SOME CHEMICAL PHYSICAL AND BIOLOGICAL STUDIES ON GHEE

by

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STUDIES ON GHEE

Merat

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ABSTRACT

Ghee is an animal fat product consumed widely in many countries of Asia and Europe. Although it is consumed in appreciable quantities little information is available concerning its chemical and physical properties and its keeping quality. Some of these characteristics may change depending on the methods of preparation. This study was carried out to investigate some of these properties and the effect of different methods of preparation on its quality.

Ghee was prepared by two different methods namely, from yoghurt and from cream. Some of the chemical and physical properties of milk, yoghurt, butter and ghee samples were determined and differences were evaluated. Biacetyl and total acidity values were found to be higher in the butter and ghee obtained from yoghurt than in the samples prepared from cream. Long chain fatty acids in milk, yoghurt, butter, and ghee samples were determined by gas-liquid chromatography. The results suggested that the amount of unsaturated fatty acids were lower in ghee than in milk. Ghee from yoghurt had a higher melting point and wider range compared to the ghee from cream. There was also a difference in the color of butter and ghee samples made by different methods.

Keeping quality was evaluated by TBA test on samples of ghee kept at room temperature with added water at two different levels (5 and 20 per cent). The results indicated that ghee from yoghurt was always more susceptible to rancidity.

As an animal fat, ghee is known to be a highly saturated fat; it seemed important then to study the rate of absorption and the affect of ghee on serum cholesterol level in rats. The results indicated that, in general, ghee was absorbed at a slightly higher rate than corn oil. Also it appeared that ghee in the diet at a level of 20 per cent increased the serum cholesterol level in the rat.

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INTRODUCTION

Ghee is a highly palatable dairy product prepared by heating and clarifying of butter. Its distinctive flavor and good keeping quality make it a valuable food ingredient which is appreciated in many countries of Europe, Asia and particularly in the Middle East.

The preparation of ghee from milk has been known for centuries and different names have been given to it. In India and Europe it is called Ghee, in the United States of America it is referred to as Butter oil, and in the Arab world it is called Semna.

There are considerable variations in the methods used for preparing ghee. Thus differences in flavor, aroma and keeping quality of the finished product are common features . Although a large number of less expensive shortenings are available on the market today, consumers, particularly in the Middle East tend to prefer ghee to any other fat for shortening. It is equally preferred to other edible fats and oils in cookery and some confectionary products.

Although ghee is a widely used product little information is available concerning its physical and chemical properties. The literature on the keeping

quality of ghee is also limited. Therefore, an attempt was made in this study to make further investigation on some chemical and physical properties of ghee, particularly when it is prepared by different methods. Also an attempt was made to study the effect of moisture content on the keeping quality of ghee.

Since it is a common belief that using ghee in cooking gives delayed digestion, its rate of absorption was compared with an unsaturated fat in rats. Ghee being a highly saturated fat product, it seemed also important to study the effect of feeding ghee on serum cholesterol level of rats.

LITERATURE REVIEW

Methods of Preparation of Ghee

The term Ghee and Butterfat are used by some authors interchangably. According to Triebold and Aurand (33), butterfat is prepared by melting butter at a temperature below 60°C, and after separation of the curd from the fat, it is filtered through a water-jacketed funnel heated to 70°C. According to Jacobs (14), butterfat is made by clarifying butter through melting and centrifuging. The term Ghee is usually used when the butterfat is obtained from Yoghurt.

Principally there are two methods by which ghee is prepared (8):

1. The Yoghurt Method
2. The Cream Method.

1. The Yoghurt Method

This is the old method used in villages for making ghee. In this method milk is brought to boiling (8), then allowed to cool to about 24°C. The starter is then added, which is usually some Yoghurt, at the rate of two per cent and mixed thoroughly. The mixture is then incubated for at least four hours at a temperature of about 24°C. The resulting product (Yoghurt) is then

churned in a skin or a churn to separate the fat globules as butter. Butter is then heated to boiling to evaporate the water content. In this heating process curd is separated by heat denaturation from the fat and forms a foamy white layer on top of the heated mass and some precipitate inside the container. In some countries, such as Lebanon, Burghul (par-boiled wheat) is added to help removal of water by absorption. Then the foam is removed and the rest is passed through a thin cloth for further removal of curd.

2. The Cream Method

This is a new method for making ghee according to which cream is used instead of yoghurt; otherwise the rest of the procedure is the same as in the yoghurt method. Cream is sometimes heated to boiling for one to two hours, depending on the thickness of the cream, to evaporate the moisture content and then it is clarified. (8).

The recovery of butterfat from milk is of great importance to ghee producers. The churning of yoghurt is very wasteful and there is a considerable loss of fat in this process. According to Gill (8) the cream method has the advantage of giving 30 to 35 per cent more recovery of ghee as compared to the yoghurt method; also less time is required in the cream method.

Organoleptic Characteristics

Mallette *et al.* (18) stated that the unique flavor of butterfat is attributed in part, to the presence of biacetyl which is produced during the ripening of the cream. According to the Wilster's review (36), Wright mentioned that ghee was judged by its appearance, texture, taste, and aroma. The product should be light yellow, have a fine granular texture, be neither greasy nor waxy, taste slightly acid, having about twice the acidity of butter. Davies in the same reference (36) reported a difference of opinion as to the ideal flavor for ghee, but common agreement is that ghee must have some strength of flavor and not be bland like pure butterfat.

Physical and Chemical Properties

Bailey (2) stated that butterfat[1] was distinguished from other fats and oils, except from those of the lauric acid group, by a low average molecular weight of its fatty acids manifested in a high saponification value and a low refractive index. And it was also distinguished from oils of the lauric acid group, such as coconut oil, by its high content of steam volatile acids as indicated by a high Reichert-Meissl value. He further stated that investigations on the physical and chemical constants of ghee from different sources revealed

1. The terms ghee and butterfat are used interchangably throughout the text.

a wide range of variations. According to the Deuel and Hallman's review (7) the lower and upper limits for Reichert-Meissl and Polenske values in cow ghee are reported by Katrak and Mehta to be 17.0 and 37.0 for Reichert-Meissl, and 0.5 and 3.7 for Polenske value. A report by Doctor et al. in Wilster (36), indicated that different methods of preparing ghee had no appreciable effect on the Reichert-Meissl value and other constants of ghee. The distinctive chemical and physical constants of ghee, in comparison with other fats and oils, are summarized in Table 1.

Lalitha and Noshir (16), reported that the color development in butterfat was influenced by micro-organisms. However, they found that among the common micro-organisms occurring in milk none showed any difference in influencing the color development. The color developed was not a carotenoid in nature. They also stated that the color development did not occur in aseptically collected milk samples unless a starter was added. It was postulated, therefore, that the developing color was formed in milk by micro-organisms acting on some unidentified constituent of milk and was absorbed by butterfat present.

Ghee is also distinguished by a large variety of component fatty acids (from C_4 to C_{22}). Concentrations of these acids depend on the fatty acid composition of milk, which varies with breed, age, feed of the animal, season

Table 1. Distinctive characteristics of butterfat in comparison with other fats and oils. (2).

