions of water, until the butter granules are formed. The diluted, beaten curd, remaining over after the butter is removed, is the butter-milk. It has been estimated that 27 lbs. of butter-milk are produced for every pound of ghee. On this basis 597 million maunds of butter-milk are annually produced in India.[8] While most of this is consumed by the villager and his family, a good quantity is either given away, fed to cattle or run to waste. The reason for this is the lack of market value for the product in rural parts. It is obvious, therefore, that this defect in the dairy economy of the country demands an urgent solution. It may perhaps be remedied by evolving successful methods of preparation of " butter-milk powder " or " butter-milk casein " in an edible form, and of lactic acid and its salts for commercial exploitation.

Composition of Butter-milk

The method of preparation of the product shows that its composition is subject to considerable variation. The

[8] *Rep. Marketing of Milk in India and Burma*, Delhi, 1943, p. 71.

factors affecting composition are all those that affect the composition of dahi, and the extent of dilution of the curd during churning and the efficiency of fat removal. The available data are given in Table XL. For comparison the composition of the "butter-milk," the by-product in the creamery process of butter-making is also given.

TABLE XL

COMPOSITION OF BUTTER-MILK (PER CENT.)

Authority	Water	Fat	Protein	Lactose	Lactic acid	Ash	Calcium	Phosphorus
Davies[6] ..	90 to 91	0·1 to 1·0	3·3 to 3·5	4·7 to 5·3	0·5 to 1·1	0·70 to 0·75	0·12 to 0·14	0·09 to 0·11
Ranganathan, *et al.*[9] ..	96·2	0·8	1·29	1·2	0·44	0·4	0·06	0·04
Creamery "butter-milk"[9]	97·9	0·5	0·51	0·9	0·19	0·1	0·03	0·02

Figures given by Davies evidently refer to butter-milk resulting from mere removal of butter from the curd without diluting it. This product is the soured-milk beverage commonly sold in the north during the hot season. The composition is identical with that of dahi prepared from skimmed milk (*cf.* Table XXXIX), although the lassi from whole milk is preferred from the point of view of palatibility and texture. The lassi from whole milk has a considerable suspension stability in spite of its content of coagulated casein particles, since the protective colloids which had enveloped the fat before churning have been released during the breaking of the emulsion and are now protecting the fine particles of casein.[6] The skimmed milk lassi, on the other hand, is deficient in these colloids since they have been removed with the cream. This makes it non-homogeneous, lumpy, and rather bland to the taste, unlike the lassi proper which gives a feeling of "body" to the drink in view of its uniform texture and slightly greater viscosity and fat

[9] *Agric. and Livestock in India*, 1938, **8**, 421.

content. Undiluted lassi therefore differs from its parent, dahi, mainly in its lower fat content.

The data given by Ranganathan and Narasimhamurthy, on the other hand, are more typical of the common village butter-milk, resulting from dilution of the curd. This is therefore less rich than the undiluted lassi. The residual fat in the butter-milk is mostly made up of the smallest globules below 3·3 μ, the larger ones entering butter on churning the curd.[10]

Table XL also reveals the difference between creamery "butter-milk" of the West and the homonymous native product. The difference exists not only in composition but in the manner of production. While creamery "butter-milk" merely represents the dilute residue from the churned cream in the barrel churn, the Indian lassi is fermented whole milk from which only the butter-fat has been removed. The latter is much richer in all the three main constituents—fat, protein and lactose—and forms a valuable human food, while the former is commonly used as cattle and poultry feed.

Preparations from Dahi and Lassi

Sreekhand—This is prepared by sweetening dahi. It may or may not contain the supernatant butter-fat layer. The curd is suspended in a muslin bag until nearly all the serum has drained off. The semi-dry mass is then whisked up with sugar and then coloured and scented with saffron.

Dahi, beaten up with 14 to 20 per cent. of sugar, makes a refreshing beverage.

Diluted with 3 to 5 times its volume of water, and with a pinch of salt and other spices added, dahi forms *ghol*, a soothing drink. A *sherbet* can be prepared by diluting the curd with 10 times its volume of water, sweetening it with sugar and adding some citrus juice.

[10] Rangappa and Banerjee, *Proc.* 34*th Indian Sc. Cong.*, 1947, Sec. 10, p. 2.

Bacteriology of Dahi

The curdling of milk, as pointed out already, is caused by the fermentation, principally, of lactose by the growing organisms. Rangappa[11] has studied the rate of multiplication of bacteria in boiled milk soured with a mixed culture at 40° C. The growth rate of the organisms is given in the following table.

TABLE XLI

RATE OF MULTIPLICATION OF BACTERIA IN MILK ON SOURING

Hours after milking	Sample	Standard agar count per c.c.	Type of organism
2	Fresh milk	556,000	..
2½	Milk boiled for 10 mins.	180	Cocci (in chains)
do.	Boiled milk with seed curd	2,240,000	Streptococci and short rods
5½	do.	84,000,000	Short, thick rods, forming spores in the centre, and coli
8½	Curd	256,000,000	..
	The curd was churned at this stage		
9	Butter	52,000	..
do.	Butter-milk	Teeming at 10^8 dilution	Yeast cells, short rods, cocci

The rate of multiplication of the organisms during souring, as may be observed in the table, is typical of bacterial activity. These data will be further discussed under "Butter".

Mention has also been made that organisms of the straight lactic type like *S. lactis* are the most suitable for the preparation of dahi. Ram Ayyar[12] has examined a number of samples of curd from different parts of the country. He found that the ordinary culture contains lactic acid organisms living symbiotically with certain types of yeast cells. In summer (37°–42° C.) a long rod, *Streptothrix dadhi*, belonging to the class of *B. Caucasicus* or *Kornchenbacilli*, prevails.

[11] *Indian Med. Gaz.*, 1947, **82**, 320.
[12] *Agric. J. India*, 1928, **23**, 107.

It develops very high acidities in the curd, but gives it a smooth and firm texture. In winter, on the other hand, a short rod gives rise to lower limits of maximum acidity, and keeps the curd sweet. But the keeping quality of this curd is said to be lower than that of the summer curd, as the acidity developed by the organism is not enough to check the growth of undesirable flora in the medium. The lives of both the long and the short rods are considerably lengthened by the presence of certain yeasts of the Torula type which are always associated with them. The yeasts were found to be non-sporulating, and were unable to produce alcohol from glucose and sucrose, on which they grew easily.

Madhok and Kapoor[13] have also succeeded in isolating *S. lactis* and *cremoris*, *L. acidophilus* and a yeast, *sacch. elipsoides*, from samples of dahi. They recommend certain combinations of these for the manufacture of curd with a good aroma and texture. Karnad,[14] working on aroma-producing organisms in Indian curd, has found *S. diacetyl aromaticus* to be responsible for the richest flavour. Joshi and Ram Ayyar[15] have isolated an organism from a few samples of milk and curd which is reported to be capable of developing considerable amounts of acetyl methyl carbinol and diacetyl in milk in the presence of small amounts of citric acid. The *S. lactis aromaticus*, as they have named it, is distinguished from *S. citrovorus* and *paracitrovorus* by its capacity to produce sufficient acidity to curdle milk in 24 hours. They recommend the use of this and other aroma-producing organisms for enriching the flavour of curd and butter. Even the available knowledge of the technology and bacteriology of Indian dairy products, meagre as it is, has yet to be utilised for educating the villager to produce superior products.

[13] *Indian J. Vet. Sc. and Anim. Husb.*, 1942, **12**, 213.
[14] *Ibid.*, 1939, **9**, 439.
[15] *Ibid.*, 1936, **6**, 730.

Chapter IX

BUTTER

Statistics[1]

Butter is an important dairy product in India, although very little of it is directly utilised for consumption. It is used for melting into ghee, which is the Indian dietary equivalent of butter in the West. As all the ghee manufactured in the country passes through the butter stage, the milk, ultimately converted into ghee, goes to form butter. Thus 57 per cent. of the total production of milk, and more than 79 per cent. of the quantity converted into milk products is first converted into butter. Of this, hardly 2 per cent. is marketed as table butter, and the rest is melted into ghee either by the producer, merchant, or consumer.

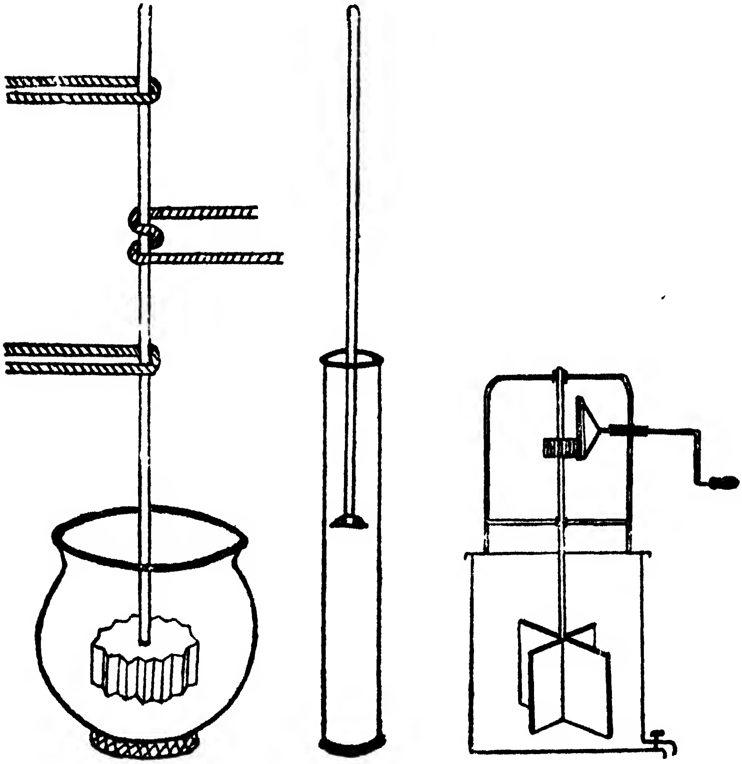
Creamery butter is also produced in India in small quantities, mostly, however, for the use of European residents. This forms about 28 per cent. of the total product marketed as butter.

Methods of Preparation

The preparation of dahi for the production of butter, usually either by self-souring of milk or addition of a culture from raw milk, has already been described. Most of the commercial butter is derived only from unprocessed milk. But in northern districts and certain parts of the Madras Presidency milk is kept warm in an earthen pot over the remains of the kitchen fire till evening when the evening milk also is added to it and a starter added from the previous run. In the early hours of the next morning the curd is churned in the pot itself with a country wooden-churner (Fig. 4 *a*). The latter consists of a circular block of wood, 3″–9″ in diameter and 1½″ to 5″ in thickness, corrugated on the churning surface. It is fixed to a thin bamboo rod,

[1] *Rep. Marketing of Milk in India and Burma*, **Delhi, 1943, pp. 67–69.**

and is rotated clockwise and anti-clockwise in turn in the mass of the curd by alternately pulling the two ends of a rope wound round the upper end of the pole.



(*a*) (*b*) (*c*)

Country Churns — Churn recommended

FIG. 4

A less common device (Fig. 4 *b*) for churning is a cyclinder of scooped bamboo or of tinned copper about 4 ft. in height and 6″ to 9″ in diameter in which plays up and down a thin bamboo with the inverted half of a coconut shell attached to the bottom end of the pole. The use of this apparatus is said to reduce the churning time considerably. Fig. 4 illustrates both the devices schematically.

Ordinarily, the villager has 2–5 lbs. of milk a day for making butter. During churning, water is frequently added to the curd. But the dilution varies from 50 to 200 per cent. of the volume of the curd. In warm weather cold water is used and in severe northern winters luke warm water is added. When the butter granules are well formed (the tendency being usually to over-churn), they are collected into a lump with the hand, and the excess water is squeezed out. The butter is rarely washed with water after collection.

The day's outturn is lumped with the previous days' and floated in the fresh butter-milk or water, these being daily renewed. The collection thus accumulates until there is enough to be converted into ghee, or to be sold to the contractor at intervals. At the butter dealer's, however, the collected butter is stored "dry" in old kerosene tins for anything upto 12 days. At the end of the period the product is lumpy, non-granular, mouldy and highly acid in flavour.

Composition of Butter

The technique of butter manufacture employed by the villager, it will be observed, is so unstandardised and imperfect that it is impossible to secure a product of uniform quality or composition. Unlike the trained dairy farmer in the West, the Indian peasant knows little or nothing of the correct processing of milk, the right type of starter, the exact time and temperature of souring, churning acidity, the washing of butter or its preservation and storage. All his knowledge of dairying is traditional, handed down to him from ancient times, like the Vedas. He knows of no improvement, and he is not educated enough to believe in it. It must not be forgotten, however, that the industry has developed according to the needs of the petty farmer peddling for small profit. Radical changes have therefore to be introduced with caution and with due regard to existing practice.

The composition of country butter is so variable that it contains moisture from 10 to 40 per cent., fat from 60 to 65 per cent. and unwarranted proportions of curd and acidity.[2] Standard creamery butter, on the other hand, contains less than 16 per cent. of moisture, and 4 per cent. of salt and curd. Moreover it is not allowed to be sold, in European countries, unless it possesses good texture and flavour. Almost the sole reason for the production and sale of a low quality product in India is that butter is not eaten as such, and the effect of low grade butter on ghee is yet to be realised.

Most of the workers in the field have dismissed the country method of manufacture of butter as defective in every way and advocate the creamery process as the only effective substitute. Wright,[3] however, correctly points out that, unlike in the West, butter and ghee manufacture in India is an uncollectivised cottage industry with no immediate prospect of following Western lines. He suggests that the indigenous industry should work out its own remedies suitable to the peculiar needs of the country.

The defects in the various country methods of manufacture and storage of butter have been extensively surveyed and studied under a scheme sponsored by the Indian Council of Agricultural Research, by Rangappa, Srinivasan and Banerjee.[4] The results have led to certain practical solutions that can be adopted by the small producer.

Defects in Country Butter—Their Causes and Remedies

It has been found that most of the defects in country butter arise from the use of low quality milk and the inefficient or lack of processing practised in its production.

[2] *Rep. Marketing of Milk in India and Burma*, Delhi, 1943, p. 70.

[3] Wright, N. C., *Rep. Development of Cattle and Dairy Ind., India, Delhi*, 1937, pp. 11–15.

[4] *Indian J. Vet. Sc. and Anim. Husb.*, 1946, **16**, 83.

Effect of Processing Milk

The workers last mentioned have shown that butter made from milk boiled for 10 mins. has a good aroma and keeps for 2–3 weeks without great deterioration or acid development. But the unprocessed milk butter lacks body, flavour and keeping quality. It acquires an alcoholic and mouldy smell, and develops a sharp acid taste in 3–4 days. Rangappa and Banerjee[5] have studied the development of acidity in the two types of butter. The results are illustrated graphically in Fig. 5.

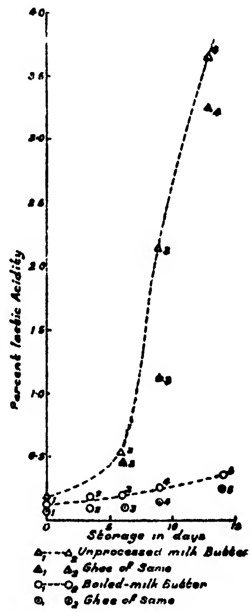


FIG. 5. Rise in Acidity on Storage of Butter from Unprocessed and Boiled Milk

The speed of acid development, though not perfectly correlated with deterioration, fairly represents the rate of spoilage also. Davies[6] has noticed that butter-fat shows signs of tallowiness at a level of about 1·4 per cent. lactic

[5] *Indian J. Vet., Sc. and Anim. Husb.*, 1946, 16, 98.
[6] *J. Ind. Chem. Soc.*, 1941, 4, 175.

acidity. The raw-milk butter, however, goes through a series of rapid changes in flavour. Beginning with a light acid flavour (0·2–0·5 per cent.), it passes on to an estery or alcoholic flavour (1·5 to 2·5 per cent.) and culminates, in about 3 weeks, in a putrefactive smell at 6·0 to 11·0 per cent. free acidity.

Rangappa and Banerjee[6] have traced the cause of development of high acidity in raw-milk butter chiefly to the activity of lipolytic organisms, rather than to autoxidation of fat. In the samples of country butter which they studied the causative organism was found to be a green mould of the *pencillium* type. Davies[7] also mentions that certain moulds belonging to the class of *pencillium* and *oidium* produce high acidities in butter.

Effect of Starter

Srinivasan and Banerjee[8] have found that a starter culture of mixed flora, containing chiefly lactic acid organisms, and free from moulds and yeasts, yields a high grade butter. With such a seed they report a 90–94 per cent. recovery of milk fat by the indigenous process. Rangappa and Banerjee[9] have been able to recover, on the other hand, only 75–85 per cent. of the fat when a mouldy, raw-milk curd was used as starter. The butter was also easily overworked, and entrained in it inordinate amounts of curd and moisture. This feature is taken advantage of by the ignorant producer who sells butter as such, and it encourages him to prefer the raw milk process.

Effect of Churning Acidity of Curd

It is well known that the churning acidity of cream determines in large part the yield, quality and storage property of butter. In certain parts of India, however, curd is ripened for 20 to 24 hours in summer, as in the arid districts

[7] *Ibid.*, p. 179.
[8] *Indian J. Vet. Sc. and Anim. Husb.*, 1946, 16, 72.
[9] To be published.

of Andhra, and for 2 to 3 days in winter in northern parts like Baroda. The level of curd acidity is thus totally uncontrolled, and sometimes allowed to reach even 2 per cent. At acidities above 1·2 per cent. although the churning time is reduced and the yield does not decrease, the butter tends to be easily overworked, and the high acidity reduces the storage property of the butter-fat. The optimum level of curd acidity for the highest yield and quality of butter and ghee has been found to lie between 0·9 and 1·1 per cent. Below the lower limit, the recovery as well as the flavour of fat suffer, though its storage property improves.

Effect of Dilution of Curd

The dairyman knows by experience that high dilutions of the curd during churning enable quick and increased recovery of butter-fat. But the dilution is also determined by the type of butter-milk, thick or thin, that he would like to have. The water added, it is to be observed, not only helps the separation of the fat from the coagulated casein, but also washes to some extent the grains of butter. Thus a proper dilution effects a removal of the butter-milk with its acids and micro-organisms from the butter and thus contributes towards a better storage quality. Rangappa[10] has noted that, on churning curd with an equal volume of water, not only 80 per cent. of the acids in curd but nearly all the microflora are washed out into the butter-milk (*cf.* Table XLI). Addition of an equal volume of water to the curd has, therefore, been found to give the highest yield of butter-fat with a good storage property.

Washing of Butter

It is well recognised that washing butter with water after churning is conducive to better keeping quality. In the creamery process washing of butter grains in the barrel churn is a routine procedure. But this practice could not be adopted by the small-scale producer in India because of

[10] *Indian Med. Gaz.*, 1947, 82, 320.

the expensive equipment, and since the resulting butter-milk, unlike in the creamery method, is an edible (and often a commercial) product which should not be too watery. Therefore, in order to retain the desired consistency of butter-milk and yet enable the butter grains to be washed in the village method, Rangappa and Banerjee[11] have devised a simple method of washing butter in the country churn. When the butter-grains in the pot are well formed, the butter-milk is carefully siphoned off from the bottom of the vessel either with a suitable hollow bamboo stick or with a rubber tube or a simple tap (Fig. 4 *c*), leaving the grains in the vessel itself. An equal volume of water is then poured into the pot, and the contents are gently re-churned for 1-2 minutes. The resulting butter, though a little lighter in colour and flavour than unwashed butter, is observed to have very low acidity and high storage property. This simple device thus enables the maximum recovery of high grade butter (which compares very well with creamery butter), by the indigenous process without undue dilution of the curd.

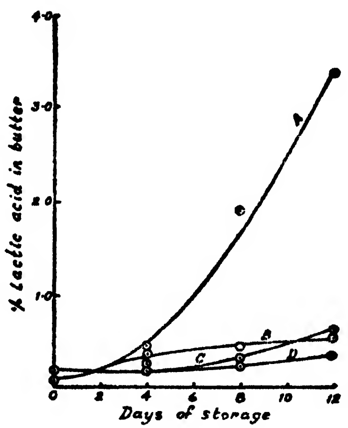
Preservation of Butter

The last major defect to be avoided in the native butter industry is improper storage of the product. The village practice is to store the day's production of butter as a lump floating in butter-milk or water. The lump is added every morning with the new yield, and the butter-milk or water replaced daily until the lump is large enough either for melting into ghee or to be sold to the collector from the town. The collector, in his turn, stores it "dry" in old kerosene tins and transports it in the same tin to the city for sale. Thus it ordinarily takes 10–15 days after the butter is made and before it is melted into ghee. Rangappa and Banerjee[12] have studied the acid and off-flavour development of butter under different conditions of storage. Under

[11] To be published.

[12] *Proc. Indian Sc. Cong.*, 1947, Sec. 10, p. 4.

tropical, uncontrolled conditions prevailing in the trade, "dry" storage of butter causes a sharp rise in acidity and off-flavour, while storage in fresh butter-milk keeps down the acidity and retains the flavour for a considerably longer period. The large lack of oxygen and the acidity in butter-milk, inhibiting micro-organic activity in butter, are responsible for the difference in storage property, as shown by the improvement in keeping quality of butter on storage in water and dilute lactic acid solution. These observations are clearly illustrated in Fig. 6.



A. Stored "*dry*"
B. Butter in butter milk
C. Butter in water
D. Butter in lactic acid solution 0·6%

FIG. 6. Acid development in butter stored in different media

The container used for storage is also a determining factor in the keeping quality of butter. Sometimes butter is stored dry even at the village producer's. The common container is the earthen pot whose pores are ever impregnated with viable organisms. Good butters which keep well for 10–14 days in tinned metallic containers, glazed jars or glass vessels show signs of off-flavour in 3 to 4 days when stored in country pots. Vitamin A destruction in butter is also more rapid on storage in country pots than in non-porous ware.[18]

It is clear, therefore, that indigenous dairy practice stands in urgent need of revision. And it can be enormously improved, to the benefit of both producer and consumer, by effecting slight alterations in current technique with necessary education and proper organisation of producers.

[18] Rangappa, Srinivasan and Banerjee, *Indian J. Vet. Sci. and Anim. Husb.*, 1946, 16, 83.

PART III

GHEE

K. T. ACHAYA

Chapter X

METHODS OF PRODUCTION OF GHEE

Introduction

Ghee is the Indian name for the clarified butter-fat, usually of the milk of the cow or buffalo; sometimes milks of other mammals, *e.g.*, goats, sheep, camel, etc., are also used in its production, but these are referred to specifically.

The word ghee is derived from the Sanskrit, *ghar*, which means bright or to make bright. It was originally used of the glittering of fat and ointment, a sense preserved in the Vedas where a priest brightens the fire by sprinkling butter on it, whence we have *ghrita*, the modern ghee. Later *ghar* came to mean brightness in general. In addition to its use as food material, it was an important element in religious or festive ceremony. The Vedas make frequent mention of the use of *Ajya* and *Ghritham* in ceremonial offerings during *Yagnyas*, and even to-day no important Hindu religious rite omits the use of ghee. It is described also as medicinal —cooling and emollient, capable of increasing the mental powers and physical appearance, and useful in eye-diseases and ulcers, while particular medicinal qualities are ascribed to the milk-fats of different animals. A belief in the curative properties of rancid ghee is still widely current in the country; specimens about ten years old are strongly odorous and cream-coloured, while those kept a century are described as hard, dry and earthy, and almost odourless. Sir T. Herbert's "Travels," written about 1665, makes mention of it, while in Fryer's account of his stay in the East (1698) is found the sentence: 'But they will drink Milk and boil'd Butter, which they call Ghee.'

The use of ghee is not confined to India alone; while exact statistics of its production in other countries are not available, scientific literature makes mention of its use under different names in various tropical and temperate climates. The Musoma district in Tanganiyaka produced 577 tons of ghee in 1938,[1] and a substantial increase was expected in subsequent years; Nigeria, in the second half of 1940, produced 1,547 lbs. of certified clarified butter-fat,[2] and the use of *samneh* or rendered butter-fat (from the Arabic, *Saman*) is extensive in Palestine.[3] The utility of the buffalo, the prime producer of ghee in India, as an economic source of wealth is also being realised[4] in Mediterranean countries like Italy.[5]

Place of Ghee in the Indian Dietary

Ghee represents an almost ideal method of preservation of most of the desirable qualities of milk-fat. A well-made ghee, in which the absence of moisture, protein and lactose almost entirely eliminates microbiological activity, can keep for long periods of time quite unspoiled; and this accounts for its popularity in India. The vegetarian habits of a great majority of the people preclude from the diet any form of animal fat. The poorer classes have therefore to consume large quantities of vegetable oils (raw, refined or hardened), while the well-to-do have recourse to ghee. Its attractive body and flavour make it popular with the consumer, while the producer benefits in that he need not dispose of his product immediately it is made through fear of spoilage. The flavour of ghee is what appears to commend it most to the average man; and hence particular local flavours have been cultivated from district to district by manufacturers catering to popular taste.

Besides these reasons for the popularity of ghee, it supplies to some extent the vitamin requirements of the people. Vitamin A and carotene (which is converted in the human

[1] *Dairy Sc. Abstracts*, 1939, 1, 320.
[2] *Ibid.*, 1941, 3, 248.
[3] *Analyst*, 1932, 57, 375.
[4] *American Chemical Abstracts*, 1923, 17, 1847.
[5] *Ibid.*, 1925, 19, 3834; 1943, 37, 6315.

body into Vitamin A) are both present in substantial quantities in ghee; and though the Indian intake of carotene from green leafy vegetables is usually adequate, no source of any vitamin, however small, can be overlooked in a country where malnutrition is so widely prevalent. Karmarkar[6] states that the highest level of intake of Vitamin A through consumption of genuine ghee never reaches the minimum daily requirement in India, emphasising the supplementary nature of the product as supplier of this vitamin. Vitamin D, which is the anti-rachitic principle, is also present in small quantities; and the third fat-soluble vitamin E, is also stated to occur in butter-fat.

As regards its nutritive value, it contains in some quantity the lower fatty acids, which are present in no vegetable oil. The presence of these greatly lessen the time required for saponification in the small intestines, and the higher fatty acids are therefore excreted in large measure as calcium soaps. This brings the calcium-phosphorus ratio to an economic level. Thus, though the percentage of glycerides that are assimilated may be lower for ghee than for other fats, the ease of digestion has also to be considered. Godbole and Sadgopal[7] found the figures for the assimilable constituents of coconut oil, cow and buffalo ghee to be 91, 63·5 and 56 per cent. respectively of the total glycerides. Moreover, Dastur and Giri[8] noticed in *in vitro* digestions that the emulsions formed with butter-fat were stable for longer periods of time than those formed with other fats. Datta and Banerjee[9] found good comparative growths with butter and ghee, the effects being more marked with male rats than with female.

The digestibility of milk-fat, however, suffers to some extent by its conversion into ghee, by reason of the large crystalline structure of the latter; for the easy digestion

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[6] *Indian Med. Gaz.*, 1944, **79**, 535.
[7] *Publ. Benares Hindu University*, 1935, No. 1992.
[8] *Proc. Soc. Biol. Chem., India*, 1936, **1**, 40.
[9] *Indian J. Med. Res.*, 1937, **25**, 857.

and assimilation of milk-fat are due to its dispersity in milk and its microscopic structure which help its precipitation and partial digestion in the stomach itself.

It might not be out of place to compare here the types of butter-fat as we know them in India and in the West, and the types of animals yielding them. Kothavalla and Doctor[10] found that the butter from a Jersey cow scored better than that from a Sindhi cow or a Surti buffalo; but when melted, the ghees obtained from the Indian animals were found superior.

To-day, dehydrated butter is becoming common in Canada, Australia and other dairy centres under the name of " butter oil". Ghee represents almost pure butter-fat in a palatable and stable form. While some of the vitamin content is undoubtedly lost in heating, especially in contact with air, the losses can be brought down to a minimum if the decantation method of melting butter is followed. Even in the boiling-method, De and Majumdar[11] record a loss of 10·5 per cent. in the Vitamin A potency when the butter is melted at 120° C. for 22 minutes, and this temperature might well be considered too high.

Statistics of the Ghee Trade[12]

Ghee is by far the most important milk-product of India; 79·2 per cent. of the milk converted into products were made into ghee in 1935. This amounts to nearly 3,589 lakh maunds of milk or over 57 per cent. of the total production (*vide* Table V). This is hardly surprising in view of its extraordinary keeping quality and consequent marketable value. The percentage conversion of milk into ghee varies from province to province according to local needs, being highest with 77 in Bihar, the Punjab and Orissa, where this is almost the only milk-product made.

[10] *Indian J. Vet. Sc. and Anim. Husb.*, 1939, 9, 151.

[11] *Indian J. Med. Res.*, 1937, 25, 857.

[12] *Report on the Marketing of Milk in India and Burma*, 1943, p. 67.

The exports and imports of ghee into India are of little significance. The average export figures for a 15-year period ending in 1940 were 58,045 cwts. per year, while the corresponding imports totalled 53,612 cwts. While the exported material went mainly to Indians in Burma and the Straits Settlements by sea, imports were all overland from the north and north-western countries.

It must be pointed out that these figures represent almost wholly the ghee from buffalo milk. Cow ghee is of little importance, and where sold, as in some northern markets, tends perhaps to fetch a slightly lower price, though a generalisation would not be strictly accurate.

Practices of Manufacture of Ghee in India

The word "manufacture" is perhaps a misnomer in connection with ghee in India inasmuch as ghee manufactories are not known here in the sense that "creameries" and "commercial dairies" are known in the West. The preparation of ghee is essentially a cottage industry which helps the farmer eke out his agricultural income to a small degree. With the advent of the hydrogenated oil industry, consumers are growing chary of buying ghee direct from retailers unless it has been passed by some accepted authority. The practice is growing, therefore, whereby central depots buy through their agents butter which they believe is genuine, and market it either as butter, or after conversion into ghee. Ghee grading stations, refineries and co-operative societies are springing up which collect good or "katcha" (partially clarified) ghee, and refine, blend, tin and market them. Such commercial centres are being actively encouraged by the Government since they ensure a quick return and sale to the producer, and a genuine ghee at a fair price to the consumer.

The practices of manufacture of butter have been dealt with in Part II, and only the methods of clarification employed in its conversion into ghee will be described here.

The process of conversion consists essentially of the separation of the fat and the water phases, whereby the water-soluble acids and the coagulated proteins pass into the latter phase. The moisture is now driven off, and the pure butter-fat isolated. The mechanism of such separation and isolation is greatly facilitated by the presence of electrically neutral particles of fat that are much larger than the charged particles of fat found as microscopic globules in milk.

In the "*desi*" or country method of clarification, the butter is heated over a low fire. It begins to boil at about 90° C. and most of the moisture is driven off up to about 120° C. Beyond this temperature the last traces of moisture are driven off, and the white casein, which has in this interval come down to the bottom, browns. The end-point is indicated by the formation of fine bubbles on the surface of the fat, and the emanation of the characteristic pleasant aroma of browned casein and charred lactose.

Butter clarification in different parts of the country, however, vary both in degree and in technique, though all aim at satisfying local taste rather than at the best possible product. Usually the butter is collected and stored in an earthen or metallic vessel for a period ranging from 3 to 15 days, until enough has been collected for melting. Often the storage vessel is used for melting as well, a procedure which results in a marked loss of keeping quality.[13] In Andhra, the ghee is generally raised to a temperature of 150° C., yielding a marked burnt flavour that is relished. The practice in Gujerat and certain parts of Baroda State is to heat the butter just to boiling, when a fair separation of the fat and butter-milk layers occur. The fat is then allowed to solidify by cooling, and the lower layer of butter-milk run out. The remaining moisture may or may not be removed by boiling. Such ghee, though low in free fatty acids, reveals a raw flavour. Moreover, the moisture and protein matter favour the growth of microflora, especially

[13] Rangappa, Srinivasan and Banerjee, *Indian J. Vet. Sc. and Anim. Husb.*, 1946, **16**, 83.

at the temperatures prevalent in these areas. In Behar, butter is prepared from raw cream on a large scale, and is then melted in large iron pans. Much undesirable metallic contamination may thus occur. Tinned pans, such as are used in Andhra, lessen this defect since tin does not promote spoilage of ghee to the same extent as does iron.

In ghee refineries and grading stations, ghee of every quality is collected from the farmers of surrounding districts, but only the "*Katcha*" (poor quality) ghee is chosen for refining. The ghee is melted in huge cylindrical vats, and clarifying agents like curd, salt, alum, etc., are added. After clarification, the clear fat is run into tinned containers, cooled, and the space left by its contraction on cooling filled with more ghee. The tins are then sealed, thus eliminating any air-gap above the ghee surface. The sediment from the clarification process accumulates till sufficient quantity has collected, when it is either discarded, or worked up for material that is sold as low grade ghee.

The Ideal Ghee: Methods of Preparation

The ideal ghee is butter-fat that retains unimpaired all the desirable properties of low acidity and the high vitamin and carotene contents, easy digestibility and high nutritive value of the original milk fat with the added virtue of perfect keeping quality. The comparative chemical and biochemical inertness of the fat constituent of milk facilitates to a great degree its isolation and preservation largely in its pristine state. Unfortunately, the lack of correct technique in manufacture and carelessness in handling lead often to a defective product. As will be discussed in detail under Chapter XIII, exposure to air, light, high temperature and microflora and contact with certain metals bring about rapid spoilage of butter-fat.

In general, "A" grade ghee is derived from "A" grade butter; and the latter has already been shown to be made from processed milk either by the creamery or by the curd process. It must here be pointed out, however, that the difference in the keeping qualities of high and low grade butters is far more pronounced than in the ghees derived from them.[14] Raw-milk butter, for instance, has a much lower storage property than boiled-milk butter. But the ghee made from fresh raw-milk butter has only a slightly shorter induction period than the ghee derived from fresh boiled-milk butter.

Kothavalla and Cox[15] state that good flavoured ghee with good yields can be made from pasteurized cream butter. Doctor *et al.*,[16] comparing the qualities of the ghees made from soured and unsoured cream with that from the curd process, report that the ghee from unsoured cream lacks the characteristic aroma which the consumer demands. Wright,[17] and Stewart and Banerjea[18] confirm these observations.

Rangappa and Banerjee[19] have studied the various methods of preparation of ghee from the point of view of yield, flavour, keeping quality, acidity and Vitamin A and carotene contents, judging the keeping quality by oxygen absorption values at 95° C. and also by storage tests. In the indigenous method, the longer the interval and the higher the temperature of souring milk, the greater the acidity of the resulting ghee. Though this holds equally for the boiling and the decantation process of clarification of butter, the acidity of ghee in the latter case is in every instance much lower. They report that the Vitamin A varies little with the method of production. From the standpoint of keeping quality, the method devised by French[20] of diluting and reseparating cream, and then boiling it into ghee, is by far the best. But such a ghee has a poor colour, and a milky flavour disliked by the average Indian customer. They

[14] Rangappa and Banerjee, *Indian J. Vet. Sc. and Anim. Husb.*, 1946, **16**, 98.
[15] *J. Cent. Bur. Anim. Husb. and Dairying*, 1927, **1**, 95.
[16] *Indian J. Vet. Sc. and Anim. Husb.*, 1940, **10**, 63.
[17] *Rep. on the Development of Cattle in India*, Delhi, 1937, p. 32.
[18] 1929, cited in the above report.
[19] To be published.
[20] *Bull. Imp. Inst.*, 1936, **34**, 33.

report also that the flavour defect is largely overcome by the addition of 0·1 per cent. of citric acid to the cream before boiling into ghee.

Ghee of excellent flavour and very good keeping quality can be made by the indigenous curd process. After preparing the butter and washing it as described on page 92, it is converted into ghee by boiling off the moisture at a temperature not exceeding 110° C., when the acidity of the ghee will be hardly 0·05 per cent. (as lactic acid). The product is sealed in air-free containers and stored in the dark. Such a product will keep for considerable lengths of time even in a tropical climate without loss of flavour, colour or Vitamins.

Chapter XI

THE COMPOSITION OF GHEE AND ITS VARIATIONS

The General Composition of Ghee

Ghee consists essentially of a single phase—the fat phase—which is composed of triglycerides of various fatty acids. This would represent the ideal case, and usually small amounts of various other constituents are present, mostly in solution. These are mainly moisture (which cannot be driven off completely without seriously impairing the quality of the product), free fatty acids, ketones, aldehydes and compounds of similar nature, and about half a per cent. of matter that cannot be saponified by alkali, and which is in turn a complex mixture of substances.

The following chart gives the general composition of cow and buffalo ghee.

CHART II

COMPOSITION OF GHEE (PER CENT.)

	Buffalo ghee	Cow ghee
Fat ..	About 99	About 99
Moisture ..	0·2	0·2
Free fatty acids, aldehydes ketones, etc. ..	In variable amount	In variable amount
Unsaponifiable matter, consisting of :–	0·4	0·4
Cholesterol ..	..	0·4
Vitamin A ..	130 γ	900 γ
β-Carotene ..	Trace (20 γ)	500 γ
Xanthophylls ..	Trace or none	30 γ
Lycopene ..	Trace or none	Trace or none
Vitamin D ..	..	0·9 γ
Vitamin E ..	..	Trace
Lecithin and cephalin ..	Trace or none	Trace or none

Inorganic constituents, like calcium, phosphorus, copper, iron, etc. Variable amounts of charred casein.

Fat—The detailed fatty acid structure of cow and buffalo ghee are discussed later. A few analyses of the glyceride structures are also included.

Moisture—The usual method of preparation of ghee in India is by direct heating of the butter to temperatures usually over 100° C. and below 150° C., when the moisture content falls to the neighbourhood of 0·2 per cent. This residual moisture cannot be driven off by heating without affecting the quality of the ghee, and hence is usually retained. The amount may differ, however, according to the temperature to which the ghee is raised, *e.g.*, if butter is merely maintained at 95° C. or so and decanted from slime, the content may be as high as 0·47 per cent, which is the solubility of water in butter-fat at that temperature.[1]

Unsaponifiable matter—This represents the small residue left over on saponifying ghee with alkali, and usually extracted with ether from the soaps thus formed. Davies[2] quotes a range of figures from 0·31 to 0·42 per cent. for cow butter-fat. Compared to this, the values reported by Dhingra and Ganesh Chandra[3] are very low indeed (0·04 to 0·09) for buffalo ghee.

Cholesterol—Here again figures are not available for either cow or buffalo ghee. While it may be fairly safe to draw inferences from foreign work on the cholesterol content of cow butter-fat, the same does not hold for buffalo butter-fat.

Vitamin A—A large volume of work on the Vitamin A content of cow and buffalo ghee and their variations on storage, heating, exposure to light, etc., have appeared, the vitamin values being given in terms of the blue colour developed with the standard Carr-Price antimony-trichloride reagent.[4-9] While this may give comparable figures, the

[1] Thiel, *J. Coun. Sc. Ind. Res.*, Australia, 1943, **16**, 139.

[2] "The Chemistry of Milk," 1939, p. 92.

[3] *Proc. Indian Sc. Cong.*, 1948, Sec. 4, p. 51.

[4] Banerjee and Sunawalla, *Indian J. Vet. Sc. and Anim. Husb.*, 1940, **10**, 335.

[5] *Ibid.*, 1941, **11**, 329.

[6] Banerjee, *Agric. and Livestock in India*, 1936, **6**, 274.

[7] Banerjee and Dastur, *Ibid.*, 1937, **7**, 24 and 697.

[8] Banerjee and Doctor, *Ibid.*, 1938, **8**, 158.

[9] *Indian J. Med. Res.*, 1938, **25**, 857.

absence of any recognized factor for the conversion of these blue values to international units of Vitamin A makes the results of somewhat limited value as an absolute standard.