Analysis	Butterfat	Coconut oil	Palm Kernel oil	Ordinary fats and oils
Saponification value	210 - 240	245 - 260	240 - 250	Below 200
Refractive index	Ca 1.4465	Ca 1.4410	Ca 1.4430	Over 1.4465
Reichert-Meissl value	22 - 34	6 - 8	5 - 7	<1
Polenske value	2 - 4	14 - 18	10 - 12	<1
Kirschner value	20 - 26	1 - 2	0.5 - 1	<0.5

of the year, and time of milking (2). The fatty acid composition of butterfats of different iodine values found by different workers are shown in Table 2. The major component acids observed were palmitic, stearic, and oleic.

Quantitative Determination of Fatty Acids by Gas-Liquid Chromatography

One of the most important applications of gas-liquid chromatography in milk lipid research concerns the quantitative determinations of fatty acid composition. James and Martin (15) were the first to report the use of gas chromatography in the separation of fatty acids. They separated methyl esters of both short and long chain fatty acids by two different columns. For the long chain fatty acids they used a column, containing Apiezon-L as the stationary phase and Celite 545 as the support, operated at 197°C. Patton et al. (24) investigated the composition of milk fatty acids by this method, using the same column for long chain fatty acids. Reference compounds were used to establish the position of various methyl esters on the chromatograms. Orr and Callen (23) reported that it was not possible in any case to resolve methyl linoleate and methyl linolenate on Apiezon-L column.

Table 2. Fatty acid composition (per cent by weight) of butterfats of different iodine values (2).

Fatty acid composition of butterfat	Iodine values of butterfat		
	32.9[+]	37.5[++]	42.9[++]
Butyric	3.5	3.6	3.7
Caproic	1.4	2.0	1.7
Caprylic	1.7	0.5	1.0
Capric	2.6	2.3	1.9
Lauric	4.5	2.5	2.8
Myristic	14.6	11.1	8.1
Palmitic	30.2	29.0	25.9
Stearic	10.5	9.2	11.2
Above C_{18}	1.6	2.4	1.2
Total saturated	70.6	62.6	57.5
Decenoic	0.3	0.1	0.1
Dodecenoic	0.2	0.1	0.2
Tetradecenoic	1.5	0.9	0.6
Hexadecenoic	5.7	4.6	3.4
Octadecenoic (oleic, etc.)	18.7	26.7	32.8
Octadecadienoic	2.1	3.6	3.7
C_{20} and C_{22} unsaturated	0.9	1.4	1.7
Total unsaturated	29.4	37.4	42.5

[+] Reported by Jack and co-workers.

[++] Reported by Hilditch and Jasperson.

Rancidity and Autoxidation

Rancidity is a major cause of fat deterioration which results in development of off flavor and odor. There are different types of rancidity; the commonest type occurring in fats is oxidative rancidity (12), which originates from atmospheric oxidation by addition of molecular oxygen to the unsaturated bonds of fatty acids. Rate of oxidation is increased by light, moisture, and heat (5, 12).

A widely accepted mechanism of autoxidation (26, 10) of fats is shown in Figure 1.

Another type of rancidity is hydrolytic rancidity (5) brought about by the activities of enzymes. In the case of dairy products butyric acid and other low molecular weight fatty acids are released from the triglycerides by hydrolysis which usually results from the action of lipase. The odor of such acids contribute largely to the smell of rancid butter.

Antioxidants presumably act to disrupt the mechanism of oxidation in the following manner (5):

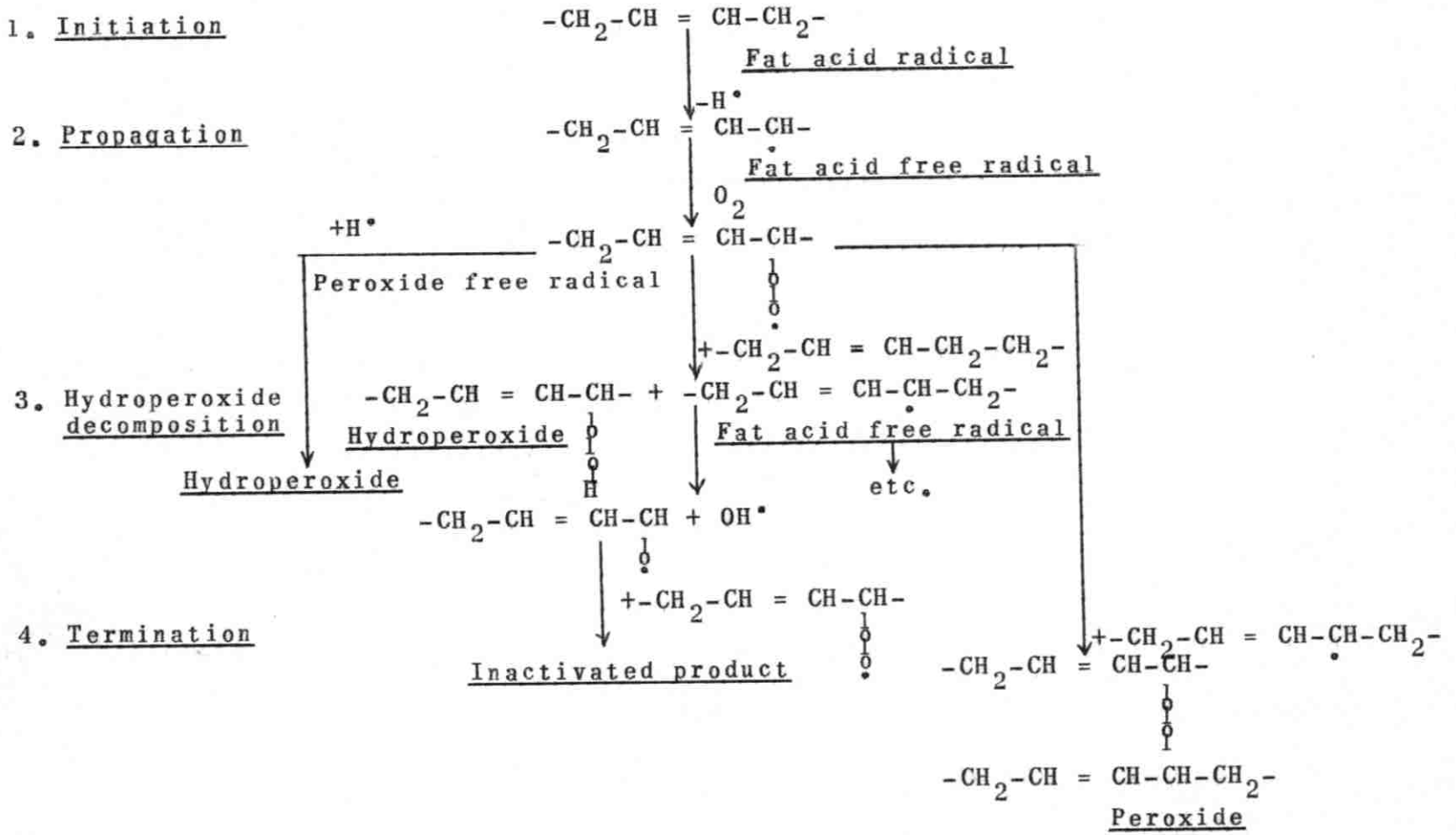
The oxidant A unites with O_2 to form a peroxide, which simultaneously reacts with antioxidant B;

$$A + O_2 \longrightarrow AO_2$$

$$B + AO_2 \longrightarrow AO + BO$$

Assuming these two oxides are mutually antagonistic, they will react with each other to regenerate the three original

Figure 1. Proposed mechanism for autoxidation of fats (10, 26).



molecules;

$$AO + BO \longrightarrow A + B + O_2$$

Tocopherols, carotenoid pigments, and vitamin C are the most important natural antioxidants occurring in butterfat (12).