Recently, however, a few assays of provincial samples of ghee have been made by accurate spectrographic methods. De and Majumdar[9] measured the Vitamin A potency of a few Madras butters and the ghees prepared from them. The figures for ghee showed that they contained about 8·3 γ per gram, and that the loss over the vitamin potency of the parent butters were from 10 to 22 per cent., depending on the time and temperature of heating. Almost simultaneously Muthanna and Seshan[10] and Majumdar[11] published further spectroscopic estimations of the Vitamin A content of ghee. The former examined samples of Bengal and Sind ghee, and did not state definitely whether they were from the cow or the buffalo, though their analytical constants indicate that they were probably genuine. The average value for Sind ghee was 12 γ per gram, and for Bengal ghee 8 γ per gram. They expressed the opinion that good ghee should contain 10–15 γ per gram of Vitamin A, and that losses are great at temperatures of clarification above 100° C.—*e.g.*, heating at 125° for 5 minutes caused a loss of 29 per cent. in one case. Exposure to sunlight was also very destructive, a 34 per cent. loss occurring in 10 minutes and a 100 per cent. loss in half-an-hour. Ultra-violet light caused an extremely rapid destruction—all the Vitamin A potency of one sample was lost on exposure for 10 minutes. Majumdar found values of 9 γ per gram for cow ghee from cross-bred animals, being the average value for 10 samples. The only spectrographic assay of Vitamin A in buffalo ghee was also made by him and yielded an average value of 1·2 γ per gram for 18 samples, though Banerjee and Sunawalla (*loc. cit.*) found that the difference in the Vitamin A contents of fresh cow

[10] *Indian Med. Gaz.*, 1941, 76, 487.
[11] *Indian J. Vet. Sc. and Anim Husb.*, 1941, 11, 329.

and buffalo ghee is not so marked as the above figures suggest, but are roughly in the ratio 1 : 0·7. The losses he found on converting butter into ghee were far less than those of the workers previously quoted and amounted in one case to 16 per cent. for 30 minutes heating reaching a maximum of 125° C. and to only 44 per cent. for a maximum of 156° C. and a time of 1½ hours. Recent work[12] shows that fresh buffalo ghee of good quality ordinarily contains 13–18 γ per gram, the lowest figure recorded being 9 γ, and the highest 24 γ. Losses on conversion of butter into ghee by the indigenous process were insignificant.

Banerjee and co-workers (*loc. cit.*) have made a series of comparative studies on the stability of Vitamin A in ghee, using as their basis the intensity of the blue value on the unsaponifiable matter of ghee with standard chloroformic antimony-trichloride. The vitamin was found to be stable in ghee for about a month, was reduced to half during ordinary storage for 6 months, and had been completely destroyed in a year. This is supported by the work of Bashir Ahmad *et. al.*[12] The vitamin was found to be fairly stable up to 125° C., but was thereafter rapidly destroyed, the destruction being greatly hastened by aeration. The effect of heat was not autocatalytic as is the case with light, *i.e.*, once the fat has been, say, heated, removal of the source of heat stops further destruction. An interesting observation, which needs more extensive study on a greater number of samples, was that the vitamin A in buffalo ghee was relatively less stable to heat than in cow ghee of the same potency, the stability depending to a large extent on other colouring matters associated with it. Acidity in ghee is a potent pro-destruction factor of Vitamin A.

The quantity of Vitamin A in the fat of cow and buffalo milk depends to a large extent on the feeding conditions under which they are reared. While carotene feeding has been attempted and the resulting fat analysed (referred to

[12] Bashir Ahmad, Ram Chand and Mansur-Hassan, *Proc. 33rd Indian Sc. Congress*, 1946.

below), the actual feeding of Vitamin A has not been done in India. Experiments in other countries, however, indicate the results to be expected. Fountaine and Bolin[13] fed two groups of Holstein and Jersey cows on a high ration of Vitamin A in the form of shark liver oil: maximum increases of 280 per cent. for the former and 305 per cent. in the latter group were reached after about two weeks. It is interesting to note that the carotene content fell by a small percentage (22 and 33 respectively) during the same period.

It may again be stressed that the above figures for vitamin potencies do not represent the finest specimens, especially of the provincial samples examined spectrographically. Dalip Singh and Fateh Mohommed[14] record a slight rise in Vitamin A and carotene of milk (fat) towards the end of lactations.

Carotene—This is the yellow pigment found in butter-fat and many varieties of fruits and vegetables. It has been extensively proved that one form of the pigment, β-carotene, has biologically twice the Vitamin A activity of the two other known forms, α- and γ-. By far the largest amount of carotene that occurs naturally is of the β-form, a rough measure of its content in foods being the intensity of their yellow colours.

While the yellow colour of ghee is a rough indication of its carotene content, to use it as a measure of carotene gives only comparable results. Unfortunately most of the results recorded so far have been in terms of such standard yellow units. While buffalo butter-fat contains no carotene at all,[15] the Indian cow ghee contains, on an average, less carotene than the milk-fat of the English cow which appears to have a better capacity to transfer carotene from its feed than its Indian counterpart.[16]

[13] *J. Dairy Sc.*, 1944, 27, 155.

[14] *Indian J. Vet. Sc. and Anim. Husb.*, 1945, 15, 57.

[15] Davies, "Chemistry of Milk", 1939, p. 215.

[16] Doctor, Banerjee and Kothavalla, *Indian J. Vet. Sc. and Anim. Husb.*, 1940, 10, 63.

Majumdar[11] has recorded the carotene contents by spectroscopic measurement of 10 samples of ghee from cross-bred cows and 18 samples of buffalo ghee. The cow ghees contained on an average 4 γ of carotene per gram, and the buffalo ghee merely a trace. Bal and Shrivastava,[17] by tintometric measurements, give a range for cow ghee of 2–5·7 γ per gram and for buffalo ghee of 0·2–0·3 γ per gram. They further state that the former varied with the carotene intake of the animal, but not the latter.

The carotene content of butter-fat is usually about 94 per cent. of the total pigments, the remaining being xanthophylls with a trace of lycopene.[18]

Carotene is rapidly destroyed by exposure to air as indicated by the loss of colour. Thus Krukovsky, Ellis and Barnes[19] found no destruction of carotene even after irradiation of butter-fat for 120 minutes at 50° C. with ultra-violet light. Seshan and Sen[20] heated a grass sample to 100° C. *in vacuo* with no loss in carotene, but in the presence of air, similar heating resulted in a 40 per cent. loss in two hours, and a 90 per cent. loss in twenty-four hours. The same might well apply to ghee.

The amount of carotene in butter-fat is roughly dependent, up to a limit, upon the quantity ingested.[21] In a remarkable study of this problem, Ray Sarkar and Sen[22] fed Hariana cows with carotene levels of 1·5–3·0 million international units, and found that a maximum potency of 10,000–11,000 I.U. per lb. of butter-fat was obtained; no point was gained by further increasing the quantity of green fodder. Initially, of course, the carotene content rose with increased carotene intake. At the maximum level of intake, the digestion of carotene was 30 per cent. of which 0·3 per

[17] *Nagpur Univ. J.*, 1940, **6**, 133.
[18] Gillam, *Biochem. J.*, 1934, **28**, 79.
[19] *J. Dairy Sc.*, 1944, **27**, 249.
[20] *Proc. 28th Indian Sc. Congress*, 1941.
[21] *Indian J. Vet. Sc. and Anim. Husb.*, 1938, **8**, 13.
[22] *Ibid.*, 1943, **13**, 219.

cent. was recovered in the fat. They had also earlier shown[23] that when the carotene ingestion was raised 18 times, a 50 per cent. increase in carotene and a similar increase in the Vitamin A potency of butter-fat occurred; when the ingestion was lowered 10 times, the carotene content of the fat was reduced 67 per cent. and the Vitamin A 19 per cent. In an earlier study, Das Gupta[24] had found that increased amounts of green fodder raised the carotene content, which fell again on reverting the animals (cows) to grass. Striking results were obtained on feeding red palm-oil to a cow: the carotene and vitamin contents rose almost by 50 per cent. when the amount of the oil fed had been 4 ozs. per day for a week. Incidentally, the Reichert-Meissl and Iodine values also increased.

Other reasons for fluctuations in the carotene content of butter-fat are diseases of the animal and such abnormal conditions. Thus Miller, Lease and Anderson[25] studied the changes brought about by inducing mastitis in one side of a cow's udder and comparing the fat of milk so secreted with that of the milk drawn from the healthy side. The carotene (and Vitamin A) content was nearly doubled in the fat from the affected part.

Xanthophylls—These are members of the carotinoid group of pigments and occur (usually together with carotene) in many green vegetables. They are also transferred to a small extent (about 6 per cent. of the total pigments[26]) to the milk-fat of the cow; they however appear to be easily destroyed in the digestive tract, since even heavy ingestion of Xanthophylls in the form of grass, which contains about twice as much of Xanthophylls as of carotene,[27] does not cause a large quantity of them to appear in the butter-fat. Neither

[23] *Indian J. Vet. Sc. and Anim. Husb.*, 1942, **12**, 179.
[24] *Ibid.*, 1939, **9**, 249.
[25] *J. Dairy Sc.*, 1940, **23**, 573.
[26] Gillam, *Biochem. J.*, 1934, **28**, 79.
[27] *A.C.S. Monograph* No. 41, 1928, p. 73.

carotene nor Xanthophylls can be synthesised by animals, but are synthesised by plants, from which source they are derived by the former.

The quantities of Xanthophylls in Indian cow or buffalo ghee have not so far been studied. The variations to be expected, however, are indicated in studies in England and America. Thompson, Kon and Mawson[28] found that in Guernsey milk-fat the total pigments consisted of larger amounts of pigments other than β-carotene, than in that of the Shorthorn. Johnson and co-workers[29] found the ratio of β-carotene : Xanthophylls : other pigments = 68 : 24 : 8, for cows receiving molasses, lucerne and silage, and β-carotene : Xanthophylls = 80 : 20 even for pasture butter. It would, therefore, not be correct to draw inferences regarding the Xanthophyll content of the milk-fat of Indian cows, and certainly not of that of buffaloes.

Lycopene—This is a pigment isomeric with carotene but not active, and occurs in large quantities in tomatoes and peppers. Though once thought to be absent in milk-fat, recent research has proved its existence in variable quantities, particular breeds of cows showing a selective action to the pigment even when fed in large quantities.[30] It may or may not be present in Indian cow and buffalo ghee.

Vitamin D—Values for Indian ghee are again lacking, nor can inferences be safely drawn. Henry and Kon[31] found that the contents were high for summer and low for winter butters; earlier they had given values of 0·35 I. U. ($= 0{\cdot}35 \times 0{\cdot}025\,\gamma$ of calciferol) and 0·28 I.U. for Guernsey and Shorthorn butter-fat,[32] which agreed well with the average of 0·36 I.U. for 75 samples given by Morgan and Pritchard[33] on the basis of biological assay. Most workers correlated

[28] *Proc. Biochem. Soc.*, 1942, 36, 7.
[29] *J. Dairy Sc.*, 1941, 24, 813.
[30] Gillam and Kon, *J. Dairy Res.*, 1940, 11, 266.
[31] *Biochem. J.*, 1942, 36, 445.
[32] *Ibid.*, 1936, 30, 776.
[33] *Analyst*, 1937, 62, 354.

the content of butter-fat with the amount of sunshine received by the cow. Thus Bechtel and Hoppert[34] gave high contents for the butter of cows grazing on pasture in the Sudan in summer. Contradictory evidence, however, is given by Sabri and Fikry[34] who were unable to show the presence of Vitamin D in cow or buffalo milk-fat even when these were fed in large amounts, and the work was done in a sunny country like Egypt.

Vitamin E—That this vitamin, necessary for growth and reproduction and in some way exerting a synergistic effect on the availability of Vitamin A,[35] exists in butter was first indicated in 1927,[36] and recent work[37] has not refuted the claim. Summer butter-fat to the extent of 20 per cent. in the diet of rats from the time of weaning was sufficient to confer fertility on them. No work has yet been done in India on the presence of this vitamin in ghee; since the vitamin is thermolabile in the presence of moisture, and since, moreover, the amount present in butter-fat is likely to be less than in butter.

Lecithin—The amounts of lecithin and cephalin carried into butter appear to be of the order of 0·05 per cent.,[38] depending on the method of manufacture of butter. Since they are fairly stable to heat,[39] similar amounts may be found in ghee also.

The fatty acids present in such phosphatide residues have recently been examined[40] in two cases; the major acids were palmitic, oleic and saturated acids higher than stearic.

Inorganic constituents—Varying amounts of these may be present in butter-fat depending upon the processing of the

[34] Quoted by Davies, "The Chemistry of Milk," 1936, p. 483.
[35] Guggenheim, *Biochem. J.*, 1944, **38**, 260.
[36] Sure, *J. Biol. Chem.*, 1927, **74**, 37.
[37] Mason and Bryan, *J. Nutrit.*, 1940, **20**, 501.
[38] Davies, W. L., "Chemistry of Milk," 1939, p. 72.
[39] Bordas and de Raczkowski, *Compt. rend.*, 1903, **136**, 56.
[40] Hilditch and Maddison, *Biochem. J.*, 1941, **35**, 24.

milk and its fat, and the cleanliness maintained in manufacture. Likely elements are copper, iron, calcium, lead,[41] etc. McIlroy[42] states that commercial samples of milk contain 0·24–0·25 mg. of copper per litre, and that very little goes into the fat. The calcium, phosphorus and iron contents of butter have been given as 0·015, 0·017 and 0·0002 per cent. respectively.[43] Figures are not available for Indian ghee.

Charred casein—On heating butter into ghee, casein is burnt, comes to the bottom as a light-brown mass, and may be poured off to greater or lesser extents with the ghee, though it usually settles as a fine powder on storing. The amounts may naturally vary according to the care with which the decantation or filtration of the product has been performed.

The Fatty Acid and Glyceride Structure of Ghee

Formerly, the fatty acid constituents of fats were determined by judicious use of the usual analytical constants for fats and oils, elaborated in the next chapter. The values thus obtained were naturally empirical. In the last twenty-five years, however, more accurate methods have come into vogue, involving usually the conversion of the acids into methyl esters and fractional distillation of these at low pressures—a technique whose usefulness has been amply proved by the results of Hilditch and his co-workers[44] at Liverpool. The analyses detailed here have been carried out by such methods and have great claim to accuracy. In every case the molecular percentages are given as being much more representative than mere weight percentages, especially where fatty acids of widely differing molecular weights occur together, as in ghee.

[41] Bagchi, Ganguly and Sirdar, *Indian J. Med. Res.*, 1940, **28**, 441.
[42] *American Chemical Abstracts*, 1936, **30**, 5668.
[43] *Nutritional Charts*, Heinz Company, Pittsburgh, 11th Edn., p. 28.
[44] Hilditch, T. P., "The Chemical Constitution of Natural Fats," Chapman & Hall, London, 1941.

TABLE XLII

MOLAR PERCENTAGES OF FATTY ACIDS IN GHEE

Acid	Buffalo Ghee						Cow Ghee	
	1[45]	2[46]	3[46]	4[46]	5[45]	6[47]	1[45]	2[45]
Butyric	10·9	15·4	13·5	11·5	10·8	3·0	8·8	6·9
Caproic	2·8	1·1	0·4	..	3·3	0·2	4·2	4·0
Caprylic	1·5	1·4	0·5	0·1	0·5	1·4	1·6	2·2
Capric	2·4	1·4	0·9	0·5	1·3	1·7	3·2	4·9
Lauric	3·3	2·0	2·4	0·8	2·4	2·6	4·4	6·7
Myristic	10·5	9·1	12·3	4·8	7·7	9·2	6·1	10·9
Palmitic	28·7	31·9	31·5	25·1	19·0	37·7	27·9	26·8
Stearic	9·3	12·4	10·1	19·0	20·9	11·5	9·4	5·5
as Arachidic	0·7	0·1	..	1·1	2·1	1·3	..	..
—Decenoic	..	0·1	0·1	{ 0·1	..	0·1	..	..
—Dodecenoic	..	0·1	0·1		..	0·1	..	..
—Tetradecenoic	..	0·6	1·0	0·5	..	0·8	..	..
—Hexadecenoic	..	3·0	3·0	2·9	..	4·9	..	..
Oleic	27·7	16·9	23·0	32·0	29·7	24·6	30·0	28·4
Linoleic	2·2	1·2	0·4	1·0	2·3	0·1	4·4	3·7
as Gadoleic	..	3·3	0·8	0·6	..	..	..	..

The figures for samples 1, 4 and 5 of buffalo ghee and in the two samples of cow ghee may be a little high for oleic acid (by 3–5 per cent.), since they include small amounts of lower unsaturated acids which were not discovered at the time the analyses were made. The samples 2 and 3 of buffalo ghee represent, in case 2, a sample of very high lower fatty acid content (R.M. value 37·4) and small unsaturation (I.V. = 27·4), and in case 3 a sample of fairly normal lower acid

[45] Bhattacharya and Hildtch, *Analyst*, 1931, **56**, 161.
[46] Achaya and Banerjee, *Biochem. J.*, 1946, **40**, 664.
[47] Anantakrishnan, Bhale Rao and Paul, *Arch. Biochem.*, 1947, **13**, 389.

content for a buffalo (R.M. value 30·7) but of low unsaturation (I.V. = 28·9). Sample 4 is a ghee of very low R.M. value (20·7), and comparatively high unsaturation (I.V. = 37·0). The content of lower acids is higher than for cow ghee, while the unsaturation is lower in each case. Samples 1 and 2 are quite normal in every respect (as are the cow ghee samples), while sample 4 is a ghee from buffaloes heavily fed with cottonseed.

Anantakrishnan, Bhale Rao and Paul[48] (with Rangaswamy[49]) studied the fat of Indian cows and buffaloes daily from the first day after parturition, and some illustrative figures are given below in mols. per cent.

TABLE XLIII

FATTY-ACID COMPOSITION OF COLOSTRUM FAT AND MILK-FAT OF COW AND BUFFALO (MOL. PER CENT.)

Fatty acid	Cow Days after calving			Buffalo Days after calving		
	1	4	10	1	3	10
Butyric ..	6·3	7·6	9·9	7·4	8·9	12·1
Caproic ..	..	3·0	1·3	0·3	0·4	0·7
Caprylic ..	1·5	3·2	2·3	1·3	1·3	2·4
Capric ..	1·8	3·2	2·5	1·5	1·4	1·5
Lauric ..	1·8	3·2	2·1	1·7	2·3	1·8
Myristic ..	10·4	11·2	11·3	9·1	12·8	12·8
Palmitic ..	29·0	26·8	27·3	19·5	25·4	28·2
Stearic ..	12·1	10·5	10·8	15·9	12·4	11·5
Arachidic ..	0·5	0·6	0·9	0·9	0·8	0·7
Decenoic ..	0·1	0·3	0·2	0·1	0·1	0·1
Dodecenoic ..	0·1	0·3	0·5	0·1	0·1	0·1
Tetradecenoic ..	0·9	1·1	0·8	0·4	0·6	0·6
Hexadecenoic ..	3·0	4·4	3·3	5·8	4·7	4·4
Oleic ..	30·3	23·1	24·7	34·1	27·8	21·6
Linoleic ..	1·3	0·7	0·3	0·7	0·1	0·2
C_{20-22} Unsaturated	0·9	0·8	0·8	1·2	0·9	1·3

[48] *Biochem. J.*, 1946, 40, 292.
[49] *J. Biol. Chem.*, 1946, 166, 31.

In cow milk-fat butyric acid rises steadily, caproic rises and then falls; caprylic, capric and lauric at first rise and then become steady, while palmitic remains more or less constant till the tenth day after calving. There is a marked fall of oleic acid up to the fourth day after which there is a slight rise; linoleic acid, however, steadily declines to about 25 per cent. of the initial content. In buffalo milk-fat, butyric, myristic and palmitic acids rise gradually, and a more pronounced decrease occurs in stearic and oleic acid contents during the change from colostrum-fat to milk fat.

Corresponding changes occur in the analytical constants of milk-fat after calving.[48-49] These are illustrated in Fig. 7.

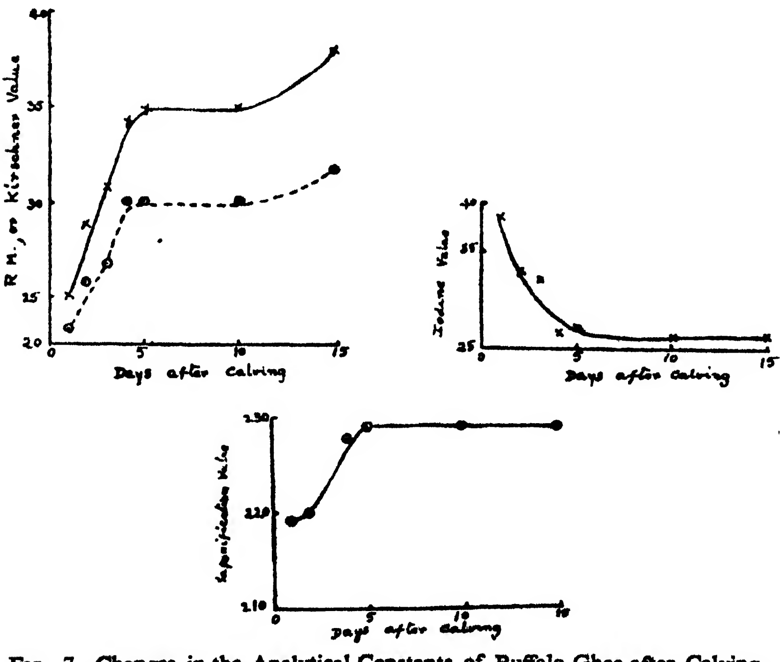

FIG. 7. Changes in the Analytical Constants of Buffalo Ghee after Calving

The Polenske value behaves generally like R.M., and the Butyro-refractometer reading declines steadily up to the 10th day after calving. These changes are characteristic of both cow and buffalo milk-fat.

The glyceride structure of a fat, by which is meant the proportions of the component triglycerides which go to make up the fat, has been studied by the same school of workers mentioned above by systematic crystallization from non-aqueous solvents, usually acetone. The information gained, while far from complete, still gives us valuable insight into the assembly of acids in the glycerides and the proportions of these glycerides themselves in terms of fully saturated glycerides (probably), mono-oleo-disaturated glycerides and (probably) di-oleo-monosaturated glycerides. The results discussed below are for cow ghee 2 and buffalo ghee 1,[50] 2[51] and 4.[51]

TABLE XLIV

MOLAR PERCENTAGES OF GLYCERIDES

	Buffalo ghee			Cow ghee
	1	2	4	2
Fully saturated glycerides ..	34	41·7	24·3	33·7
Mono-oleo-disaturated ..	42	41·2	40·1	36·5
Di-oleo-monosaturated ..	24	17·1	35·6	39·8

Moreover, it has been shown that the content of fully saturated glycerides is directly proportional to the relative proportions of saturated and unsaturated acids in the whole fat. For buffalo ghee 1, the ratio of saturated acids to unsaturated acids in the mixed glycerides $= \frac{36 \cdot 1}{29 \cdot 9} = 1 \cdot 2$, since the content of fully saturated acids in the fat is $= 70 \cdot 1$, and the content of saturated acids in the unsaturated portion $= 70 \cdot 1 - 34 = 36 \cdot 1$. Samples 2 and 4 give values of 1·32 and 1·04 respectively. For cow ghee, the same ratio works out (since the fully saturated acids make up 67·9 per cent. of the total fatty acids) to $\frac{34 \cdot 2}{32 \cdot 1}$ or 1·07.

[50] Hilditch, T. P., "The Chemical Constitution of Natural Fats," London, 1941, p. 257.

[51] Achaya and Banerjee, *Curr. Sc.*, 1946, **15**, 23.

Other Milk-fats—Goat, sheep and camel milk-fats are sometimes used in North-West India as human food. The first two are of a pale yellow or creamy tint. Banerjee and Datta[52] found that by increased carotinoid feeding the Vitamin A content of goat ghee could be enormously increased to a value more than twice that found usually in good cow ghee. The amount of pigmentation, however, was very small and not due to β-carotene; the authors suggest that since these act as anti-oxidants for vitamin A,[53] the destruction on storage of such ghee would be rapid. However, the hardy qualities of the animal and its easy maintenance should well-repay its wider use as a source of Vitamin A—rich milk-fat in India.

Camel ghee is little used, though its milk is of some value. It has also a creamy tint and a buttery smell.

The fatty acid and glyceride structures of three specimens examined by Dhingra[54, 55] are given below.

TABLE XLV

MOLAR PERCENTAGES OF FATTY ACIDS IN GHEE

Acid	Goat	Sheep	Camel
Butyric ..	7·6	8·4	5·9
Caproic ..	4·5	5·4	1·9
Caprylic ..	6·2	5·8	1·1
Capric .	11·1	10·1	2·1
Lauric ..	5·1	6·0	5·7
Myristic ..	11·2	11·8	7·9
Palmitic ..	21·5	20·4	28·3
Stearic ..	7·3	5·4	9·7
as Arachidic ..	0·1	1·3	..
Oleic ..	24·2	22·2	34·1
as Linoleic ..	1·2	3·2	3·3

[52] *Agric. and Livestock in India*, 1938, 8, 563.
[53] Banerjee and Dastur, *Ibid.*, 1937, 7, 24 and 697.
[54] *Biochem. J.*, 1933, 27, 851.
[55] *Ibid.*, 1934, 28, 73.

The lower acids are present in quite as large amounts as in buffalo or cow ghee for the first two as also oleic acid. Camel ghee has a greater amount of Polenske acids but a smaller amount of lower acids than cow or buffalo ghee. The oleic acid content is high, but not abnormally so.

TABLE XLVI

GLYCERIDE STRUCTURE OF INDIAN GHEES

	Goat	Sheep	Camel
Fully saturated glycerides ..	39·3	36·8	25·6
Mono-oleo-disaturated ..	45·5	50·2	36·6
Di-oleo-monoseturated ..	15·2	13·0	37·8
Fully saturated acids in whole fat ..	74·6	74·6	62·6
Ratio of saturated to unsaturated acids in unsaturated part ..	1·40	1·49	0·98

Commenting on his work, Dhingra remarks that "there is no reason why they (goat and sheep milk-fats) should not be used as substitutes for the latter (cow and buffalo milk-fats)". Considering the enormous lack of milk-fat in India, it may be pointed out that goat ghee would form an effective supplement, rather than a substitute, to the cow and buffalo ghee used at present.

Chapter XII

ANALYTICAL VALUES OF GHEE

Introduction

Fats and oils are characterised by certain physical and chemical properties which have been the basis of their analysis from the point of view of either purity or quality. Many of these constants are arbitrary and depend upon the utilization of strictly controlled conditions, so that the results are not of absolute but of only comparable value. By far the greatest use to which they are put is to judge the purity of any sample of the fat or the oil, by comparison of the values yielded on analysis with values from samples of known purity.

With butter-fat especially, where the temptation to adulterate is great and almost as often yielded to, such analytical constants are of great value to the analyst. This chapter indicates the usual ranges to be expected for the numerous values developed from time to time, endeavouring as far as possible to give data for both cow and buffalo ghee of attested purity. Methods of examination are not given since they are found in well-known books of fat analysis. Much information here has been drawn from the observations of Godbole and Sadgopal,[1] Brahmachari[2] and Ghose,[3] and further matter has been incorporated from an analysis of about 200 samples of ghee carried out by Katrak, Achaya and Banerjee.[4]

Physical Methods

Flavour, texture, colour: These are the tests used by consumers in estimating the quality of a product, and the

[1] "Butter Fat (Ghee)," Benares, 1939.
[2] *Indian Med. Gaz.*, 1927, **62**, 318.
[3] *Analyst*, 1920, **45**, 447.
[4] Partly published, *Curr. Sc.*, 1946, **15**, 107.

preferences naturally vary from district to district. Cow ghee has a characteristic sweetish flavour, like that of freshly made bread. Sometimes, due to overheating in preparation, the charred casein and sugar impart to the ghee a burnt or caramelised flavour which is appreciated in certain localities. Buffalo ghee has a distinct aroma which through long usage is preferred in India to that of the cow.

On cooling ghee without stirring, it crystallizes as fine granules, the size of which are supposed to depend on the rate of cooling. In this way, cow ghee gives granules which are slightly smaller than those from buffalo ghee. A characteristic "grainy" feel is an important criterion of quality for most buyers.

The colour of cow ghee of good quality is a bright beautiful yellow and usually of the order of 8–10 Lovibond yellow units per gram; it is well known that this is almost wholly due to the presence of the yellow carotinoid pigment, β-carotene. The Indian buffalo, however, transfers very little of the pigment from its feed to the fat, and yields butter-fat of a pale yellow colour, of the order of 3–5 Lovibond yellow units per gram.

On standing, ghee usually separates into two layers—the top, a clear liquid layer, and the lower mass consisting of closely packed granules. The process is somewhat analogous to the settling of "stearine" in oils, since a similar partial separation of glycerides occurs. Thus Daji[5] states that the physical constants of these layers are often different, and emphasizes the need for thorough mixing before sampling.

Melting point—Being by nature "mixtures of mixed triglycerides", butter-fats do not melt sharply as do pure chemical compounds. Further, triglycerides exhibit a property of melting at two different temperatures, especially if the temperature of fusion is determined soon after melting and re-solidification. Bömer[6] has suggested that the lower

[5] *Poona Agric. Coll. Mag.*, 1939, **30**, 141.
[6] *Analyst*, 1907, **32**, 357.

melting point obtained in this case is due to the change of the labile into the stable form. Usually, however, the values are implied for ghee not more than a few days old maintained at 0° C. for 24 hours before determination, and for the point of complete fusion as against the temperature of incipient fusion.

Values for melting varied from 28°·5–43°·5 C. for 200 samples examined by Godbole and Sadgopal.[7] Samples examined by the authors gave the same upper limit, but some of the samples were liquid at a room temperature of about 23° C. Buffalo ghees on the average gave higher melting points than did cow ghees, and were distinctly harder to the feel. Godbole and Sadgopal's figures for cow ghee are 28°·5–42°·0 C. and for buffalo ghee 32°·0–43°·5 C. Ghose[3] records figures for buffalo ghee from 34°·0–38°·0 C., with the vast majority at about 36° C.

Solidifying point: (*a*) *of butter-fat*—This does not usually represent the same point as the melting point. Polenske,[8] in fact, suggested that the difference between the two should be used for the estimation of the purity of butter-fat—a method which later proved useless.[9] Values for cow ghee are from 15°·0–23°·5 C. and for buffalo ghee from 16°·0–28″·0 C.[10]

(*b*) *Of the fatty acids of butter-fat*—When the mixed fatty acids of ghee are allowed to cool from a molten condition, at the point of solidification the latent heat of fusion is liberated and considerable rise of temperature occurs. This observation was standardized as the 'titre test', and the results were expressed as the highest temperature recorded. Lewkowitsch[11] gives the values for a number of oils and fats, but not for butter. Values for ghee are not available, except for one value of 33°·5 C. for buffalo ghee.[10]

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[7] "Butterfat," Benares, 1939, p. 48.

[8] *Analyst*, 1908, 33, 476.

[9] Bömer and Limprich, *Zts. Unters. Genussm.*, 1913, 25, 367.

[10] Godbole and Sadgopal, "Butterfat," Benares, 1939, p. 17.

[11] "The Chemical Technology and Analysis of Oils, Fats and Waxes," London, 1913, Vol. 1, p. 506.

Viscosity—This depends on the resistance to motion of the fat flowing through a tube, and is the friction of the liquid to the displacement of the different layers relative to one another during the flow. Figures are not available for ghee probably because they are of little value except from an academic standpoint.

Specific gravity—This is usually expressed by comparing the specific gravity of ghee at 15°·5 C. with that of an equal volume of water at 15°·5 C. Alternatively other temperatures may be chosen, 4° C. being one of the more common for water because it possesses its maximum density at that temperature. Values are given by Godbole and Sadgopal as lying between 0·9358 and 0·9443 for cow ghee and between 0·9340 and 0·9444 for buffalo ghee.

Fluorescence—By convention this has come to mean the colours, whether characterized as wave-lengths or not, exhibited by ghee on exposure to ultra-violet light. The usual source of such light has been a mercury vapour lamp of one of the numerous varieties marketed.

Baker and Taubes[12] reported in 1932 that any sign of blue in the fluorescent light was definitely suspicious. Jha,[13] working seven years later, found a green colour for ghee by daylight and a lemon-yellow colour in the dark; this was confirmed the next year by Muthanna and Mukherji[14] who gave as the colours of both cow and buffalo ghee a deep green. The same year, however, this was refuted by Narasimhamurthy and Suryanarayanamurthy[15] who found a variety of colours from green to blue. Achaya and Banerjee[16] have shown that the colours are often masked by the presence of carotinoid pigments (and perhaps by Vitamin A). When these are removed with animal charcoal, a light green colour is obtained, which changes progressively on aging

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[12] *Analyst*, 1932, **57**, 375.
[13] *J. Indian Chem. Soc.*, 1939, **1**, 159.
[14] *Curr. Sci.*, 1940, **9**, 120.
[15] *Ibid.*, 1940, **9**, 334.
[16] *Indian J. Vet. Sc. and Anim. Husb.*, 1945, **15**, 261.

into a blue colour. Both cow and buffalo ghee gave this light green tint. Later quantitative examination of the fluorescent spectrum of various fats gave band spectra of almost the same limits, the different colours being therefore due to respective portions of bright intensity. Simple viewing of fluorescent light is therefore to be preferred.

Refractive index—This is usually measured on one of the two standard instruments—the butyro-refractometer or the Abbe refractometer, readings of which are mutually convertible.

The following table gives the refractive index for ghee at 40° C.

TABLE XLVII

REFRACTIVE INDEX OF GHEE (B.R. READING) AT 40° C.

Observers (*loc. cit.*)		Cow	Buffalo
Katrak, Achaya and Banerjee	..	42·5–47·7	39·2–46·9
Brahmachari	..	39·6–44·6	..
Ghose	..	..	40·0–42·0
Godbole and Sadgopal	..	40·0–43·0	40·0–43·5
Hogan and Griffith-Jones[17]	..	..	40·0–44·0
Mehta (Jodhpur)[18]	..	42·0–45·5	41·0–45·0

The results of the first set of workers seem distinctly high. For every degree centrigrade rise of temperature, the B.R. reading at 40° C. falls by 0·55. The presence of 1 per cent. moisture lowers the value by about 0·7 B.R. degree.

Colour fringes—Godbole and Sadgopal[10], using an uncompensated Butyro-refractometer, found that just where, the dark field separates from the bright half, colour fringes were found which were characteristic for the oil. Ghee gave a colourless or a light violet fringe in this region which the authors explain as being due to the differing dispersions of

[17] *Analyst*, 1916, 41, 307.
[18] *Proc. Indian Sci. Cong.*, 1947, Sec. 10, p. 2.

the glycerides in ghee, and which was found to be characteristic for ghee. It is hoped that this colour fringe of ghee will be more widely studied after fuller details have been published than are done in the second edition of their volume on ghee. Very recently, some workers have reported that the test gives varying colours and is largely dependent upon the refractive index.[19]

Refractive Dispersion—This represents the difference in the refractive indices for two different lines of the spectrum. Athavale and Jatkar[20] used the green (5460) and violet (4358) lines of the mercury arc and gave the dispersion values at 40° C. for cow ghee as lying between 0·00838 and 0·00853, and for buffalo ghee as ranging from 0·00851 to 0·00856. Achaya and Banerjee,[21] working on buffalo ghee, found that for the highest class of samples the value lay around 0·00874 to within a few units of the last place of decimals quoted. Rancidity in ghee caused a great fall in the value; and neutralization of free fatty acids a further fall. They did not work on cow ghee and the only values available are those of Athavale and Jatkar.

Raman Spectra—This has been measured for buffalo ghee,[22] though not for cow ghee. The lines recorded were one at 1300 indicating a C-O-C grouping, and the line characteristic of the usual C-H aliphatic linkage at 2900. Van den Hende and Fonteyne[23] state that a study of Raman spectra shows that both monomeric and polymeric molecules exist in the lower fatty acids; it is only possible to distinguish acids below undecylic (C_{11}) acid by this means.

Chemical Constants

As above, the full methods for carrying out these determinations have not been given, since they are to be found in

[19] Dastur, Kashyap and Kothavalla, *Indian J. Vet., Sc. and Anim. Husb.*, 1944, **14**, 49.

[20] *J. Indian Inst. Sc.*, 1938, **21-A**, 15.

[21] Awaiting publication.

[22] Joga Rao, *Proc. Ind. Acad. Sc.*, 1937, **4 A**, 459.

[23] *American Chemical Abstracts*, 1944, **38**, 3908.

well-known treatises on the subject of analytical chemistry. Sometimes differences are found between the methods recommended by the British Standards Institution and those of the Association of Official Agricultural Chemists, America, and it is hoped that some definite standard will be laid down and adopted in common.

Reichert-Meissl, Polenske and Kirschner values. These values all depend on the volatile acids present in ghee and are only comparative, so that strict adherence to the conditions of the experiment are necessary for concordant results to be obtained. The fat is saponified with glycerol-soda, the soap diluted and acidified and the fatty acids distilled in steam A definite volume of the distillate is titrated against alkali and the Reichert value (it has been proposed that this simple term supersede other cumbrous names associated with the process) calculated as the number of c.c.s of N/10 alkali required to neutralize the water-soluble volatile acids distilled from 5 gm. of the fat.

At the same time it is found that there occurs volatile acids insoluble in water which collect as white flakes or oily drops. These are usually filtered off, dissolved in neutral alcohol and titrated; the number of c.c.s of N/10 alkali required to neutralize the water-insoluble volatile acids distilled from 5 gm. of fat is called the Polenske value, after the name of its introducer.

A further modification, purporting to furnish a measure of the butyric acid was later introduced by Kirschner. The butyric acid is converted into its soluble silver salt, the solution is filtered free of insoluble silver salts of the higher acids, and distilled, and the distillate titrated against alkali, after which the value is calculated.[24]

These values represent by far the most useful routine method for the examination of ghee. It is only the fat of the milk of herbivora that are characterised by appreciable content of lower water-volatile acids, and hence this is a

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24 *Analyst*, 1936, 61, 404.

most important characteristic both from an absolute and an analytical point of view.

The Reichert-Meissl (R.M.) values for cow and buffalo ghees are known with some degree of certainty. As a rule it has been found that cow ghees vary over a much smaller range than do buffalo ghees, and are *usually* lower in value than the latter.

TABLE XLVIII

R.M. AND POLENSKE VALUES FOR COW AND BUFFALO GHEE

Observers (*loc. cit.*)	Cow		Buffalo	
	R.M.	P.V.	R.M.	P.V.
Godbole and Sadgopal ..	21·0–34·4	0·7–2·0	24·6–35·5	0·8–2·2
Ghose ..	..	..	29·0–42·0	..
Katrak, Achaya and Banerjee	17·0–28·0	0·9–3·7	14·0–40·0	0·4–5·3
Brahmachari ..	19·5–42·4	..	..	..
Hogan and Griffith-Jones ..	..	..	24·5–37·0	1·0–2·8
Mehta (Jodhpur) ..	18·0–37·0	0·5–2·5	16·6–31·0	0·8–2·5

The average R.M. reported by Brahmachari for fifty-one samples is 24·5 for cow-ghee, and this low value is also apparent in the figures of Katrak, *et al.* Samples examined over a period of two years from a dairy in Bangalore by the authors, gave for buffalo ghee figures between 25 and 31. The figures given by Mehta clearly indicate the wide variations in the constants of genuine ghee that can occur in different parts of the country.

The Polenske figures vary little. The figures for cow ghee of Godbole and Sadgopal seem to be low, especially considering the corresponding high R.M. figures (*cf.* next chapter). The majority of the P.V. figures for the buffalo ghee samples examined by Katrak, *et al.*, was inclined to be low, the average value being 1·8. It would be best to consider the variations of R.M. and P.V. in relation to each other, as the two figures are closely related.

The Kirschner values for ghee have not been so extensively studied as the other two companion values. Bhattacharya and Hilditch[25] quote about 25·0 for buffalo ghee, and Doctor, Banerjee and Kothavalla[26] 26·7; while for cow ghee the values are distinctly lower—the figures of Bhattacharya and Hilditch being 20·6 and 20·9 for the ghees examined; and those of Doctor, *et al.*, form 19·0 to 22·4. The figures run parallel to the R.M. figures; this will be discussed later.