Keeping Quality

Persai and Barnicoat in Wilster (36) stated that some of the main factors influencing the keeping properties of ghee were moisture content, acidity, heat treatment, and type of bacterial culture used in souring milk (when ghee is prepared from yoghurt). Of these factors, heat treatment was reported to play the major role. They came to the conclusion that, in general, the higher the final temperature reached during heating the butter (110°C or higher) the better the keeping properties of the product. Certain flavoring ingredients, such as Curry leaf (Murraya) and Moringa leaf, which in some countries are added to ghee, were found to improve the keeping quality of the product to some extent (30). Cow ghee was also found to absorb oxygen less rapidly than buffalo ghee (34), possibly due to the presence of the antioxidant carotenoid pigments in the cow ghee.

Intestinal Absorption

The intestinal absorption of dietary fat consumed

in physiologically moderate amounts is almost complete (37). However, when the same quantities of hydrogenated cottonseed oil were given to rats of different weights, considerably greater amounts of oil were absorbed by the larger rats (7). Also the rate at which fats are absorbed depends on their chemical composition. Absorbability of natural oils and fats, of plant and animal origin, have been investigated by many workers (7, 19, 31, 32).

A satisfactory way to determine the fat absorption quantitatively, is to measure the rate of disappearance of the fat under consideration from the gastrointestinal tract. This could be done by measuring directly the amount of fat remaining in the gastrointestinal tract (6, 13). According to this method, the animals are fed the fat after a period of fasting. Deuel (6) and Thomasson (32) force fed the animals without anesthetizing them. Irwin (13), however, concluded that light anesthesia was much less disturbing to the animal than the excitment caused by forced feeding. He found that prolonged etherization will have an effect on absorption, but a period of one to five minutes had no significant effect.

Thomasson (32) reported that the rate of absorption of butterfat in the rat was faster than that of corn oil. Deuel et al. (7) observed no difference in the rate of

absorption of cottonseed oil, butterfat, and coconut oil in rats. They further reported that the rates of absorption of cottonseed oil, butterfat, coconut oil, and hydrogenated cottonseed oil were between 40 to 50 mg/100 cm^2/hour.

Effect of Saturated and Unsaturated Fats on Serum Cholesterol Level

It has been well established that saturated and unsaturated fats in the diet can influence serum cholesterol level, both in man and in animals. Hegsted et al. (11) concluded that highly unsaturated oils cause lower serum cholesterol levels in rats. Rademeyer and Booyens (25) found that addition of butterfat to the diet of rats caused hypercholesterolemia, whereas the addition of corn oil caused hypocholesterolemia. However, Olson et al. (22) reported that butterfat, lard, and corn oil fed at 40 per cent level for three weeks had no effect on serum cholesterol in rats. Another report by Olson (21) indicates that the type of dietary fat was apparently of low effect on serum cholesterol level.

MATERIALS AND METHODS

Preparation of the Samples

A batch of 152 kilograms of cow's milk was obtained from the Agricultural Research and Education Center of the American University of Beirut at the end of January 1965. In July of the same year another batch of 160 kilograms was taken from the same source. Each of the two samples was divided into two equal parts; one part was heated in a water bath to 38°C and the cream was separated by means of a cream separator. The other portion was made into yoghurt by boiling the milk and cooling it to around 24°C; starter was then added at the rate of one per cent and incubated at about the same temperature for at least five hours. Both cream and yoghurt, prepared as mentioned above, were churned in an electric wooden churn of 12 gallon capacity. After the separation of fat globules as butter was obtained, the buttermilk (remaining liquid from yoghurt after churning will also be called buttermilk) was drained off and the butter washed with cold water three times, each time with five minutes of churning. A portion of each batch of butter was kept for analysis and the rest was heated to evaporate the moisture. To remove the curd and obtain pure ghee, the melted mixture was filtered through a

thin cloth. The final temperature in the heating of butter was 115°C in all cases.

Figures 2 and 3 show the specifications for different steps of preparation of the butter and ghee from the first and the second samples respectively.

To minimize chemical and physical changes, both butter and ghee samples were kept in a freezer at -18°C for later analysis. For shelf life studies 100 gram samples of each of the two lots of ghee from the first preparation were kept in 500 milliliters white glass jars on an open shelf at room temperature. To study the effect of moisture on keeping quality, moisture levels of different lots of ghee were raised to 5 and 20 per cent. This was done by mixing the melted ghee with the calculated amount of water. Other samples (100 grams each) were stored as mentioned above, except that jars were wrapped in aluminium foils. Samples of butter were kept under similar conditions.

Composition of Milk and Yoghurt

Samples of milk and yoghurt were analyzed for fat content, using the Babcock method (14). Total nitrogen was determined by a modified Kjeldahl method (1); protein was calculated by multiplying N x 6.38. For the determination of lactose the Munson and Walker method (14) was used; the method employs the reduction of copper hydroxide

to copprous oxide by the lactose, and the copprous oxide is measured gravimetrically. Total solids were determined by difference in weight before and after heating a sample at 99°C; this value subtracted from 100 gives the water content (14).

Samples of butter and ghee were analyzed for fat, curd, and moisture content according to the Jacobs Rapid Crucible Method (14).

Chemical and Physical Methods Used for Analysis of Butter and Ghee

Iodine Value:

The iodine value was determined by addition of a known amount of iodide (Hanus Solution) to the fat. Iodide reacting with the unsaturated linkages was calculated by the titration of the excess iodine (14). Iodine value is expressed as gI/100 g. fat.

Saponification Value:

For obtaining saponification value, a sample of fat was saponified by KOH and the excess KOH was titrated (14). Saponification value is given as mg KOH/g. fat.

Hehner Value:

To find the Hehner value, the fat was saponified and the soap was treated with an acid and filtered. Free

fatty acids remaining on the filter were washed with hot water to remove the water soluble fatty acids. The water insoluble fatty acids were gravimetrically determined (14). Percentage water insoluble fatty acids is known as the Hehner value.

Reichert-Meissl and Polenske Value:

Reichert-Meissl value was found by saponifying the fat, adding an acid, and distilling to collect steam volatile fatty acids. The distillate was filtered and the filterate titrated. The number of milliliters of 0.1N NaOH required to nutralize the water soluble fatty acids, distilled from five grams of fat was calculated as Reichert-Meissl value (14). The slightly water soluble fatty acids remaining on the filter were washed with alcohol and titrated for Polenske value, expressed as milliliters of 0.1N NaOH required to nutralize the water insoluble, but alcohol soluble, fatty acids distilled from five grams of fat (14).

Kirschner Value:

To measure the Kirschner value the Reichert-Meissl distillate was nutralized; silver sulfate was added and filtered to obtain silver soaps of the water soluble fatty acids. Sulfuric acid was then added and distilled to collect the fatty acids (14).