"A" and "B" Values—These values, introduced by Bertram, Bos and Verhagen[27] in 1923, and modified slightly by Godbole and Sadgopal (*loc. cit.*) have been very highly commended for use by the latter authors. They yield further information regarding the distribution of the lower fatty acid fraction of ghee and have the advantage of being more restricted in their range of values. The "A" value represents a measure of the fatty acids yielding water-soluble-magnesium and water-insoluble-silver soaps in terms of c.c.s of N/10 silver nitrate. The "B" value gives a content of the acids with silver- and magnesium-soluble soaps in terms of c.c.s of N/10 alkali. The values quoted by Davies[28] are from 6·2–6·9 for the "A" value, and from 33–37 for the "B" value; and Godbole and Sadgopal state that the values for Indian ghee are strikingly in agreement with those for European butter-fats. It would certainly be of much help to study the variations of these values under the many conditions experienced in the ghee trade, especially from the point of view of the much-vexed question of detection of adulteration. Perhaps its only drawback is the tediousness of the determination, at any rate, in the light of our present limited knowledge of the range of values. In view of the wide range of R.M. values, we anticipate that wide ranges for these two values are inevitable.

[25] *Analyst*, 1931, **56**, 161.
[26] *Indian J. Vet. Sci. and Anim. Husb.*, 1940, **10**, 63.
[27] *Ch. Weekblad*, Bd., 1923, **20**, s. 610.
[28] Davies, W. L., "Standards and Analytical Processes for the Examination of Ghee," 1944, pp. 16–17.

Cadmium Number—This depends again on the fact that the cadmium salts of butyric and caproic acids are soluble in water while those of the higher acids are not. Values from 70 to 90 are given for butterfat by Elsdon[29]; but results for ghee are not available, and this is perhaps to be expected after the statement by Revis and Bolton[30] that the method was soon abandoned by them " on account of its extreme laboriousness and questionable value ".

Barium Value—This is sometimes called the Ave-Lallemant value after its founder. It represents for the total acids what the R.M. and Polenske values do for the volatile acids. The insoluble Barium value represents the content of acids forming insoluble Barium salts from one gram of fat, calculated as Barium oxide, and is usually represented by b. The total barium value, a, represents the saponification value (see below) of the fat for one gram, also calculated as Barium oxide, from which is obtained the soluble Barium value $a-b$, usually expressed as c. The most general way of recording the results is as $b - (200 + c)$, and the value is chiefly used in the detection of adulteration. Ave-Lallemant[31] gave an average value of 9·6 for butter, and the value for fifty samples was always negative. Bolton and Revis[32] gave negative values for 32 samples collected from all over the world, yielding an average of about 6·4. Parthasarathi and Banerjee[33] showed, however, that under conditions when oil-seed feeding was the rule, as is very common in India, positive values were almost invariably obtained, the figures rising to as high as + 10·0. Full ranges of figures have yet, however, to be given and may still prove characteristic for ghee, since most oils and animal fats give high Positive figures above + 40, as recorded by Ave-Lallemant.[31]

[29] " The Chemistry and Examination of Edible Oils and Fats," London, 1926, p. 165.

[30] *Analyst*, 1911, **36**, 334.

[31] *Ibid.*, 1907, **32**, 382.

[32] " Fatty Foods," London, Vol. II.

[33] Private communication.

Hehner Number—This expresses the percentage of water-insoluble fatty acids obtained from a fat. It is obvious that it will hardly serve as a characteristic of much importance for any fat, including ghee. The value for butter-fat has been recorded as lying between 86·5 and 88; its limited usefulness is obvious from the fact that for coconut oil the value lies between 94·5 and 96, and for other oils in general tends to approach 100. Frequently the border-line between insoluble and soluble acids is not rigid and the values for ghee are too high owing to the presence of the difficultly soluble lauric acid, which may be washed out in greater or smaller amounts.[34]

Saponification Value—This gives a measure of the mean molecular weight of a fat, and in fact may be expressed as a molecular weight, or saponification equivalent preferably. The usual form in the case of natural fats is the saponification value, while with acids the saponification equivalent gives a better idea of the product under examination.

The saponification value represents the number of milligrams of potash required to saponify one gram of fat; while the saponification equivalent is the number of grams of oil saponified by a litre of N alkali—the equivalent weight, as it were, of the fat.

The lower glycerides (theoretically assuming unmixed triglycerides to occur in fats—which is usually not the case) give high saponification numbers—*e.g.*, 557 for tributyrin. Since fats contain *on the average* similar types of glycerides of similar molecular weight, the saponification numbers are very similar. Their value as characteristics, is therefore small. Since, however, they are easily estimated, they form one of the routine determinations. The following are some figures for this constant (Table XLIX), as observed by the workers previously quoted.

[34] "The Chemical Technology and Analysis of Oils, Fats and Waxes," London (J. Lewkowitsch), 1913, Vol. 2, p. 855.

TABLE XLIX

SAPONIFICATION VALUE OF GHEE

Observers (*loc. cit.*)		Cow ghee	Buffalo ghee
Godbole and Sadgopal	..	225·5–236·0	228·5–236·0
Ghose	..	..	226·0–240·0
Katrak *et al.*	..	201·9–229·7	200·8–248·0
Brahmachari	..	213·9–236·4	..
Hogan and Griffith-Jones	..	..	218–235

The range is wide for both types. This is perhaps to be expected in the case of a physiological product like ghee and the very general nature of the determination. The relationship between these constants is discussed later, as also their variations.

Juckinack Difference—This is merely an empirical relationship and is equal to R.M. + 200–S.V., and is based on the direct relationship between the R.M. and the saponification values. Reference to Tables LIII and LIV will show that the figures would amount to + 3 for cow ghee, and from − 3 to + 1 buffalo, in an average case.

Free Fatty Acids—During the process of preparation of ghee, some fatty acids are liberated, and these when extracted with a suitable solvent, usually neutral alcohol, yield a definite titre value against alkali. The value is diversely expressed as percentage of oleic acid, lactic acid, acid value or as points of acidity.

The value is of some importance in determinations of rancidity and indicates in a rough measure the condition of ghee. Naturally the value differs very widely: suffice it to say that a good ghee should have as low an acidity as possible, about 0·1 per cent. (as lactic acid) indicating a product of fair quality.

Neutralization Value—Suggested in 1940 by Hawley,[35] this represents the milligrams of potash necessary to neutralize

[35] *Curr. Sci.*, 1940, 9, 337.

one gram of the insoluble fatty acids in ghee under certain definite conditions. Losses of the lower acids are brought to a minimum during clarification of the acids by boiling on a water-bath under reflux, after the acids have been liberated following saponification. Further results have not been reported after the author's work. He obtained values above 209 for genuine ghee, but the number of samples were few in number and from a limited area. Since this value gives a measure as it were of the acids of ghee excluding butyric acid, and since it is not merely differences in the amount of butyric acid that differentiate samples of ghee, it is anticipated that little is to be gained from this method: it would break down, for example, with samples from cotton-seed feeding areas (*cf.* Table XLII).

Enzymic Hydrolysis Colours[36]—This is a chemical method yielding physical results of an empirical nature. Ghee is hydrolysed by an enzyme preparation from pigs' pancreas, and the fatty acids are flooded with dilute copper sulphate and iodine solutions successively, when a dirty yellow colour is obtained. The authors attribute this to the presence of butyric acid after tests with pure acids. Rancid ghees showed the same colour as fresh ones. The authors hoped to standardise the test using a tintometer to yield results more truly quantitative, but so far no results have been reported. It is possible that an enzyme, specific for butyric acid, would yield more definite results than the mixed enzyme used for the test.

Iodine Value—Some of the fatty acids present in ghee contain unsaturated linkages at which halogen addition can take place under certain conditions. The usual halogen chosen is iodine and the reagent differs according to the method used. The number of grams of iodine absorbed by 100 grams of ghee represents the iodine value of the sample, and is a measure of the degree of unsaturation of it. It serves as an important analytical characteristic of ghee;

[36] Giri and Bhargava, *Ind. Eng. Chem.* (*Anal.*), 1937, 9, 395.

the range is smaller than for the other constants, and the value is sensitive to small changes that do not affect the other constants. Since the unsaturated portion of ghee is almost wholly oleic acid (see previous chapter) it forms nearly a quantitative measure of its content, probably to the extent of 80 per cent.

The following are some figures for the iodine value of ghee.

TABLE L

IODINE VALUE OF GHEE

Observers (*loc cit.*)		Cow ghee	Buffalo ghee
Godbole and Sadgopal	..	31·5–45·0	26·5–44·0
Katrak, *et al.*	..	32·3–45·6	24·6–43·8
Brahmachari	..	25·6–44·1	..
Hogan and Griffith-Jones	..	..	23·0–39·7

The agreement is excellent between the sets of values. The average for cow ghee tends to be distinctly higher (at about 35) than that for buffalo ghee, which is usually in the neighbourhood of 29. The values are affected by a wide variety of causes, a few of which will be considered later.

Thiocyanogen Value—This represents the degree to which thiocyanogen combines with ghee, and is a measure of the unsaturated acids with one, two, and four double bonds. Thiocyanogen combines with oleic acid almost exactly quantitatively, but with linoleic acid to slightly greater than half the extent that iodine does and to which it quantitatively should by theory. Since the unsaturated acids of ghee are composed almost wholly of these two, the percentage of linoleic can be calculated if the iodine value is known. The iodine and thiocyanogen values for oleic acid are the same and theoretical.[37] The following formulæ give (approximate) calculations for expressing the distribution of fatty acids in the glycerides and the mixed fatty acids. If I = I.V., T = Thiocyanogen value, S = percentage saturated, O = percentage oleic and L = percentage linoleic acids respectively, then

[37] Hilditch and Murti, *Analyst*, 1940, 65, 437.

(1) $L = 1{\cdot}223\,(I - T)$
$O = 1{\cdot}176\,(1{\cdot}712\,T - I)$
$S = 100 - (L + O)$

in the case of glyceride distribution.

(2) $L = 1{\cdot}169\,(I - T)$
$O = 1{\cdot}245\,(1{\cdot}891\,T - I)$
$S = 100 - (L + O)$

in the case of fatty acid distribution.

Budhalakoti and Mukherji[38] studied 10 samples of ghee, of which 8 were probably genuine. The values were from 32·0–45·0 for cow ghee and from 25·1 to 36·8 for buffalo ghee. Godbole and Sadgopal's figures are 25·5 to 40·8 for cow and 22·7 to 40·0 for buffalo ghee which are probably more representative. The values seem to indicate that on the average the content of two double-bond acids is slightly higher in cow ghee than in buffalo ghee.

Acetyl Value—If a fat be acetylated, then saponified exactly, and the soap acidified with a calculated quantity of acid and filtered, the titre values of the filtrate against N/10 potash multiplied by 5·61 and divided by the weight of fat taken gives its acetyl value. It is a measure of the hydroxy acids present in a fat, which have been converted into acetic acid and are washed down. Naturally the figures for ghee include the lower water-soluble acids, but these are separately determined without acetylation and subtracted from the other titre. The value for butter-fat should be zero since it has been shown to contain no hydroxy-acids, and this is the value recorded by Godbole and Sadgopal. They also found that the value rises with increasing acidity on aging. Woodman[39] gives the figure 5·2 for butter-fat, which certainly seems high.

The Constants of Other Ghees

Goat, sheep and camel ghee have been studied by Dhingra[40, 41] and Mehta[42] in India, though reports are found

[38] *J. Indian Chem. Soc.*, 1935, **12**, 455.
[39] Woodman, A. G., "Food Analysis," McGraw Hill, N.Y., 1939, p. 80.
[40] *Biochem. J.*, 1933, **27**, 851.
[41] *Ibid.*, 1934, **28**, 73. [42] *Proc. 33rd Indian Sc. Congress*, 1946.

in the literature giving the constants of ghee from animals bred elsewhere, especially goats. The following are their results, from single samples of each type by Dhingra, and an average of 40 samples in the latter case from Jodhpur animals.

TABLE LI

CONSTANTS OF INDIAN GOAT, SHEEP AND CAMEL GHEES

Constant	Goat		Sheep		Camel
	1	2	1	2	
R.M.	31·7	23·2	33·9	26·5	16·4
P.V. ..	8·2	4·5	8·0	3·1	1·6
K.V. ..	19·9	..	22·8	..	14·3
I.V. ..	28·8	33·6	32·1	35·9	40·8
Acid value ..	2·9	..	2·2	..	0·2
S.E. ..	238·1	242·5	232·4	243·0	259·0
B.R. at 40° C. ..	39·9	42·8	40·3	43·2	44·4

The following values are given for goat and sheep ghee by other workers, and are quoted by Dhingra.[40]

TABLE LII

ANALYTICAL CONSTANTS OF GOAT AND SHEEP GHEE

	R.M.	P.V.	K.V.	I.V.	S.E.
1. *Goat ghee*					
Trimen (Egyptian)	20·8–22·9	4·9–6·5	..	..	..
Atkinson (Egyptian)	20–28	4·0–8·7	..	32·5–41·2	228–238
Knowles and Urquhart (English)	24·5–27·8	4·9–8·7	16·8–19·0	24·7–36·9	..
Bardisian (English)	..	..	..	28·1	241·3
2 *Sheep ghee*					
Bardisian (English)	..	..	..	32·3	241·3
Faltin and Dedinzky (European)	22·5–39·4	2·2–6·9	15·8–21·0	..	..

The difference in the figures in Table LI of the two workers clearly indicates the possibility of wide variations due to region, breed and other factors. In the absence of sufficient data no definite conclusions can evidently be drawn regarding the average characteristics of these Indian milk-fats.

Chapter XIII

INTERRELATIONSHIP AND VARIATIONS OF CONSTANTS OF GHEE

Interrelationship

Individually the various "constants" of butter-fat vary within wide limits; when they are considered with respect to a fixed value for any one constant, the variation is usually much smaller. In other words, the constants of ghee vary in a very definite way with each other, and the range of individual variation for each constant is smaller corresponding to a fixed value for any other. It would be of extreme interest if values for such interrelationship were available for all the known constants; and in sufficient number. Since however some of the methods are of recent inception, and others too cumbersome for routine estimation the commoner values recorded in the scientific literature are the R.M., P.V., I.V., S.V. and Refractive Index. The relationships between these will be discussed here. The sources for the figures are the publications of Ghose,[1] Brahmachari,[2] and the work of Katrak, Achaya and Banerjee.[3] Other figures have unfortunately not been of much help since they have been recorded in the form of averages, maxima or minima. Ghose's figures are for samples of buffalo ghee from all over India; Brahmachari's for cow ghee from Bengal; and the latter workers' for both types from various parts of India. The figures must not be taken too literally when all the causes which upset them are considered, a few of which are detailed later, with cow ghee. Brahmachari records fifty-four analyses with R.M. values ranging from about 20 to 35. These were divided into four groups, yielding

[1] *Analyst*, 1920, **45**, 447.
[2] *Indian Med. Gaz.*, 1927, **62**, 318.
[3] Partly published, *Curr. Sci.*, 1946, **15**, 107.

average R.M. figures of 21, 24, 27 and 30, and the corresponding figures for the other constants were worked out. Katrak, *et al.*, record forty-four analyses, of wider range, which were similarly analysed. Except for the refractive index which tends in the former figures to be low, there is good agreement between the correlations. The following table shows the average, normal interrelationships between the constants of cow ghee. Fig. 8 illustrates this.

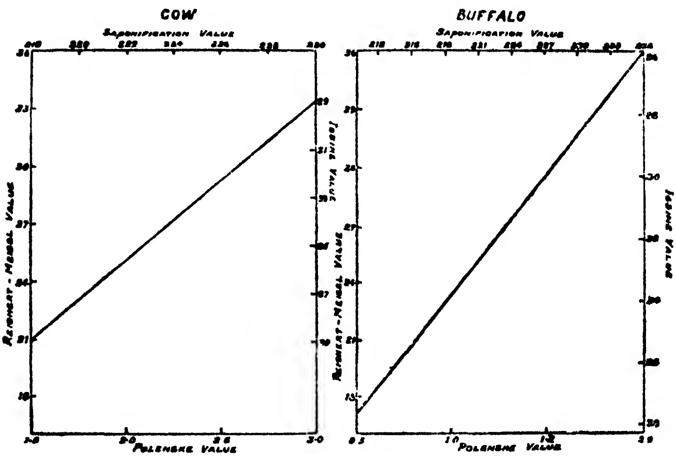

FIG. 8. Correlation of the Analytical Constants of Ghee

The following table brings out the interrelationship between the various constants of cow ghee.

TABLE LIII

ANALYTICAL VALUES FOR COW GHEE

R.M.	P.V.	S.V.	I.V.	B.R. (40° C.)
21	1·5	218	39	46·2
24	1·9	221	37	44·7
27	2·3	224	34	43·1
30	2·7	227	32	41·5
33	3·0	230	29	40·5

Particularly striking is the inverse relationship between the R.M. and Iodine values. It has been shown by Smith and

Dastur[4] that during fasting, the R.M. value decreases markedly, and the I.V. rises at the same time in an exactly similar way: in fact the sum of R.M. and I.V. varies within very narrow limits. For cow ghee, the sum may lie between 62 and 60. However, it is interesting to observe the remarkably low unsaturation of these milk-fats compared to those of Western origin[1]; the R.M.–P.V. relationship of the two appear to be of the same order. The rows of constants in the above table are linked by an empirical relationship, $\frac{\text{S.V.}}{\text{R.M.} + \text{P.V.} + \text{I.}} = 3\cdot53.$

Buffalo Ghee—Similar analyses of the 233 figures of Ghose and the 125 of Katrak, *et al.*[5] yielded the following correlative analytical values for buffalo ghee; these are graphically shown in Fig. 8.

The interreactionship between the various constants of buffalo ghee are also given in Table LIV.

TABLE LIV

ANALYTICAL VALUES FOR BUFFALO GHEE

R.M.	P.V.	S.V.	I.V.	B.R. (40°C)
18	0·6	212	37	44·9
21	0·8	217	36	44·2
24	1·0	222	34	43·7
27	1·3	226	32	43·2
30	1·6	230	30	42·7
33	1·8	233	28	42·1
36	2·0	235	26	41·6

Two differences are apparent from the corresponding figures for cow ghee: (i) the mean unsaturation for the same R.M. value is lower, to the extent of about 3 units in each case. The sum of R.M. and I.V. is lower and lies between 62 and 55; (ii) the Polenske acids, caproic and caprylic, are very small in proportion to the R.M. acids.

[4] *Biochem. J.*, 1938, **32**, 1868.

[5] Achaya and Banerjee, *Curr. Sci.*, 1945, **15**, 23.

Besides these, the S.V. has almost the same range; the B.R. readings for buffalo ghee are lower and indeed of a surprisingly small range. The value of the empirical relationship, $\frac{S.V.}{R.M. + P.V. + I.V.}$, is again constant for each row, and equal to 3·74.

Voriations in the Constants of Ghee

Many factors influence the constants of butter-fat, both before it is secreted and later in the various stages which it passes through before conversion into ghee. Milk is a physiological product of the milch animal: and any factor which tends to affect any one of the processes involved in its production will affect the milk and probably also the fat it contains. Among such causes for variation in the composition of ghee, may be mentioned the method of manufacture, the breed of the animal, the season of the year, the stage of lactation of the animal, the degree of its nourishment, the effect of hormones (native or foreign), the influence of disease, the feed of the animal and the state of freshness or rancidity of the sample of ghee examined. An attempt is made to study each of these effects separately, with two reservations: first, that the work has mostly been done in other countries, though there is no reason why the results should not hold good for Indian conditions as well, and second, that all the work has been done on cows, and it would not be safe to extend the conclusions to embrace buffalo ghee also.

Method of Preparation—These have already been described in Chapter X. The effect of the method of preparation on the constants of the resulting ghee is one of degree, and that too to a small extent. Rangappa, *et al.*[6] have found that cow ghee made directly from cream shows the same intensity of colour as that made by the curd process. But buffalo ghee, which is always much lighter in colour

[6] Rangappa, Srinivasan and Banerjee, *Indian J. Vet. Sc. and Anim. Husb.*, 1946, 16, 83.

than cow ghee, is twice as yellow when made by the curd process than when made direct from unsoured cream. It would appear that a part of the colour is water-soluble and is removed with the skim milk in the cream process requires further elucidation.

The acidity of ghee is affected to a slight degree by the method of preparation. Ghee prepared by the *desi* process from fresh butter usually has an acidity of about 0·2 per cent. expressed as lactic; if cream be used, and washed repeatedly before boiling into ghee, this figure may be brought down to 0·02 per cent. for the ghee, or about ten times less. In the indigenous method, which normally involves the practice of storing butter for a period, increasing proportions of acidity are transferred to ghee with storage. This is more pronounced in unprocessed—and inefficiently processed—milk butter, as illustrated in Fig. 9.

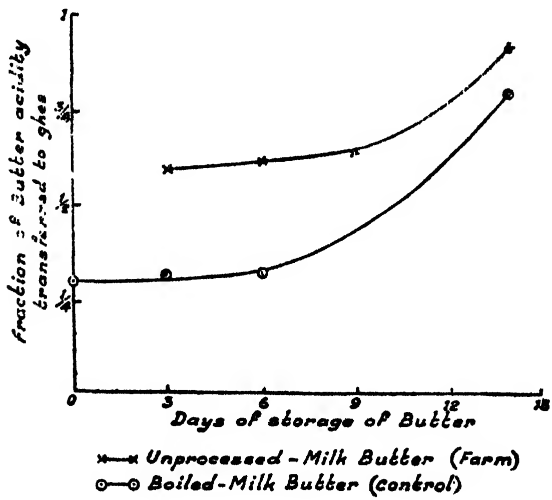

FIG. 9. Rise of the Ratio, $\frac{\text{acidity in ghee}}{\text{acidity in butter}}$, on storage of Butter

It is generally supposed that overheating during production lowers the Reichert-Meissl value of ghee, but no proof is obtainable for this: further, the fall, if any, is less than the latitude for R.M. determinations usually allowed, and the question becomes merely academic in character.

Breed—Doctor, *et al.*[7] have examined the ghee from the milk of four varieties of cows and one type of buffalo under identical conditions. They found that the iodine value for the four breeds of cow, Ayrshire, Cross-bred, Sindhi and Gir, were almost constant at 39, but that the R.M., P.V. and S.V. rose in the order of animals stated. The refractive index fell steadily in the order of the breeds. As proof that the variations were genuine breed variations, they point out that individuals among breeds showed very little difference in the analytical characteristics of ghee from their milk. The only buffalo ghee studied showed all the features associated with it: high R.M. and S.V., and low I.V., B.R. reading and P.V. Naturally, breed variations are of little significance when mixed milk is the starting-point of manufacture.

Similar variations are met with among English cows. The well-known idiosyncrasies of the cows of the Channel Islands, especially in excluding colouring matters to a smaller extent from their feed than do other types is a case in point.[8, 9]

Season—It would be misleading to say that the season of the year had any effect on the quality of the ghee produced; more correctly, the type of food and its amount, the quantity of water available and the humidity of the atmosphere all affect the cow and in turn the ghee. Since however each season is associated with a certain type of climate and food, it would be convenient to study the variations in ghee with respect to season, as distinct from factors accompanying it. Thus Das Gupta[10] found that if the summer and winter rations of cows were interchanged, all the changes associated with "season" occurred to the same degree.

[7] Doctor, Banerjee and Kothavalla, *Indian J. Vet. Sc. and Anim Husb.*, 1940, **10**, 63.

[8] Gillam and Kon, *J. Dairy Res.*, 1940, **11**, 266.

[9] Thompson, Kon and Mawson, *Proc. Biol. Soc.*, 1942, **36**, 17.

[10] *Indian J. Vet. Sc. and Anim. Husb.*, 1939, **9**, 249.

Plymen and Aiyer[11] have recorded such seasonal effects in two herds of cattle from Telenkheri area, one of cows and one of buffaloes. The R.M., P.V., S.V. and Refractive index figures were all low in the hot and dry summer months, while the Iodine values were high. The changes were less marked with the buffalo herd, where the figures for I.V. and Refractive index tended to be constant, even while the other three values went down. Similar seasonal fluctuations are reflected in the month-to-month figures of Katrak, Achaya and Banerjee,[12] though the differences were small as the samples were all from dairy herds in excellent condition and management. Das Gupta's values for a herd of cows in Bengal show a marked inverse relationship between the R.M. and I.V.—the sum being almost exactly 61·5 in every case. The ghee produced in winter is usually softer than summer ghee, the effect being less marked with the hardier buffalo.

Stage of Lactation—With progress of lactation, definite changes are observed in the composition of the milk-fat. It is naturally not easy to sort out these differences from those due to season and feed even under the best of conditions, especially in India where marked changes in the climate are obvious the year round.

Elsdon[13] quotes a fall of figures for the R.M. from 30·9 in the first month to 22·0 in the tenth, though this fall is not steady. Kuhlman and Gallup[14] state that the butter-fat became harder as lactation proceeded, and that its Iodine value increased—which is in line with the consistent fall of R.M. Channon, Golding and Drummond[15] attribute the fall in R.M. and rise in I.V. in their feeding studies to an advance of lactation in the animals and quote figures by Nielson for Swedish butter showing a fall from 33·4 for

[11] *Mem. Dept. Agric. India* (Chem. Ser.), 1921, 6, 155 and 187.

[12] Unpublished observations.

[13] "The Chemistry and Examination of Edible Oils and Fats," London, 1926, p. 386.

[14] *J. Dairy Sc.*, 1939, **22**, 424.

[15] *Analyst*, 1924, **49**, 311.

the first to 25·4 for the fourteenth month. Arup[16] noticed that advance of lactation increased the I.V. of butter-fat.

Dhingra and Ganesh Chandra[17] have observed the changes in the fatty-acid structure and analytical constants of buffalo ghee in early and late lactation. The following table gives the changes in fatty-acid composition.

TABLE LV

CHANGES IN FATTY-ACID COMPOSITION OF BUFFALO GHEE WITH LACTATION (PER CENT.)

Fatty acid	Early lactation	Late lactation
Butyric ..	2·61	2·08
Caproic ..	0·70	0·93
Caprylic ..	0·99	0·72
Capric ..	1·76	0·68
Lauric ..	8·04	4·27
Myristic ..	11·60	14·39
Palmitic ..	28·55	31·63
Stearic ..	9·14	5·45
Arachidic ..	4·31	2·02
Oleic ..	32·21	34·17
Linoleic ..	..	2·29
$C_{20}-C_{22}$ ..	..	2·23
Unsaponifiable matter ..	0·09	0·04

It also follows that the R.M. and P.V. are slightly higher and the I.V. lower in early than in late lactation.

The Effect of Hormones and Other Substances—The hormonal control of lactation from the point of view of increased milk production with or without successive breeding has attracted much attention of late. Investigators have mostly concerned themselves with the yield and the characteristics of the whole milk, while little attention has been paid to the fat.

Thyroxine has long been known to produce marked increases in the secretion of both milk and milk-fat, either when injected into the blood or when taken orally. Smith and

[16] *Analyst*, 1932, 57, 610.
[17] *Proc. Indian Sc. Cong.*, 1948, Sec. 4, p. 51.

Dastur[18] studied the composition of the milk-fat from four cows receiving thyroxine injections. They found that, temporarily, a slight disturbance occurred in the milk-fat—an increase in the R.M. value and corresponding decrease in the I.V. After this very slight variation, the fat returned to normal even when the milk-production was at its peak. The changes due to the hormone were therefore very slight and only temporary.

Besides hormones, dyes, drugs, stimulants, etc., have been injected or fed to cows and the fat studied. Since these are not practices usually followed, the results have little practical bearing from the analyst's point of view. Graf, Ludwick and Petersen[19] found that small doses of dinitrophenol caused a rise in R.M. and I.V., and a considerable fall in S.V. consistent perhaps with the rise in the other two values. It should be difficult to say if these changes could be permanently affected. Shaw[20] used oxytocin and observed variations in the I.V. of the fat. These facts are consistent with a temporary interference of the processes involved in milk-fat secretion, but little further can at present be said on the matter.

Disease—Any diseased condition could naturally be expected to influence the milk and milk-fat of a lactating cow, and such indeed is the case. One of the commonest of such affections is mastitis. Miller, Lease and Anderson[21] induced mastitis in one portion of a cow's udder and compared the milk and fat with that secreted by the healthy side. The R.M., I.V. and R.I. all showed a fall, while the Saponification value, as is to be expected, increased. The same fluctuations were noticed by Kon and Thompson[22] in naturally occurring mastitis also. Marked alterations in the fat due to disease in the milch animal cannot be expected unless there is (i) direct infection of the mammary gland and (ii) a considerably lowered metabolic rate as a whole.

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[18] *Biochem. J.*, 1940, **34**, 1093.
[19] *J. Dairy Sc.*, 1940, **23**, 539.
[20] *Ibid.*, 1942, **25**, 1051.
[21] *Ibid.*, 1940, **23**, 573.
[22] Quoted by Smith, *J. Dairy Res.*, 1943, **13**, 216.

Degree of Nourishment.—Eckles and Palmer[23] noticed that underfeeding lowers markedly the R.M. and S.V., and raises the I.V. Moreover they also stated that various factors influenced the degree to which these variations occurred—the degree of underfeeding and the type of rations allowed, and the physical state and flesh of the cow. The results do not appear to have been taken very seriously till

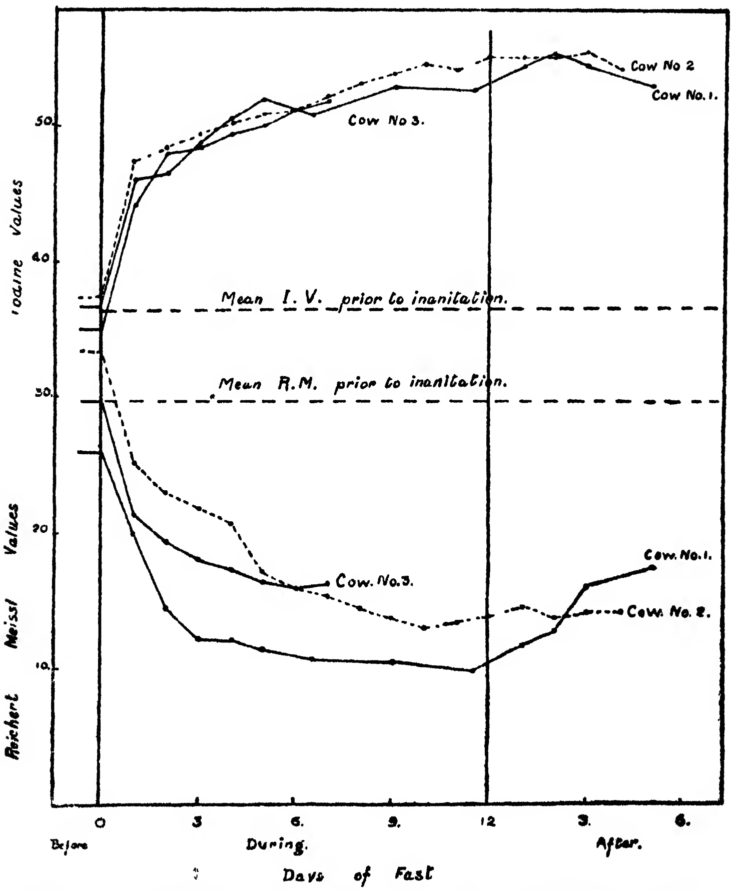

FIG. 10. The effect of inanition on the Iodine values and Reichert Meissl values of the milk-fat

[23] *Miss. Agric. Expt. Sta. Res. Bull.*, 1911, **25**, 107.

1938, when Smith and Dastur[4] studied the effect of total starvation on the milk-fat of cows, using the method of detailed ester-fractionation to study the fat.

The lower acids were markedly decreased in amount; at the same time the loss is almost wholly made good by a rise in the oleic acid content, while the proportions of other acids tend to remain constant. This fact, as the authors remark, is of considerable practical interest when it is realized that inanition is only an extreme form of malnutrition, and that considerable degrees of undernourishment exist in many countries. This is especially true in India where droughts and scarcity of fodder are very common. Fig. 10 illustrates the changes in R.M. and I.V. of milk fat during starvation of the animal.

Locality—Here again, as with the variations labelled seasonal, it is clear that variations from normal found in ghee from some specific locality may be attributed to breed variations or to the character of the rations, availability of water, etc. Since, however, it is usual to associate the place with the type of abnormal ghee produced, it would be convenient to class such variations as localized, and consider them as such without heed to the factors contributing to the abnormality.

As far as cow ghee goes, such variations have not received much attention, since the amount of cow ghee marketed is small. The cows of Assam, especially in the Shillong area, appear to produce ghee of uniformly low R.M. value, very high I.V. and abnormally high R.I. as well. In a study of twelve such samples over a period of one year, Katrak, Achaya and Banerjee (unpublished results) found a range of 17–26 for R.M. (only one sample giving this figure 26 and all the others yielding below 22), of 40–46 for I.V. and 45·3–47·7 for the B.R. reading, with the averages at 20·4, 42·6 and 47·0 respectively. Other such striking local variations have not come to the notice of the authors.

Striking variations are found among buffalo ghees. Especially noticeable is the case of ghee from the hot and arid Kathiawar area. Values of 14–20 are usual for the

R.M., 36 for the I.V. and 46 for the B.R. reading, with the P.V. at about 0·8 and the S.V. at 210. Intensive cottonseed feeding has been the practice for generations. Quite in contrast, samples from Lyallpur gave values for the R.M. well over 30, Iodine values of 30, and a high S.V. of 230 or so.

Feed—Of all the factors that go to influence the composition of the fat, that of feed is the most common and most intensively investigated. It is the common practice of producers to state that any abnormality of their ghee is due to feeding and to ward off in many cases the suspicions of the analyst. They have in most cases a large volume of experiments on feeding to back them up, and it was therefore thought necessary to examine the question from the purely objective and critical points of view. Accounts are given of work done on a detailed or a comprehensive scale to include many possibilities; the common oils or meals used for feeding in India are discussed next, as far as possible in order of importance. Finally, results are given of other meals of theoretical interest only, in India at any rate; and this is followed by other experimental feeding of perhaps only scientific interest. Most of the work in this direction has been done on cow milk-fat; and that similar information regarding the buffalo would be of immense interest to the industry and science.

(1) *General Results*—Hilditch and co-workers[24, 25, 26] made a series of tests on the effect of diet on the fat of cow milk. The experiments embraced a number of oils in common use, and the fat was in every case submitted to detailed fatty analysis. The results obtained were as follows: coconut oil caused an increase in lauric and myristic acids, but little other change, especially in the Polenske, C_8 and C_{10} acids which are commonly supposed to be affected. Soyabean cake and linseed oil caused little variation, even in the C_{18} unsaturated acids which they contain in some quantity.

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[24] Hilditch and Sleightholme, *Biochem. J.* 1930, **24**, 1098.
[25] Hilditch and Thompson, *Ibid.*, 1936, **30**, 677.
[26] Hilditch and Jasperson, *Ibid.*, 1943, **37**, 238.

Rape oil had no effect either beyond a slight increase in oleic acid content. Groundnut and hydrogenated groundnut oil (of I.V. 45) caused an increase of oleic acid and a decrease in the lower acids. When an almost completely hydrogenated fat (I.V. = 18) was used, there was little change in the fatty acid make-up, probably because most of it was unabsorbed at the temperature of the digestive tract. Cod-liver oil produced remarkable fluctuations: a 50 per cent. decrease in the lower acids and a corresponding increase in oleic acid, and a large increase in acids more unsaturated than oleic. The authors remark that it stands in a class by itself amongst the oils in the effects it has on milk-fat characteristics.

Anantakrishnan[27] and co-workers have repeated the above experiment with the indigenous oils and fats on the buffalo, and arrived at similar conclusions.

Brown, Dustman and Weakley[28] found that a period of about three weeks was necessary for peak variations in milk-fat, when coconut, soyabean and hydrogenated soyabean were used as supplement. Reduction of I.V. obtained was of the order of 5 units with coconut oil, but very large increases resulted with the soyabean oil. Exactly similar figures for coconut oil were given by Hill and Palmer,[29] who also gave the following results: increased I.V. with maize, cottonseed and linseed oils, the latter especially increasing the content of acids less saturated than oleic as indicated by the greater differences between I.V. and thiocyanogen value. Hansen and Steensberg[30] studied the effects of feed on the I.V. of butter and summarized their results briefly: I.V. over 35—feed consisted of soyabean oil, sesame oil, linseed oil and rape seed oil; I.V. 30–35—feeds giving these normal values were peanut, cottonseed and soya-bean cakes; low I.V.'s were obtained with coconut, corn, babassu and rye cake. The results are all in good general agreement.

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[27] Anantakrishnan, Bhale Rao and Paul, *Arch. Biochem.*, 1947, **13**, 389.
[28] *J. Dairy Sc.*, 1941, **24**, 265.
[29] *Ibid.*, 1938, **21**, 529.
[30] Cf. *American Chemical Abstracts*, 1932, **26**, 1674.

Doctor, Banerjee and Kothavalla[7] made similar studies in India, using an Indian breed of cow and keeping the animals for some time on the diet before collecting samples. The results obtained after three weeks of such feeding are given below; the rise is indicated by + and the fall by −, with respect to the controls.

TABLE LVI

Feed	R.M.	P.V.	I.V.	B.R.
Groundnut cake ..	−4·2	−0·2	+1·0	+2·7
Gingelly (sesame) cake ..	const.	−0·3	+4·0	+2·3
Linseed cake ..	−4·5	−0·3	+4·4	+2·6
Coconut cake ..	const.	+0·5	−9·5	−2·7
Cattonseed (whole) ..	−2·3	−0·2	+3·3	const.

It is interesting to see that falls in the R.M. are almost balanced by rises in the iodine value. It may also be noticed that in many cases the values first showed increases and then came back to normal, and in other cases the tendency to come back to normal was indicated (these are shown in the figures in the original paper). It is therefore probable that intensive feeding of specific oil seeds or cakes would so accustom the animal to them that no effects on the constants, either individually or in relation to each other, would be noticed.

Generally, it has been found that the character of the rations—whether it is composed of fat, or of carbohydrate, or of protein—has a certain definite action on the nature of the butter-fat. A diet low in fat causes a hardening of the butter-fat and a low iodine-value; with diets rich in fat the results are somewhat capricious, since various factors such as the availability of the fat, the protein level of the diet, the carbohydrate level, etc., all come into play. Usually, however, the fat tends to be a little soft and leaky and of high iodine value when oils are used.

A diet rich in protein usually results in ghee of high iodine value,[31] and a low protein diet in one of low iodine value.

[31] Brouwer and Frens, *American Chemical Abstracts*, 1938, 32, 3033.

Excessive feeding of green grass, a rich source of protein, gives a soft butter-fat of high iodine value,[32] and in fact the iodine value closely follows the amounts of green feed taken by an animal. The extreme unsaturation of grass fatty acids[33] must also however be considered in this connexion.

(2) *Coconut Oil or Cake*—This is a form of concentrate feeding very common in India since it represents great economy in food for the peasant to feed his cattle on waste coconut material. Much confusion has arisen regarding the effect of this feed, since the results of experiments vary considerably among themselves. Cranfield[34] observed a slight rise in R.M. and a considerable rise in the P.V. of butter-fat. Channon, Golding and Drummond[35] noticed a slight temporary rise in R.M. and S.V. and a corresponding temporary fall in I.V. and R.I. But Doctor *et al.*[8] found only a small fall in R.M., R.I. and P.V., and a marked fall in I.V. An interesting summary is given by Paraschtschuk[36] who states that if the cows are normal in condition, no rise in Polenske value (the value most disputed in such feeding) occurs; but if the cows are below normal in health or nourishment, as may well be the case in India, an increase is usual. This may explain the divergent results obtained by the other workers, and may perhaps be generalized to extend to all other oil feeding as well.

(3) *Cottonseed*—More information is available on this aspect of feeding than on any other; and this is quite in keeping with the widespread use of whole cottonseed as a concentrate the world over. It is commonly used in India as a buffalo feed as well, and more information on its effects with that animal would be very welcome information to the industry. Very recently Patel, Patel and Dave[37] have shown that intensive cottonseed feeding produced much greater

[32] Platon, Hermansson, Edin and Hansson, *American Chemical Abstracts*, 1935, **29**, 6965.
[33] Hilditch and Jasperson, *J. Soc. Chem. Ind.*, 1945, **64**, 109.
[34] *Analyst*, 1911, **36**, 445.
[35] *Ibid.*, 1924, **49**, 311.
[36] Cf. *American Chemical Abstracts*, 1926, **20**, 2212.
[37] *Indian J. Vet. Sc. and Anim. Husb.*, 1944, **14**, 97.

effects on buffalo ghee than on cow ghee. The R.M., P.V. and S.V. fell markedly while the B.R. was raised. In a fatty-acid analysis of such ghee, Achaya and Banerjee[38] showed that the lower acids were very small in amount, and that the stearic acid content shot up to a remarkably high figure—probably due to bio-hydrogenation of the ingested oleic or linoleic acid from cottonseed oil.