Saturated and Unsaturated Fatty Acids:

The concentrations of saturated fatty acids were determined by saponifying the fat and hydrolysis of the soaps to release fatty acids. After cooling and filtering, the saturated fatty acid residue remaining on the filter was further treated with an acid and extracted with ether. The same ether extraction was applied to the filterate for the unsaturated fatty acid fraction. Iodine value of both fractions was determined and used for correction in the calculations (14).

Free Fatty Acids (Acid Value):

The fat was heated in alcohol to dissolve the free fatty acids for titration to obtain the percentage of free fatty acids (14).

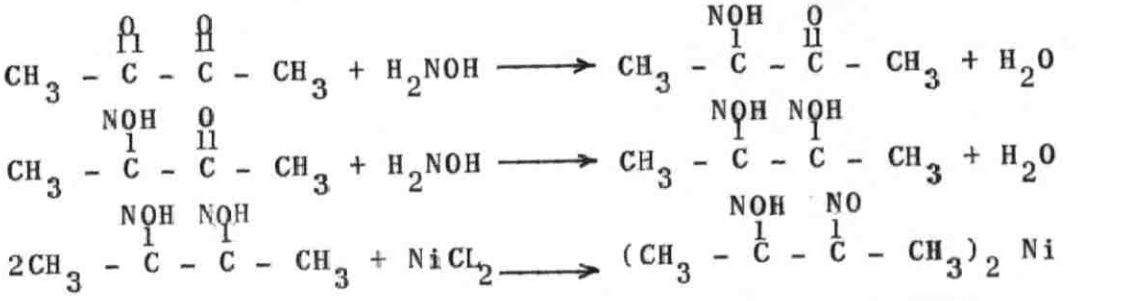
Peroxide Value:

Peroxides developed as a result of autoxidation of fats, were measured iodometrically according to the method of Lea (12). The peroxide value is expressed as milliliters of 0.002N $Na_2S_2O_3$/g. fat.

Biacetyl Value:

The modified Barnicoat Method (14) was employed to measure the biacetyl value (expressed as parts per million). The method uses a steam distillation of the fat, and collecting the biacetyls of

the distillate in a trap containing a mixture of hydroxylamine hydrochloride solution, sodium acetate, and nickel sulfate. The biacetyl will precipitate as nickel dimethyl glyoxime with the following proposed mechanism:



Nickel Dimethyl Glyoxime

Total Acidity:

To extract the total acids, fat was nutralized to form the salts of free fatty acids and lactic acid which are water soluble. The fat was then extracted by ether; the residue was hydrolyzed by an acid to release the fatty acids and lactic acid. The free acids were extracted by liquid - liquid extraction and titrated to find the percentage total acids (14).

Melting Point:

The melting point was measured by using a Fisher Johns Melting Point Apparatus.[2] A drop of fat was put on a plate connected to a thermometer. By heating the plate, melting of the fat could be seen through a magnifier and

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2. Fisher Scientific Co. Pittsburgh, N.Y., U.S.A.

the temperature could be taken immediately from the thermometer at the side (14).

Color:

Color determination was done visually. The colors were graded by comparing the samples with one another; the color having the least intensity was scored yellowish; yellow was the score for the more intense color and yellowish green for the ghee sample prepared from yoghurt.

Refractive Index:

Refractive index was measured by use of an Abbe Refractometer[3] at 40°C (14).

Chromatographic Determination of Fatty Acids

The fatty acids were esterified with methanolic HCl according to the method of James in Glick (9), except that nitrogen was used for evaporating the chloroform, excess methanol, and petroleum ether. The petroleum ether, in which the esterified fatty acids were kept, was evaporated under nitrogen just before the injection of the sample in the chromatograph. Use was made of a Pye Argon Chromatograph[4] with an Apiezon-L column, having Celite as

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3. Bausch and Lomb Optical Co. Rochester, N.Y., U.S.A.
4. W G Pye and Co. Ltd., Granta Works, Cambridge England.

the support for the stationary phase. The voltage and the temperature were kept constant at 1,250 and 197°C, respectively; a rate of 70 milliliters per minute for gas flow was maintained throughout .

Keeping the column, temperature, and the rate of gas flow constant, a given fatty acid always emerges at the same time. Therefore, for the identification of the fatty acids according to the method of James in Glick (9), a sample of standard methyl palmitate (as it is frequently the major fatty acid in lipids) was initially run[5] to identify its position on the chromatogram. Samples of milk, yoghurt, butter, and ghees from yoghurt and cream were run and the retention volumes of the component fatty acids relative to the standard methyl palmitate were determined; these values were found by measuring the distance from the center of the air peaks to the injection point on the chromatogram. The values so obtained were compared with those given in a table by Beerthuis et al. in Glick (9), and the fatty acids were identified according to these values in the same table.

To measure the quantity of the fatty acids appearing on the chromatogram, the product of peak height and retention time for each fatty acid was taken as the peak area according to the method of Carrol (3). By equating the sum of peak areas to 100, the percentage of

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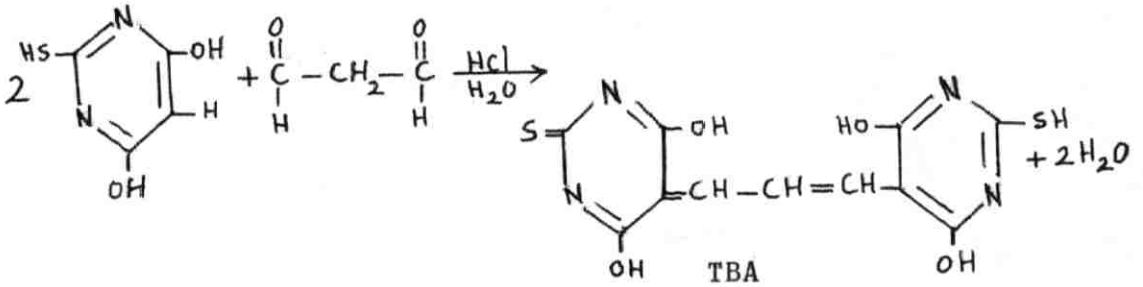
5. This was done in the Biochemistry Department of School of Medicine, American University of Beirut.

each fatty acid on the chromatogram was calculated.

2-Thiobarbituric Acid Test (TBA test)

Malonaldehydes which are known to be the major final product of autoxidation of fats, were measured according to the method of Yu and Sinnhuber (38). The method is based on the reaction of 2-Thiobarbituric acid with malonaldehyde, forming a red pigment. Sinnhuber et al. (29) have proposed the mechanism of formation of TBA pigment from malonaldehyde as follows:



TBA pigment

The TBA pigments were extracted by applying the aliquot to a base-exchange column (with the barrel of 0.7 x 15 centimeters dimention), used for thiamine determination, packed with cellulose powder. Interfering yellow pigments were washed by 0.1N HCl and the TBA pigments eluted by 0.1N NaOH. The intensity of the red color was measured colorimetrically, using a Unicam Spectrophotometer[6] at 532 mu. The TBA value was calculated by using the following formula given by Yu and Sinnhuber (38):

$$\frac{O.D \times 2}{Wt.\ Sample} \times 46 = \text{mgs. of malonaldehyde/1000 g. fat.}$$

6. Unicam Instruments, Cambridge, England.

Organoleptic Test

Rice (150 grams) was cooked with 25 grams of ghee samples prepared from yoghurt and cream. The kind of rice used, time, and the temperature of cooking were the same for all samples.