Eckles and Palmer[39] found that cottonseed wholemeal decreased the R.M. and S.V., while the I.V. and melting-point of the butter-fat were raised, the effects being most marked when the cottonseed was given with dried hay as supplement. Sanarens[40] noticed that such butter-fat gave a positive Halphen reaction equivalent to the addition of 2–4 per cent. of added cottonseed meal. Geisler[41] found that cottonseed meal produced a high R.M., I.V. and M.pt., and a lower P.V.; Neasham and Gelp[42] got almost exactly the opposite effects—lower R.M., I.V., S.V., and P.V. with whole cottonseed. Herzer[43] found that cottonseed meal decreased the R.M., but increased the I.V. and R.I., while cottonseed oil decreased the R.M. definitely, and slightly increased I.V. and R.I. The bulk of evidence therefore appears to indicate the following effects for regular cottonseed feedings; lowered R.M., S.V. and P.V. (slight) and slightly increased I.V. and R.I. The effects seem to be at best very slight; and this fact should not be forgotten when highly aberrant results come up for criticism, though regular and intensive cottonseed feeding coupled with arid conditions has been found by the authors to yield ghees of extremely low R.M. (14–20 for the buffalo and 18–23 for the cow) and S.V., and high I.V. and B. R.

(4) *Groundnut Oil*—This is commonly used in India, as well as in the West under the name of arachis or peanut oil.

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[38] To be published.
[39] *Miss. Agric. Exp. Sta. Bull.*, 1916, **27**.
[40] *Ann. Fals*, 1910, **4**, 72.
[41] *J. Oil and Fat Ind.*, 1926, **3**, 115.
[42] *American Chemical Abstracts*, 1934, **28**, 1420.
[43] *Miss. Agr. Exp. Sta. Rep.*, 1932, **45**, 23.

In a study in India, it was found[44] that this type of feeding produced greatly increased I.V. and a slight fall in R.I.; Doctor, *et al.*,[7] found that this increase in I.V. was only temporary, but that the R.M. fell by nearly 4 units though it showed signs of rising again. Increased I.V. were similarly obtained even with decorticated groundnut cake by Cranfield.[45] The need for caution is however emphasized by the results of Channon *et al.*,[17] who found that the two cows used behaved differently with the same feed—*e.g.*, with arachis oil, the I.V. in one case fell and in the other rose sharply. In general, it would perhaps be reasonable to expect a decrease in the iodine value and a rise in R.I. with little variation in the other constants.

(5) *Palm-kernel Oil*—On the South-West coast of India, the abundance of palm trees makes this a popular feed for cattle. Das Gupta[46] obtained an increased R.M. and P.V. with cows using red palm-oil, while Crowther and Woodhouse[47] obtained in addition a fall in I.V. and R.I. Elsdon's remarks,[48] that the experiments are too limited to be of value, would be in place for the first mentioned worker as well. A decrease in I.V. would be quite in keeping with the character of this feed.

(6) *Linseed, Soyabean, Sesame, Maize and Corn Oils*—In India, most of these oils, excepting perhaps sesame and linseed, are of little more than theoretical interest but have been included here for the sake of completeness. Linseed oil gives a fall in R.M., a great depression in P.V. and a marked rise in R.I.;[34, 7] it produces a greater quantity of acids less saturated than oleic as noticed by Hill and Palmer.[49] Soyabean cake or oil produces a high I.V. and thiocyanogen

[44] Dutta, Frincisco, Kamal, Mackijani and Soundararajan, *Agric. and Livestock in India*, 1937, 7, 503.

[45] *Analyst*, 1916, 41, 336.

[46] *Indian J. Vet. Sc. and Anim. Husb.*, 1939, 9, 299.

[47] *Analyst*, 1918, 43, 62.

[48] "Edible Fats and Oils," 1926, p. 401.

[49] *J. Dairy Sc.*, 1938, 21, 529.

value and a high m.pt. as well.[50] Sesame oil produces effects similar to groundnut oil, as Dutta, *et al.*[44] have proved: great rise in I.V. and a slight rise in R.I. Maize oil and corn oil, widely used in America, appear to cause marked diminution in R.M. and rise in I.V.[51]

(7) *Poppyseed and Babassu Oils* appear to act very differently. Annett and Sen[52] obtained no differences in composition of butter-fat with poppyseed cake; babassu oil[53] on the other hand caused a very marked rise in the P.V., a figure of 3·0–3·3 being obtained for an R.M. figure of 28·8, while the normal in Europe would be about 2·6.

(8) *Jowar, Beets and Turnips*—The first named has no untoward effect on the butter-fat as shown in an extended study by Plymen and Aiyer.[54] Beets and turnips have been much experimented with in Europe and England; excessive feeding of beet appears to lower very markedly the I·V.,[32] a result also confirmed by other workers.[5] Turnips tend to raise the Polenske value, and lower the Iodine value.[55, 56]

(9) *Fish Meals*—These appear to cause only small changes. Cod-fish meal gave a hard butter; lupine fish meal raised the oleic acid content,[57, 58]—results which apparently contradict each other. The quantity of residual and generally highly unsaturated oil in the meal would no doubt play its part (*cf.* page 160).

(10) *Other Feeds*—Lower fatty acids or salts did not raise the R.M. value of butter-fat when fed to a cow,[59] while a ration high in bases was apparently tolerated by the cow with no effects on the butter-fat.[60] Glucose even at high

[50] Williams, Cannon and Espe, *Ibid.*, 1939, **22**, 442.
[51] Sutton, Brown and Johnson, *Ibid.*, 1932, **15**, 209.
[52] *J. Agric. Sc.*, 1919, **4**, 416.
[53] Bengtsson, *Z. Nahr. Genussm.*, 1922, **44**, 336.
[54] *Mem. Dept, Agric. India* (Chem. Ser.), 1921, **6**, 155.
[55] Magi, *American Chemical Abstracts*, 1938, **32**, 253.
[56] Schmidt-Neilsen and Astad, cf. *Ibid.*, 1937, **31**, 2309.
[57] Dibbern and Eichstadt, cf. *Ibid.*, 1930, **24**, 4098.
[58] Morozck, Schlag and Eichstadt, cf. *Ibid.*, 1930, **24**, 5892.
[59] *Rep. Nat. Inst. Dairy.*, Reading, 1940, p. 19.
[60] Cf. *American Chemical Abstracts*, 1936, **30**, 1888.

levels has also no action on the composition of the butter-fat.[61] Rice bran is found to decrease the R.M. alone,[62] but to increase it when fed with linseed oil.[63] It would be unsafe to draw any inferences from the meagre information available.

(11) The *altitude* appears to have some effect on milk-fat secretion, since differing values for the analytical constants were obtained as a herd moved up an Alpine hill—a decrease in R.M. and increase in I.V. as the altitude rose,[64] but the results must be viewed with caution in view of the many factors that may be responsible for the change.

In conclusion, it must be emphasized that the changes in the composition of butter-fat are in most cases only temporarily affected by the composition of the feed, especially when the animals are in good condition, and that spectacular deviations can safely be excluded from the limit of feeding possibility and must always viewed with suspicion.

The Problem of Adulteration of Ghee—Ghee, while providing adequately the fat requirement of an individual with the additional advantages of palatability and attractive flavour, is a costly product; and the temptation to adulterate it with cheaper foreign fats is an obvious one. The risk involved is also small since detection is not easy when the similarity of the composition of ghee and the other fats, and the wide variations in genuine ghee itself, are considered. The Provincial and Central Governments have no doubt fixed certain standards of purity (see Appendix), but the wide range of constants of ghee allow of fairly copious adulteration even within those limits. Municipal or provincial analysts have no easy task in detecting these adulterated samples. Moreover, scientific adulteration is practised on a large scale, adding to the difficulties of the

[61] Knodt, *J. Dairy Sc.*, 1941, **24**, 401.
[62] *Monti, Lait*, 1935, **609**, 15.
[63] Iyer, quoted by Davies, "Chemistry of Milk," 1936, p. 68.
[64] Koestler, Kügi, Lehmann and Wegmüller, cf. *Dairy Sc. Abstracts*, 1944, **6**, 92.

makers and upholders of the law. The numerous causes for variation in the constants of ghee have been already reviewed and these add enormously to the difficulties of prevention and detection of adulteration.

So far, methods in common use are the determination of the Reichert-Meissl, Polenske, Kirschner values and the phytosteryl acetate test of Bōmer[65] which enables the detection of added vegetable oil by the fact that it contains a sterol different from that found in animal fats. The need for a " quick " test is urgent, but its realization is a doubtful possibility when the difficulties in its way are considered. Formerly, the " graininess " of a ghee was often used as a test of its quality, but with the coming of hydrogenated oil industry, grains of any size are made to order and this has eliminated most effectively this line of detection.

No attempt is made to indicate here the tests to be used for detection of adulteration since this is beyond the province of this book. The perusal of these chapters it is hoped will give some idea of the variations to be expected in the constants of ghee and the measures to be taken to circumvent them in the judging of purity of an unknown sample. There is still need for a large-scale analysis of genuine butter-fat on a provincial or regional basis, while from the legal standpoint laws based on the analytical relationships shown on pages 148 and 149 would appear to offer a more reasonable standard of purity than the maxima and minima type of standard now in force.[66]

[65] *Z. Nahr. Genussm.*, 1898, **2**, 81.
[66] Achaya and Banerjee (awaiting publication).

Chapter XIV

THE GRADING, RANCIDITY AND PRESERVATION OF GHEE

Ghee grading

Like the grading of butter, this is more a skilled art than a standardized science. The qualities looked for in a good ghee are granularity, colour and flavour.

Granularity—Melted butter crystallizes on cooling, forming visible granules which give a distinct sandy feel on the tongue. The crystals of cow ghee are slightly larger than those of buffalo ghee. When stored in a vessel the main mass of ghee consists of crystals with a thin liquid layer at the top.

Colour—Cow ghee is deep yellow, but buffalo ghee is much lighter. This property however cannot always be used as a distinguishing feature because of the fairly common practice of colouring ghee with turmeric powder.

Flavour—This is tested both by smell and taste. A small portion of the ghee is rubbed on the back of the hand before smelling. A light but pleasant cooked flavour is usually looked for, though this depends on individual preference. In some parts of the country, for instance, a slightly raw, buttery flavour is preferred. The taste of the ghee should be the characteristic flavour imparted by lightly browned casein; it should be quite free from butteriness or 'oiliness'.

Apart from these organoleptic tests, certain standards of chemical quality, like R.M., P.V., R.I. and acidity are prescribed by the Agricultural marketing board for grading genuine ghee (see Appendix).

The limit of acidity of 3·2 per cent. (as oleic) fixed for ghee calls for certain modifications. Rangappa, Srinivasan and Banerjee[1] have shown in their experiments on storage of ghee that a well-prepared ghee does not exceed the prescribed limit even when it goes rancid with time. After six months of storage, samples exhibited a slightly oily taste and possessed an acidity of 0·6 per cent. oleic acid; at the end of one year rancid samples with a bitter taste had an acidity of only 1·2 per cent. as oleic. On the other hand, fresh ghees prepared from stored, raw-milk, butter, and which had an acidity of 4 per cent. as oleic showed no signs of off-flavour. The present standard of high acidity fixed by the Board has therefore no warrant in either case. The workers mentioned above urge the institution of a lower limit of acidity, and the inclusion of some standard of keeping quality, like the induction period during oxygen absorption at elevated temperatures. These would cause a marked improvement in the Technology of Ghee Manufacture, and give an impetus in the right direction to the industry as a whole.

In this connection they have also shown that the acidity and age of a sample give a fairly accurate indication of the method employed in the preparation and preservation of the parent butter and of the ghee itself. As these have a direct bearing on the quality and storage property of the resultant product, the information will be of immense value to the trade. Their conclusions are summarised in the table below.

The Table shows clearly not only the importance of the correct technique of manufacture of ghee, but also of its preservation under fairly sterile conditions, without access to air. It also brings into prominence the factor of rancidity in ghee. The causes of development, the prevention and the elimination of rancidity are obviously of cardinal importance to the ghee trade and industry.

[1] *Indian J. Vet. Sc. and Anim. Husb.*, 1946, **16**, 83.

TABLE LVII

AGE AND ACIDITY OF GHEE AS AN INDICATION OF THE METHOD OF PREPARATION AND PRESERVATION OF THE SAMPLE

Age	Flavour	Acidity (per cent. oleic)	Method of preparation and preservation (at 20–30° C.) of butter and ghee
Fresh	Good	0·3	Fresh and boiled or raw-milk butter boiled into ghee
Fresh	Good	0·75	Boiled-milk butter stored for about 2 weeks, or raw-milk butter stored for 3 days, and boiled into ghee
Fresh	Fair or very faintly off	1·0–1·5	Raw-milk butter stored for 5–7 days and boiled into ghee
Fresh	Slightly off	3·0–3·5	Raw-milk butter stored for about 10 days and boiled into ghee
Fresh	Off	exceeding 10·0	Raw-milk butter stored for over 14 days and boiled into ghee
1 month	Good	0·08–0·15	Fresh boiled-milk butter, boiled into ghee and stored in non-porous vessel without air gap.
5 months	Fair	0·3–0·6	Same as above
1 month	Fair	0·6–1·0	Boiled-milk butter stored for about 2 weeks and boiled into ghee, stored with an air gap
3–5 months	Fair	0·3–0·4	Fresh boiled-milk butter, boiled into ghee and stored with an air gap
3 months	Fair	1·0–1·5	Boiled-milk butter stored for 2 weeks before boiling into ghee.
6 months	Fair	0·35–0·5	Fresh boiled-milk butter boiled into ghee.
1 month	Neutral or no flavour	0·15–0·3	Fresh boiled-milk butter, boiled into ghee, and stored in a clean, porous earthenware vessel with free access to air
1 month	Incipient off-flavour, or slight oiliness	0·3–0·75	Similar to above, but preserved in a contaminated, porous earthenware vessel

Rancidity in Ghee and its Effects on Trade and Industry—While ghee represents an excellent method of preservation of milk-fat, it is far from being an ideal method, and the deterioration which sets in so rapidly in a tropical climate like that of India is a serious problem alike to the vendor and the industry as a whole. Rancid ghee has little market value, and the question therefore of preventing the spoilage of ghee, either by ensuring a good product by care in the intervening stages of its preparation from milk, or by the use of suitable agents which prevent the onset of rancidity, cannot be too strongly overrated in an agricultural country where the conserving of every source of livelihood becomes imperative. Moreover, it has been shown that destruction of Vitamin A also runs parallel with the spoilage of fat, and both are accelerated by unhygienic conditions, traces of moisture, exposure to air and light, and the use of metallic vessels and porous earthenware for storage. The loss of income to the ghee industry by spoilage has been computed at 8 crores of rupees,[2] much of which could perhaps be avoided by a little care and foresight.

Rancidity—With most oils and fats, the term rancidity merely implies any undesirable flavour of the fat, different from the normal, brought about as a result of changes within the fat itself or in compounds associated with it. The off-flavours are judged by taste and smell. With milk-fat, several distinct types of such (organoleptic) changes can be perceived, and hence the terms used are different.

" Rancidity " is the term generally applied to those changes brought about by hydrolysis, and are very marked in butter; this contains the lower acids, which have strong odours, in some quantity. These are released even on slight hydrolysis. With ghee, this type of hydrolysis is not common, since the moisture-content is only a fraction of one per cent.

The term tallowiness is reserved for those changes brought about by oxidation, usually by the oxygen of the atmosphere, at least in the first instance. The site of oxidation is the double bond of the various unsaturated acids, chiefly oleic acid, present in ghee. It is easy to understand the extreme susceptibilty of butter-fat to go bad, since it contains in appreciable amounts both the lower fatty acids,

[2] Hare Duke, J. A., " Notes on the Vegetable Oil Industry in India," Allahabad, 1931, p. 18.

giving rise to rancidity, and oleic acid, giving rise to tallowiness. The extent to which these changes occur is naturally dependent largely upon the conditions of storage.

Fishiness is a term applied to the smell occurring in butter-fat of high moisture content, and was formerly believed to be due to hydrolytic action on lecithin and choline producing trimethylamine which has a marked fishy flavour. Evidence is steadily accumulating, however, to show that the compound responsible for the taint is at least not usually trimethylamine, and various compounds have been suggested. The earlier view was mainly based on the work of Supplee,[3] Cusick,[4] and Davies[5]; among the latter workers who differ from this view may be cited Mohr and Arbes[6] who failed to recover trimethylamine from strongly fishy butters. In any case, the phospholipid content of ghee, and its moisture content, are so small as to rule out almost completely the development of fishy odours in ghee; and in practice this is found to be true.

Another type of rancidity found in butter-fat, caused usually by the action of moulds, is termed ketonic rancidity. It is interesting to note that this type of action affects saturated acids as well, unlike ordinary oxidation. These act on the surface of butter or ghee, and grow at the expense of the lower fatty acids, converting them into methyl ketones with one carbon atom less. These ketones possess strong odours, and are characteristic of ketonic rancidity. That these are responsible for this type of rancidity has been shown by Starkle[7] by growing the moulds on pure triglycerides They possess very little lipase activity and therefore cannot liberate fatty acids in quantity from fat.

Chemical Changes during Spoilage of Butterfat—A variety of reactions occur in the spoilage of fats, leading to a large

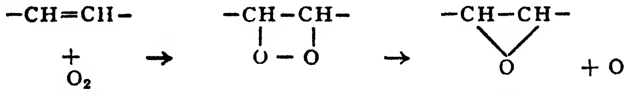
[3] *Cornell Univ. Expt. Sta. Memoirs*, 1919, No. 29.
[4] *Ibid.*, 1920, No. 30.
[5] *Proc. Ass. Econ. Biol.*, 1930, 17, 167.
[6] *Fette u. Seifen*, 1939, 46, 678.
[7] *Biochem. Z.*, 1924, 157, 371.

number of end-products, many of them odoriferous. While some of the main reactions have been understood, the possibility of innumerable side-reactions is so large that one can at best only indicate the nature of these. The changes following on the above types of rancidity are discussed below.

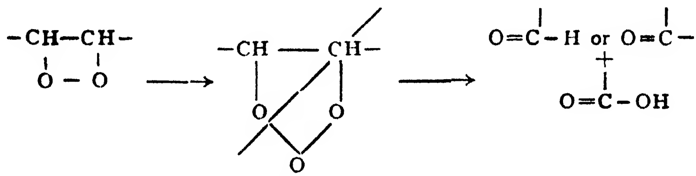
In hydrolytic rancidity, the main agents are either lipolytic enzymes or free fatty acids, especially in the presence of water. These result in the breaking-off of fatty acids from triglycerides, especially the lower acids and oleic acid. The former being strongly flavoured, characteristic off-odours are produced in the butter-fat. In the case of ghee, lipase action is non-existent since the product has been usually heated to a high temperature; concurrently, however, free fatty acids are present and these catalyse the production of further fatty acidity, especially in the presence of moisture.

Tallowiness has been stated to be due to the oxidation of oleic and other unsaturated acids by atmospheric oxygen. Butter-fat does not absorb oxygen immediately on coming into contact with it, but passes through a period during which little or no absorption occurs. This is called the "induction period", and varies with the quality of the fat, the temperature, the pressure of oxygen (air), the presence of anti- or pro-oxidants, and other factors. Experimentally, it is measured at elevated temperatures, using the variation of the pressure of oxygen as an index of its absorption. After the induction period, a rapid absorption occurs at a logarithmic rate in a way characteristic of an auto-catalysed reaction, as indeed it is. The period of induction is a valuable experimental index of the time during which a fat will keep whole and sweet.