A panel of nine persons from the Food Technology and Nutrition division tasted the samples each time. Equal amount of samples were presented for evaluation on scoring sheets. Scoring was done from 9 to 1, 9 being excellent and 1 very poor. Aroma and flavor were evaluated in each sitting. The evaluation was repeated three times and therefore, each figure in the results is the average of 27 evaluations.

Animal Experiments

Male albino rats of Sprague-Dawley strain[7] were used as the experimental animals. Three groups of six weanling rats (50 to 60 grams) were used for serum cholesterol determinations; they were kept on stock diet[8] for 5 days. Seven groups, of five rats each, were used for fat absorption study. These animals weighed 80 to 90 grams when they arrived and were kept on stock diet for 12 to 16 days to gain weight, as the experiment required larger animals. The animals were individually housed in

7. Obtained from Animal Suppliers (London) Ltd.
8. Obtained from Vitasni Feed Company, Beirut, Lebanon.

mesh-bottom cages in an air-conditioned room held at 21± 1°C and relative humidity of about 60 per cent; food and water supplied ad libitum. Food consumption and weight gains were recorded weekly.

Experiment 1:

One control group of animals was sacrificed with ether at the beginning of the experiment for serum cholesterol determination. About one milliliter of blood was taken by heart puncture from each of the animals with a syringe (using needle No. 20). After removing the needle, the syringe was immediately emptied, to prevent hemolysis of red blood cells. Blood samples were then centrifuged at 3,000 r.p.m. for five to seven minutes and the serum decanted into individual tubes and stored in a freezer for later analysis.

The other two groups were kept on two experimental diets, different only in the kind of fat included. The control diet had the following composition:

Ingredient	Percentage
Corn starch	55
Casein	20
Vitamin mixture[9]	1
Mineral mixture (USP XIV)[9]	4
Corn oil	20

9. Obtained from Nutritional Biochemicals Cooperation, Cleveland, Ohio, U.S.A.

The third group received the same diet as the control group, except that 20 per cent of ghee was incorporated in the diet in place of corn oil.

Animals were fed the experimental diets for three weeks. At the end of this period one milliliter blood samples were taken from the animals by heart puncture without sacrificing the animals; the blood samples were treated as described above.

Serum Cholesterol Determination

The method employed for the total cholesterol determination in serum was that described by Zlatkis et al. (39). The method consists of adding a constant amount of concentrated sulfuric acid, acetic acid, and ferric chloride solution to 0.1 milliliter of serum. Purple color develops during the reaction and it requires approximately one minute and remains stable over a period of several hours. The intensity of the color was determined colorimetrically at 560 mu by use of a Unicam Spectrophotometer[6]. A standard curve was also prepared with pure cholesterol (USP)[10] using five different concentrations.

Experiment 2:

This experiment was designed to determine the rate of fat absorption. The animals were fasted for 48 hours,

10. General Biochemicals. Chagrin Falls, Ohio, U.S.A.

water was available during the fasting period; and the fat to be investigated was administered orally at a dosage of 250 milligrams per 100 centimeter square of the body surface, by means of getatin capsules. Body surface was calculated according to the Lee method (43), using the following formula:

$$S = KW^{0.60}$$

where K is a constant equal to 12.54, and

W is the weight of animal in grams.

For the determination of the fat content in the gastrointestinal tract a modified method of Deuel et al. (31) was used. The animal was sacrificed with ether; the abdomen was opened; and the intact gastrointestinal tract was removed. The stomach, small and large intestine were separated; each of these three parts was flushed twice with 20 milliliters of diethyl ether, using a syringe with blunt needle. The three extracts obtained in this way were dried overnight with sodium sulfate and filtered. The residue was washed with 100 milliliters of ether; then the ether was evaporated and the residue weighed. A group of fasted animals were used as a blank to determine the endogeneous fat, present in stomach, small and large intestine, which would interfere with the determinations; these values were subtracted from their respective values found from the experimental animals. In two groups, each of five rats, the content of the gastrointestinal tract

were analyzed respectively three and six hours after administering the fat. Using the following formulas (28), from these values it was calculated at what time 50 per cent of the fat administered would be absorbed from the gastrointestinal tract.

$$AT_{50} = \bar{X} + \frac{50 - \bar{Y}}{b}$$

X = Time interval between administering the fat and the analysis (in minutes).

Y = Percentage of fat recovered in the gastrointestinal tract after absorption time X.

$\bar{X}$ = Mean of all X values.

$\bar{Y}$ = Mean of all Y values.

b = Regression coefficient of Y upon X =

$$\frac{\Sigma XY - \frac{\Sigma X \cdot \Sigma Y}{n}}{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}$$

in which n = number of observations.

The value so obtained (AT_{50}) was applied to a third group of rats for each fat (corn oil and ghee).

RESULTS AND DISCUSSIONS

Methods of Preparation of the Samples

The two different methods used for the preparation of ghee samples are described in Figures 2 and 3. It can be observed that the cream method has the advantage of giving a 20 to 25 per cent higher recovery of fat from milk than that of the yoghurt method. This observation is in agreement with the report given by Gill (8). Both butter samples in the first trial had high moisture contents (about 28 per cent in the butter from yoghurt and 35 per cent in the other one). The moisture levels of the butter samples kept for analysis were reduced to about 20 per cent for butter from cream and 15 per cent for butter from yoghurt by leaving them on a shelf for one or two days. Figures 2 and 3 show that longer heating time of butter was required in the cream method than that of yoghurt method. The difference was thought to be due to the higher moisture content of butter prepared from cream. Also, in the yoghurt method longer time was required per unit weight of ghee than in the other method because of the time needed for fermentation.

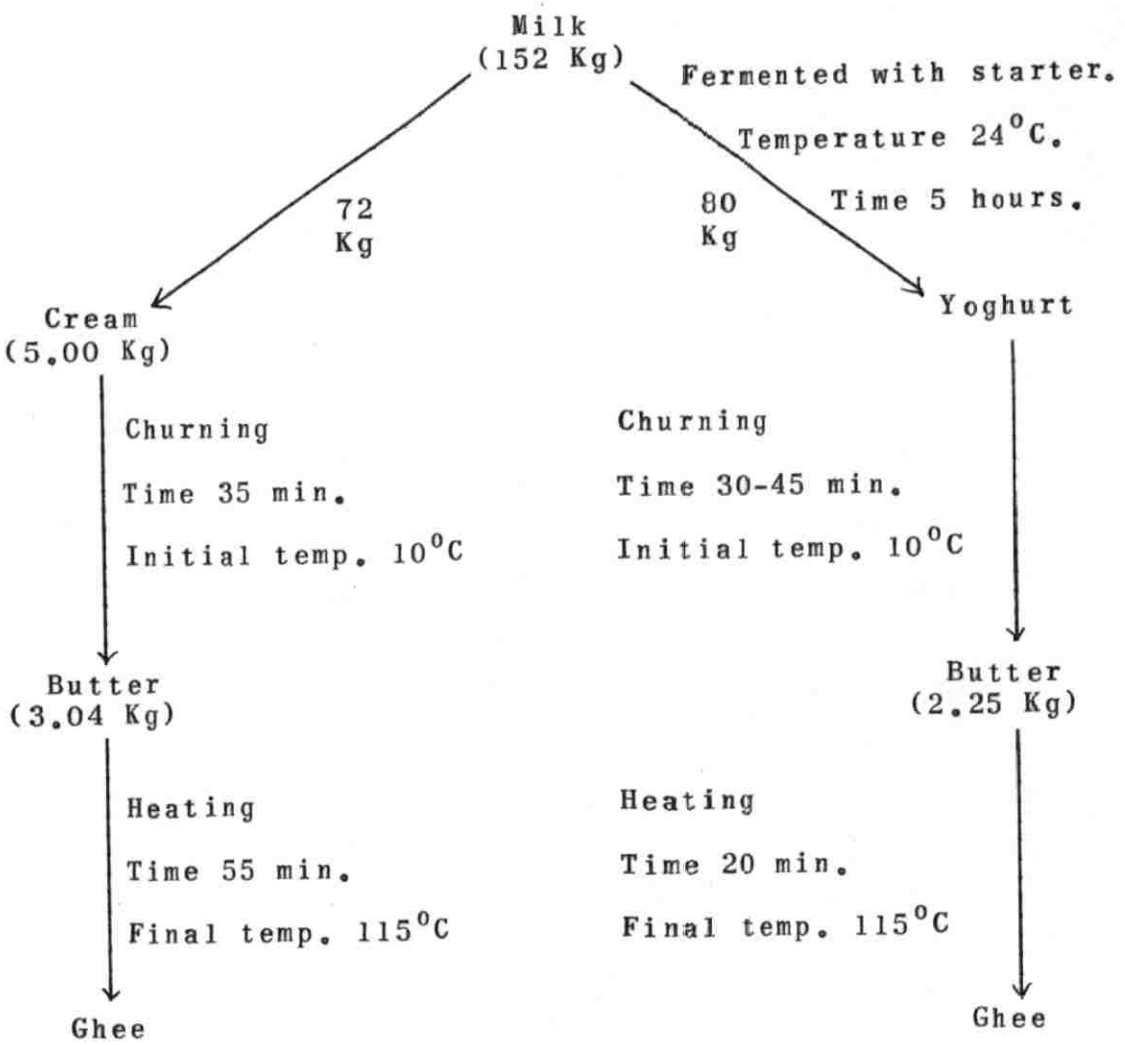


Figure 2. The steps of preparation of butter and ghee samples from the first batch of milk.

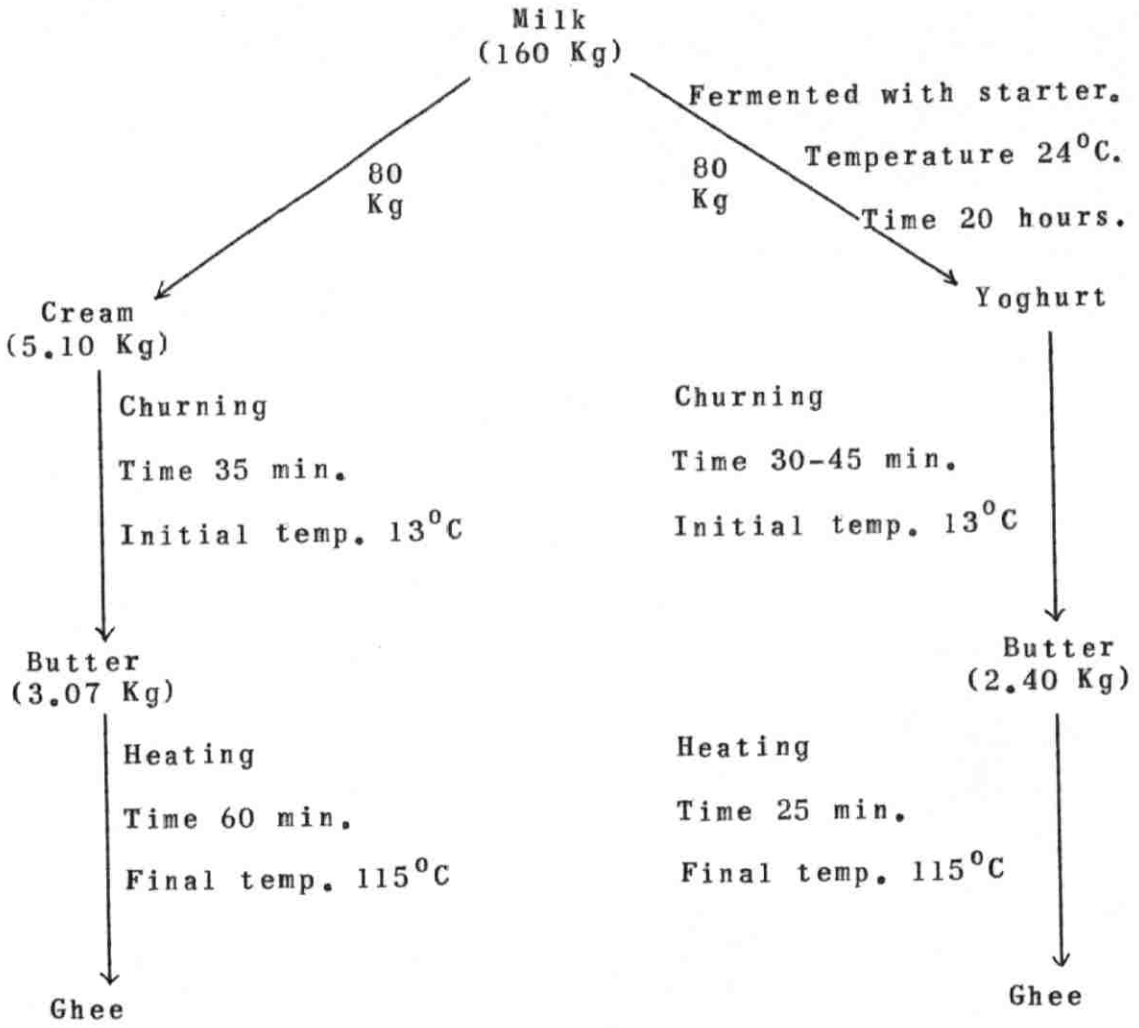


Figure 3. The steps of preparation of butter and ghee samples from the second batch of milk.

Chemical Analysis on Milk and Yoghurt

Results of the chemical analysis of milk and yoghurt samples are given in Table 3. Yoghurt was found to have a lower lactose content than milk. This may be attributed to break down of some of the lactose by microorganisms active in fermentation. Fat content of yoghurt was slightly higher than in milk, while the protein was found to be higher in milk.

Chemical and Physical Determinations on Butter and Ghee

Results of the chemical and physical analysis made on butter and ghee samples are given in Tables 4, 5, and 6. Values for butter samples are calculated on fat basis in order to compare them with the values found for ghee samples.

The iodine and saponification values which are indicative of the degree of unsaturation and the molecular weight of the component fatty acids, were not found to be appreciably different in samples from the same preparation, as the data in Tables 5 and 6 suggest. Peroxide value which is a measure of oxidative rancidity is lower in butter than its respective ghee sample in the second trial. This is attributed to the heat treatment given to ghee which is known to be an important factor in oxidation. The first preparation, the

Table 3. Determinations on milk and yoghurt samples prepared from the first[x] batch of milk.