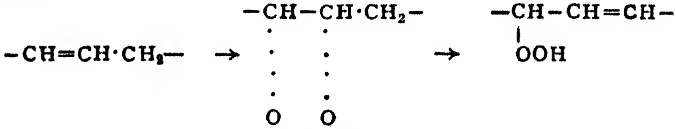
During the period of induction, it is possible that the small quantity of oxygen absorbed reacts, probably by double-decomposition, with the natural anti-oxidants, and creates a system not so resistant to the action of oxygen. This goes on till all the anti-oxidant is destroyed, when rapid oxidation of oleic acid occurs. It was earlier implicity believed[8, 9] that the double bond is attacked and that a peroxide is formed, which decomposes into an oxide and nascent oxygen as follows:



Tsirch and Barben[10] suggest the further formation of an ozonide, which then decomposes into an aldehyde, an acid or a ketone:



Morrell *et al.*[11] believe that the peroxides rearrange themselves to give ketohydroxylic derivatives of the type, $-CH_2 \cdot CH(OH) \cdot CO \cdot CH_2-$, which then either polymerise or give rise to aldehydic compounds, and in fact at high temperatures of 100° C. this has been experimentally demonstrated to occur. At ordinary temperatures, however, the mechanism of oxidation is now believed to be the formation of a hydroperoxide group, $-\underset{\displaystyle OOH}{\underset{|}{CH}} \cdot CH = CH-$. Farmer[12] thought it was the CH_2 group adjacent to the double bond that was attacked, the bond itself remaining unaffected, while Hilditch[13] proposed that the reaction took the following course:



[8] Holm and Greenbank, *Ind. Eng. Chem.*, 1923, **15**, 1051.
[9] *Ibid.*, 1924, **16**, 518.
[10] *Schweiz. Apoth. Ztg.*, 1924, **62**, 293.
[11] *J. Oil. Col. Chem. Ass.*, 1929, **12**, 83; 1936, **19**, 264, 359.
[12] *J. Chem. Soc.*, 1943, p. 119, 122, 541.
[13] *Ibid.*, 1944, p. 105; 1945, p. 836.

According to this scheme oxidation occurred actually at the double bond, with subsequent migration of a hydrogen atom or proton from an adjacent CH_2 group and creation of a new double bond.

Moreover the hydrogen atoms necessary to complete some of the above reactions may come from other fatty radicals, and create fresh centres of unsaturation that may again be oxidized. The intermediary compounds are all catalytic, and the reaction therefore proceeds at a logarithmic rate. It is of the greatest importance, therefore, to prevent the initial oxidation, whereby the subsequent trail of reactions are not allowed to occur.

Yet another type of reaction that may arise is by the reaction of hydrogen peroxide with the fatty acids, by β-oxidation, a principle familiar in fat metabolism in the animal body. This results in the final formation of ketones and carbon dioxide:[14]

$$R{\cdot}CH_2{\cdot}CH_2{\cdot}COOH \rightarrow R{\cdot}CH(OH){\cdot}CH{\cdot}_2COOH \rightarrow$$
$$R{\cdot}CO\ {\cdot}CH_2{\cdot}COOH \rightarrow R{\cdot}CO{\cdot}CH_3$$

Moreover, Raper[15] and Cahen and Hartley[16] have demonstrated the occurrence of α-, γ- and δ-oxidations, so that the compounds that may occur are immense. Glycerol may react with hydrogen peroxide to give aldehydes and hydroxyl acids.[17]

This is by far the commonest type of rancidity encountered in ghee, and hence the necessity of preventing access of air cannot be too strongly emphasized.

The ketonic rancidity due to moulds occurs by the β-oxidation mechanism indicated above. It is of fairly common occurrence in ghee, and a black or brown coating of the mould is often encountered on the surface of samples.

Fishiness in ghee is uncommon, though it may be carried over from the butter state if that has previously been allowed to develop it.

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[14] Dakin, *J. Biol. Chem.*, 1908, **4**, 419.
[15] *Biochem. J.*, 1914, **8**, 320.
[16] *Ibid.*, 1917, **11**, 161.
[17] Davies, *Ind. Chemist*, 1928, **4**, 269.

Polymerisation commonly results from storage of the more highly unsaturated oils, and may also occur in butterfat by some type of condensation of unsaturated molecules leading to compounds of high molecular weight (though low saponificaton equivalents) and medium unsaturation.

The products of decomposition of ghee have been theoretically enumerated above. In actual practice, Starkle[7] has isolated ketones from methyl-amyl to methyl-nonyl; and Mundinger[18] has precipitated ketones from a steam-distillate of rancid butter-fat. Godbole and Sadgopal[19] have found an increased content of hydroxy-acids and have suggested their determination by the acetyl value method as a test for rancidity. Rangappa and Banerjee[20] have shown that the free fatty acidity of high acid ghees consists largely (about 50 per cent.) of oleic acid, with the lowest acids, constituting the Reichert-Meissl value, comprising 20 per cent., and the Polenske acids making up about 3 per cent. Similar results have been recorded by Davies[21] for butter, and indeed it seems probable that in the other case the free acids were carried over from butter. It is to be expected that the products would vary depending upon the conditions of spoilage.

Aggarwal[22] found that on storage of ghee, the peroxide values increased for a period of 6–9 months, and then declined, probably because the rate of turn-over of peroxides was less than their rates of further decomposition.

Achaya and Hilditch[23] have made a study of the changes in the analytical characteristics of ghee on storage, and have also studied the fatty products of rancidity, using three lots of ghee of widely differing initial character. The changes, except for the refractive index which was erratic, were quite

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[18] *American Chemical Abstracts*, 1930, **24**, 4098.
[19] "Butterfat," Benares, 1939, 149.
[20] To be published.
[21] *J. Indian Chem. Soc. (Ind. and News Edn.)*, 1941, **4**, 175.
[22] *Ibid.*, 1942, **5**, 121.
[23] Partly published. *Nature*, 1947, **159**, 274.

well defined and parallel, resulting in a rise of R.M., P.V. and S.V., and a fall in I.V. and R.I. The free acidity paralleled closely the fall in iodine value, and pointed to a very largely oxidative mechanism of rancidification giving rise to free fatty acids, while lower fatty glycerides appeared to remain almost intact. The free fatty acids contained the saturated acids of normal ghee in roughly equal amounts (Ca, 0·5% mol. each), while the unsaturation (I.V.=20–30) was largely composed of apparent hexadecenoic acid and 15–20% (of the total) of a polymerised residue of S.E. 215 and I.V. 55. They also characterised nonanoic acid and a dibasic acid which was largely azelaic but contained traces both of pimelic and suberic acids.

Factors influencing the spoilage of Ghee

Various factors influence, either adversely or favourably, the spoilage of butter-fat. Pro-catalysing factors are initial bad quality, heat, light, acidity, enzymes and moulds, metals, oxygen, etc.; anti-catalysing factors are anti-oxidants (natural or artificial), nitrogen,[24] etc.; moisture has certain advantages and disadvantages depending upon the point of view from which it is regarded.

I. (*a*) *Initial bad quality*—It cannot be too strongly emphasized that the initial bad quality of a ghee consisting chiefly of high acidity, low Vitamin A, carotene and moisture, is by far the greatest pro-oxidant factor in its spoilage. Not only does it impair the induction period but certain forms of rancidity may be carried over into ghee from the butter stage, which will catalyse at an increasingly rapid rate its further spoilage. The causes of butter spoilage have already (p. 89) been discussed, and it is only necessary to stress here the importance of preventing it at an early stage in the preparation of ghee.

(*b*) *Heat*—The effect of heat is very marked: a rise of 10° C. in the temperature of storage has been found by

[24] Banerjee, *Agric. and Livestock in India*, 1938, 8, 153.

Overman and Wright[25] to double the reaction velocity of spoilage. Bevis[26] showed that up to 50° C., the catalytic effect of temperature was not great, but above 100° C. the deterioration was rapid, in fact more rapid than if it had been exposed to air. Ghee can be well stored at 0° C.

(*c*) *Light*—Though it is debatable whether decomposition does not occur in the dark, the marked effects of sunlight on oxidation of ghee has never been in doubt. The process is auto-catalytic, and once the reaction has commenced, removal of the source of light does not reduce the rate of decomposition to that of the unexposed fat.[27] Moreover, light alone can produce organoleptic rancidity even in the absence of air.[28] The shorter wave-lengths of light cause greatest deterioration. Below 540 $m\mu$ light is absorbed only to the extent of 5 per cent., but above this wave-length absorption is rapid and is almost complete for blue light.[29] Green light has almost the effect of total absence of light.[30] Direct sunlight is much more effective than diffuse daylight, though the catalytic activity is not directly proportional to the intensity of the light.[31]

(*d*) *Acidity*—Free acidity in ghee plays a small part in rancidification processes,[18] but is certainly a catalyser.[9] Rogers and co-workers found that butters from high acid creams were far inferior in keeping quality to those from fresh creams, and this was no doubt due to the acidity carried over from cream. Similar results have been obtained by the authors for butter and ghee, and all other conditions being equal, an increased free acidity results in a conspicuous lowering of the oxygen absorption value, which is a good measure of keeping quality.

[25] Lait, 1931, **11**, 564; cf. *Ind. Eng. Chem.*, 1935, **27**, 1287.
[26] *J. Soc. Chem. Ind.*, 1923, **42**, 417 T.
[27] Lea, *Proc. Roy. Soc., Lond.*, 1931, **108 B**, 175.
[28] Wagner, *Z. Natur. Genussm.*, 1913, **25**, 704.
[29] Grenbank and Holm, *Ind. Eng. Chem.* (*Ind. Edn.*), 1941, **33**, 1058.
[30] Coe and LeClerc, *Ibid.*, 1934, **26**, 245.
[31] Lea, C. H., "Rancidity in Edible Fats," 1930, 1029, p. 30.

(*e*) *Enzymes and Moulds*—As has been already mentioned, lipolytic enzymes are completely destroyed at the high temperature of 120° C. to which ghee is usually raised. Moreover, the acidity of curd inhibits them in large part even before the butter stage is reached. But this plays a great part in butter storage and if deterioration has occurred to any great extent, the resulting changes are carried over into the ghee.

Moulds like the Pencillium, Aspergillus, Cladosporium Spp. all grow on ghee on the surface at the expense of the lower free fatty acids, and since their lipolytic activities are small, have little scope for action in the absence of initial free acidity. The importance of preventing free fatty acidity, therefore, is again emphasized.

(*f*) *Metals*—Traces of heavy metals in ghee render it much more liable to decomposition. The reason for this has been stated by Ingle and Marking[32] to be due to the capacity of these finely divided metals to form oxides, and the greater the number of these oxides and the more their stability, the more effective is the metal as a procatalyst. Vanadium, Copper and Manganese are pro-catalysts.[33] Iron has a slight activity (about one-twelfth that of copper) in the ferric state,[17] while in the ferrous state it has no effect; tin and aluminium are non-active and much used for lining copper and iron equipment used in the dairy industry. Incidentally zinc has a stabilizing effect on the onset of deterioration.

(*g*) *Oxygen*—The availability of oxygen naturally has an inverse relationship with the progress of spoilage. Godbole and Sadgopal[34] found that in the presence of moisture and light, the acid value of a sample of buffalo ghee was 9·3 compared to the value of 15·1, when air, moisture and diffused light were allowed play. Clearly the presence of oxygen, on which depend the series of changes called tallowi-

[32] *J. Soc. Chem. Ind.*, 1917, **36**, 317.
[33] Briggs, *J. Dairy Res.*, 1931, **3**, 61.
[34] "Butter-fat," Benares, 1939, 125.

ness, catalyses spoilage, even if it is not absolutely essential for it.

(*h*) *Carbon dioxide*—Holm, Wright and Greenbank[35] showed that this gas can be regarded as a pro-catalyst, since it creates on acid condition which favours the spoilage of fat.

II. *Anti-oxidant factors*

(1) *Anti-oxidants, natural and artificial*—In 1927, Moureu and Dufraisse[36] showed that natural fats contain small quantities of non-fatty compounds, which could be concentrated in the unsaponifiable matter, and which lengthen the induction period for a considerable time.

A few compounds associated with the fat are also anti-oxidants. These are carotene, Vitamin A, lecithin, etc.

To-day, the fortification of natural fats with anti-oxidants, either isolated from natural sources or otherwise, is an easy and profitable means of preventing the onset of rancidity. The quantities of such compounds required are extremely small, and it is far easier to use pure organic compounds than to concentrate and utilize natural anti-oxidants.

For fats in general, poly-phenols, *i.e.*, organic benzenoid compounds containing more than one hydroxyl group, have been eminently successful. Lea[37] found that 0·005 per cent. of ethyl gallate was highly efficient as an anti-oxidant. Moreover, it retarded the loss of carotene when used with butter-fat. Sometimes a synergistic effect is observed. While oleic acid itself is a fairly good anti-oxidant, in combination with a polyphenol like hydroquinone it has an activity far surpassing that of each taken individually.[38] The same workers recommend the use of the indigenous Kamala dye as an anti-oxidant which has the advantage (not shared by ethyl gallate) of acting synergistically with the free fatty acids of ghee. Rangappa and

[35] *J. Dairy Sc.*, 1927, **10**, 33.
[36] *Chem. Rev.*, 1927, **3**, 113.
[37] *J. Soc. Chem. Ind.*, 1944, **63**, 107.
[38] *Indian J. Vet. Sc. and Anim. Husb.*, 1940, **10**, 361.

Banerjee (unpublished) have recently shown that for market (high acid) samples of ghee, the concentration of anti-oxidant required for protection is much higher (0·05–0·1%) than for fresh samples.

The Role of moisture in Ghee Spoilage

Holm and Greenbank,[39] and French, Olcott and Matill[40] showed that where moisture was present, the end-products of spoilage were fatty acids with little odour, while in its absence, odoriferous aldehydes and ketones were formed. From the point of view of flavour, therefore, moisture acts as an anti-catalyst, but from the point of view of hydrolytic rancidity, it favours spoilage, so that moisture might, from the absolute point of view, be regarded as a pro-catalyst. Of course, it is only in ghee with a high percentage of moisture that the above condition arises.

Preservation of Ghee

From what has been said, the importance of protecting ghee from the bane of spoilage and loss is obvious. Various methods, preventive and curative, can be adopted, though the old adage regarding prevention comes naturally into mind. The initial good quality of the product, which is the result of sterilized milk, controlled souring, effective washing of butter and its immediate clarification into ghee at a low temperature cannot be too strongly emphasized. Storage in a tinned vessel without an air-gap, and maintenance at as low a temperature as possible is conducive to perfect preservation of ghee. The use of earthenware vessels has been shown by Rangappa, Srinivasan and Banerjee[1] lead to quick deterioration. Storage in small lots rather than in bulk, and the use of anti-oxidants like ethyl gallate in suitable concentrations may be recommended.

Should the ghee be initially rancid, it may be refined by neutralization. While the refining of vegetable oils is now

[39] *Ind. Eng. Chem.*, 1924, 16, 598.
[40] *Ibid.*, 1935, 27, 724.

firmly established, the renovation of rancid ghees is yet to be attempted on a large scale in India. Rangappa and Banerjee[41] have standardized a procedure for the elimination of rancidity from ghee. It consists of careful neutralization, by instalments, of the free acidity, using sodium hydroxide of a calculated strength and quantity. The soap is thoroughly washed off by repeated additions of boiling water. They have noted that the resulting ghee is entirely free from any off-flavour, although it does not regain the characteristic flavour of fresh ghee. The R.M. and S.V. of the renovated ghee are slightly lower than those of the original, but the keeping quality is very small. If such ghee is fortified with anti-oxidants, it can be successfully marketed as low grade ghee, or utilized for blending with higher grade ghees. The consequent gain to the industry by the adoption of a successful method of renovating rancid ghee is at once obvious.

[41] *Indian J. Vet. Sc. and Anim. Husb.*, 1946, **16**, 98.

APPENDIX

A. Legal Standards for Fresh Milk under the Food Adulteration Acts[1]

Province or State	Minimum %age of milk-fat	Minimum %age of S.N.F.	Minimum %age of lactose	Sp. Gr. at 15° C.
Cow milk—				
North-West Frontier Province and Ajmer-Merwara	3·5	8·0	..	..
The Punjab, United Provinces, Bihar, Bengal, Assam Bombay Province, Sind, Baroda City and Hyderabad (Dn.)	3·5	8·5	4·0 (U.P.) 4·4 (Bengal and Sind)	1·028–1·030 in U.P. and Bengal
Central Provinces, Madras Province,* Mysore State* and Cochin State*	3·0	8·5	..	..
Buffalo milk—				
Madras Province,* Mysore State,* Cochin State*	4·5	9·0	..	..
North-West Frontier Province, the Punjab, United Provinces, Ajmer-Merwara and Delhi Province	5·0	9·0	4·0 (U.P.)	1·028–1·030 in U.P.
Central Provinces ..	5·0	8·5	..	..
Bihar, Bengal, Assam, Bombay Province, Sind, Hyderabad (Dn.), and Baroda City	6·0	9·0	4·4 (Bengal and Sind)	1·028–1·030 in Bengal. Not less than 1·028 in Assam
Mixed milk—				
North-West Frontier Province, Delhi Province, Ajmer-Merwara and Central Provinces	3·5	..	4·0	..
The Punjab ..	3·5	8·6–9·0	..	..
Bihar ..	5·0	9·0	4·0	..
Skimmed milk—				
United Provinces, Sind and Bombay Province	..	8·7	..	..
Central Provinces ..	..	8·5	..	..

[1] *Rep. Marketing of Milk in India and Burma*, 1943, Manager of Publications, Delhi.

* In Madras Province, Cochin State and Mysore State, cow milk should have a minimum of 0·5% and buffalo milk 0·53% of nitrogen. Both the milks should not have more than 5 parts of sediment per 10,000 parts of milk on 24 hours standing.

B. Legal Standards for Ghee

I. Standards for Cow Ghee

Authority (Govt.)	Moisture (maximum %)	On butter-fat B.R. at 40° C.	S.V.	R.M.	Remarks
1 North-West Frontier Province	..	40·0–42·5	..	24–32	Less than 2·8% free fatty acids as oleic
2 The Punjab ..	..	,,	..	Not less than 24	,,
3 United Provinces	1·0	48·0–51·0 (at 25° C.)	Not less than 220	,,	..
4 Bihar ..	..	40·0–43·0	..	,,	..
5 Bengal ..	..	40·0–42·5	Not less than 220	,,	..
6 Assam ..	..	,,	,,	,,	..
7 Central Provinces	..	40·0–46·0	..	19–36	..
8 Madras ..	1·0	..	..	..	..
9 Bombay ..	,,	40·0–44·5	..	Not less than 24	..
10 Sind* ..	,,	40·0–42·5	Not less than 220	,,	..
11 Central Govt. (Agmark)	0·5	,,	222–226	26–28	Polenske V. 1·5–2·5 Kirschner V. 20–25 Less than 1·5% free fatty acid as oleic

* The Sind Government prescribes Polenske values not higher than those corresponding to the Reichert values in the following table :

Reichert	30	29	28	27	26	25	24
Polenske	3·5	3·4	3·2	2·9	2·5	2·3	2·2

II. Standards for Buffalo Ghee

Authority (Govt.)	Moisture (maximum %)	On butter-fat: B.R. at 40° C.	S.V.	R.M.	Remarks
1 United Provinces	1·0	48–51 (at 25° C.)	Not less than 220	Not less than 30	..
2 Bihar ..	..	40·0–43·0	,,	,,	..
3 Bengal ..	..	40·0–42·5	Not less than 222	,,	..
4 Assam ..	..	,,	Not less than 224	,,	..
5 Sind ..	..	,,	Not less than 222	,,	Pol. Vals. not more than those shown against R. Vals. for cow ghee
6 Central Govt. (Agmark)	0·5	,,	226–234	,,	Pol. Val. from 1·0 to 1·75 Kirs. Val. nol less than 25 Less than 1·5%

III. Standards for Mixed Ghee

Authority (Govt.)	Moisture (maximum %)	On butter-fat: B.R. at 40° C.	S.V.	R.M.	Remarks
1 United Provinces	1·0	48·0–51·0 (at 25° C.)	Not less than 220	Not less than 28	..
2 Bihar	..	40·0–43·0	..	,,	..
3 Bengal	..	40·0–42·5	Not less than 220	,,	..
4 Assam	..	,,	Not less than 224	,	..
5 Sind	1·0	,,	Not less than 220	,,	Pol. Vals. as for pure cow and buffalo ghee standards
6 Central Govt. (Agmark)	0·5	,,	222–234	,,	Pol. Vals. 1·0–2–0 Less than 2·5% f.f.a. as oleic

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