Composition	Milk	Yoghurt
	%	%
Fat	3.7	3.9
Protein	3.6	3.4
Ash	0.79	0.79
Total solids	13.1	13.3
Lactose	5.1	3.9
Water	86.9	86.7

x The milk and yoghurt from the second batch of milk was only analyzed for the fat and lactose content; the results were as follows:

Fat = 3.4% in the milk and 3.5% in the yoghurt.

Lactose = 5.18% in the milk and 4.6% in the yoghurt.

Table 4. Percentage fat, curd, and moisture content of butter and ghee samples prepared from the first and second preparation.

Analysis	B.C.[1]	B.Y.[2]	G.C.[3]	G.Y.[4]
		First Preparation		
Fat	78.71	84.24	99.64	99.61
Curd	0.81	0.71	0.07	0.04
Moisture	20.48	15.05	0.29	0.35
		Second Preparation		
Fat	80.70	80.45	99.40	99.28
Curd	0.80	0.65	0.01	0.02
Moisture	18.50	18.19	0.59	0.70

1. B.C. Stands for Butter from Cream.
2. B.Y. Stands for Butter from Yoghurt.
3. G.C. Stands for Ghee from Cream.
4. G.Y. Stands for Ghee from Yoghurt.

The same abbreviations are used throughout the text.

Table 5. Chemical and physical determinations on butter and ghee prepared from the first preparation.

Analysis	B.C.	B.C. on fat basis	B.Y.	B.Y. on fat basis	G.C.	G.Y.
Iodine value	24.7	31.4	26.6	31.6	32.1	31.7
Saponification value	160.2	215.0	183.3	217.6	214.7	215.7
Peroxide value	2.81	3.57	3.78	4.48	3.41	3.34
Acid value	1.8	2.3	1.0	1.2	1.2	0.8
Hehner value	68.4	86.9	73.4	87.1	87.6	86.3
Kirschmeir value	15.20	20.10	16.31	19.93	20.00	19.94
Reichert-Meissl value	19.41	24.66	20.74	24.66	24.62	24.55
Polenske value	2.33	2.96	2.51	2.98	2.99	2.99
Total acidity	0.22	0.28	0.37	0.43	0.27	0.44
Biacetyl value	31	40	186	221	4	15
Saturated fatty acids (%)	14.2	18.0	15.3	18.1	18.4	18.3
Unsaturated fatty acids (%)	38.1	48.4	40.6	48.2	48.2	48.2
Melting point (°C)					24-34	23-40
Refractive index					1.4551	1.4551
Color	Yellowish		Light yellow		Yellow	Greenish yellow

Table 6. Chemical and physical determinations on butter and ghee prepared from the second preparation.

Analysis	B.C.	B.C. on fat basis	B.Y.	B.Y. on fat basis	G.C.	G.Y.
Iodine value	18.1	22.4	17.2	20.9	22.2	23.5
Saponification value	180.0	223.0	179.0	223.0	221.0	220.0
Peroxide value	2.23	2.77	2.10	2.64	3.78	3.50
Acid value	1.1	1.3	1.0	1.3	1.1	1.3
Reichert-Meissl value	17.17	21.29	17.65	22.00	21.90	19.93
Biacetyl value	30	37	223	277	8	21
Total acidity (%)	0.24	0.30	0.37	0.46	0.31	0.45
Melting point (^{o}C)					24-35	23-38
Refractive index					1.4551	1.4551
Color	Yellowish		Light yellow		Yellow	Greenish yellow

butter samples had higher peroxide values than their respective ghee samples. This was thought to be due to the fact that the butter samples were kept at room temperature for one and two days (butter from yoghurt kept for one day and the other butter was kept two days) and within this period peroxide level has increased as a result of oxidative rancidity. Butter from yoghurt had a higher peroxide value than that made from cream (Table 5). The cause of it may be the heat treatment before fermentation of milk. The butter samples which were kept at room temperature to reduce their moisture content had acid values higher than their respective ghee samples possibly due to the higher rate of oxidative rancidity taking place in them (Tables 5 and 6). Ghee was always found to have a higher total acidity than its respective butter. This may be due to the removal of water and curd from butter which gives rise to the concentration of the acids present in ghee. Higher biacetyl value in butter was observed compared to its respective ghee samples. This indicates that in the heating process of butter to prepare ghee, most of the aromatic compounds which are known to be volatile were driven out. Color of the samples was always deeper in ghee than in butter which may be modified by higher concentration of pigments, brought about by removal of the water from butter. Ghee obtained from yoghurt had a greenish tint which did not

show very much in the butter because of the lower color concentration. The range of the melting point, as indicated in Tables 5 and 6, for ghee from yoghurt was found to be wider and higher than that of ghee from cream.

Analysis by Gas-Liquid Chromatography

The samples used for gas-chromatographic analysis were selected from both samples. Although methyl linoleate and methyl linolenate, according to Orr and Callen (23), do not dissolve on Apiezon-L column, still it appears to be the best column for long chain fatty acids. This column has been used for long chain acids in butterfat by many workers (15, 23, 24). It was, therefore, preferred to use Apiezon-L column in this study.

Results of the chromatographic analysis are tabulated in Table 7. It is apparent that the amount of palmitic acid ($C_{16:0}$) in all samples was the highest, followed by oleic ($C_{18:1}$), myristic ($C_{14:0}$) and stearic ($C_{18:0}$); stearic acid being the least of the four. In general, going from milk to butter and ghee, there was an increase in the amount of lauric ($C_{12:0}$) acid. Oleic acid content was lower in ghee, higher in butter and yoghurt, and highest in milk. This could be explained by the breakdown of oleic acid due to the oxidation of its unsaturated linkage as a result of heat treatment. Linolenic (trienoic) and linoleic (dienoic) acid content,

Table 7. Gas-chromatographic analysis of long chain fatty acids using Apiezon-L column at a temperature of 197°C.

Sample	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{15:0}$ a.i.br.	$C_{15:0}$	$C_{16:1}$ Cis.	$C_{16:0}$	$C_{17:0}$ a.i.br.	$C_{17:0}$	$C_{18:1}$	$C_{18:0}$
Milk	-	0.64	11.64	0.70	1.10	1.83	41.54	1.33	0.83	29.74	10.86
Yoghurt	0.06	1.44	14.60	0.74	1.10	1.60	52.30	1.16	0.58	18.30	8.01
B.C.	0.41	2.66	14.58	1.00	1.30	1.54	47.20	1.40	1.00	19.45	9.23
B.Y.	0.07	2.08	13.43	0.74	1.14	1.70	43.70	1.20	0.80	25.10	9.92
G.C.	0.01	2.00	14.44	0.80	1.32	1.68	46.30	1.34	0.91	20.60	10.30
G.Y.	0.36	2.25	15.80	0.64	0.90	1.20	50.00	1.00	0.70	18.50	8.10

Values are percentages of fatty acids appeared on the chromatogram.

which did not appear on the chromatogram, as confirmed by Orr and Callen (23), were also thought to be oxidized in the course of heat treatment. Being polyunsaturated fatty acids, linolenic and linoleic, are known to be more liable to oxidation than oleic acid which is a mono-unsaturated fatty acid.

Keeping Quality

In studying the shelf life, degree of oxidative rancidity was measured in different samples using 2-thiobarbituric acid (TBA) test. TBA values for the two ghee samples prepared from cream and yoghurt, each with two levels of moisture (5 and 20 per cent) are given in Table 8. Graphical presentation of the results is also shown in Figure 4. The samples were kept for eight weeks and TBA test was run biweekly. In order to compare shelf life of ghee samples with that of butter samples having 20 per cent moisture, the moisture content of samples of ghee were raised to 20 per cent by addition of water. Initial and final free fatty acid values of all samples were determined and results are included in Table 8. The data in this table show that the rate of oxidative rancidity for the first two weeks was higher in the samples having 5 per cent moisture than those with 20 per cent. However, oxidative rancidity in the samples of 20 per cent moisture exceeded that of 5 per cent levels after two

Table 8. TBA values of samples used for shelf life studies.

Sample	Initial	Two weeks	Four weeks	Six weeks	Eight weeks	Initial acid value	Final acid value
G.C. 5% moisture	1.2	5.0	6.5	7.8	9.5	1.2	1.4
G.C. 20% moisture	1.2	3.8	7.6	9.0	11.3	1.2	1.5
G.Y. 5% moisture	1.1	4.5	6.1	8.2	11.4	1.3	1.6
G.Y. 20% moisture	1.1	3.6	8.5	9.9	13.6	1.3	1.8

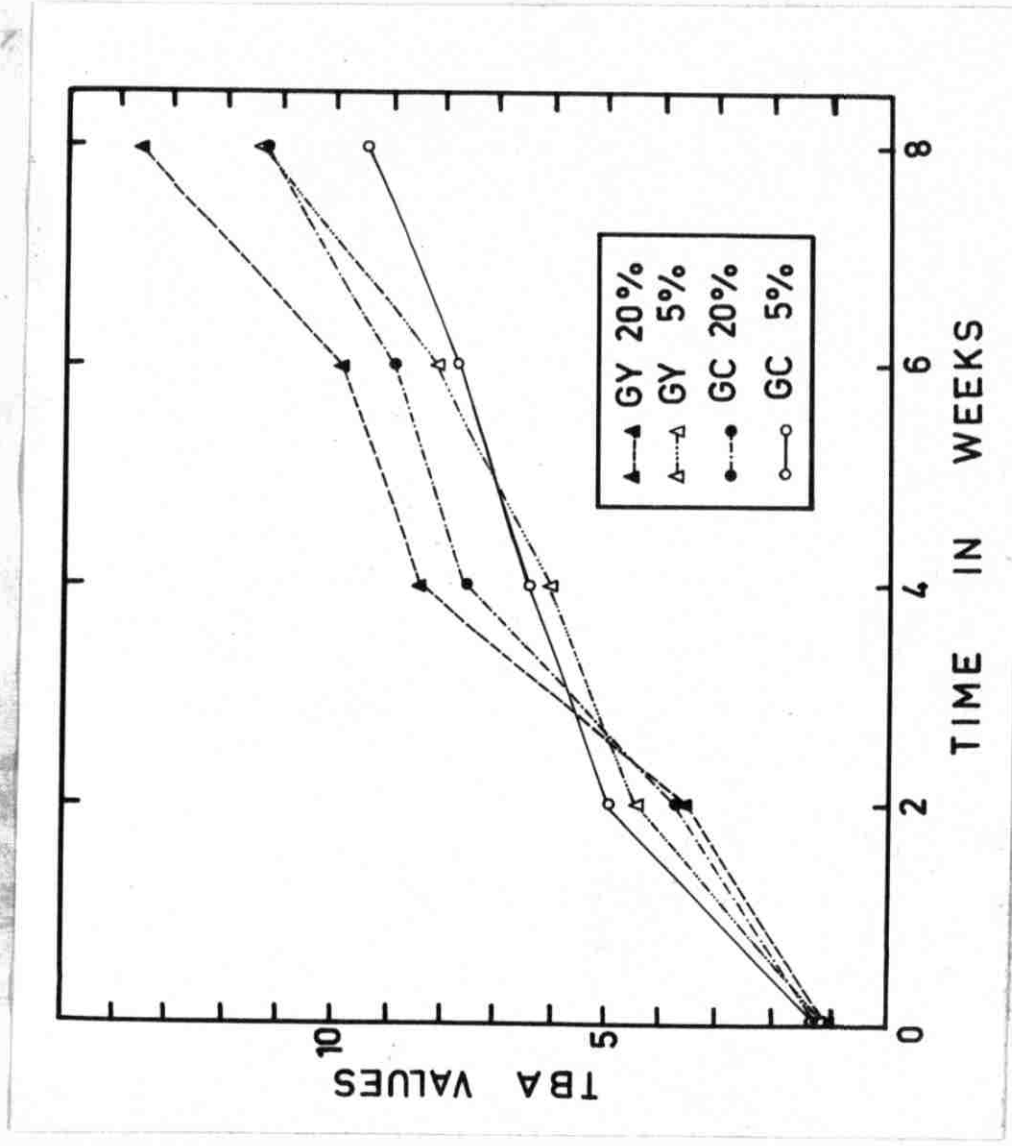


Figure 4. Graphical presentation of development of rancidity in samples used for shelf life studies.

weeks. It is also shown that ghee obtained from cream had a lower TBA value than that of ghee from yoghurt. Free fatty acid value (acid value) was determined to estimate the degree of hydrolytic rancidity which is known to occur at a noticeable rate in dairy product. Comparing the initial and final acid values (Table 8), it is shown that there was a direct relation between acid and TBA values. Thus, it can be concluded that ghee from cream had a better keeping quality than ghee from yoghurt under the experimental conditions used.

Butter samples kept on shelf were excluded from the study after two weeks due to the occurance of mold contamination.

Organoleptic Tests

The difference between biacetyl values of the two ghee samples, prepared by different methods, was not found to be as great as that between the two butter samples as reported in Tables 5 and 6. Organoleptic test was performed for the ghee samples obtained from cream and yoghurt. The values obtained, using scoring sheets, were 7.87 for the aroma and 8.00 for the flavor of ghee from cream, and 7.25 and 7.00 for the ghee from yoghurt, respectively. In spite of the lower biacetyl value (Tables 5 and 6) ghee from cream was scored higher than ghee from yoghurt.

Animal Experiments

Serum cholesterol levels were determined on three groups of rats, one for the initial level of cholesterol in serum, a second group receiving a diet containing corn oil, and the third group given a diet containing ghee as the only source of fat. The serum cholesterol levels of the three groups of animals were found to be 31 milligrams, 33 milligrams, and 39 milligrams per 100 milliliter of serum, respectively. These results are in agreement with the findings of Rademeyer and Booyen (25), and Hegsted et al. (11). The average food consumption of the group fed the diet containing corn oil was 7.9 grams per rat per day and that of the group received ghee was 9 grams per rat per day. The animals fed corn oil as the fat ingredient of their diet had an average gain in weight of 89 grams, while those given ghee as their dietary fat gained 106 grams in weight during the experimental period. This increased gain in weight may be attributed to higher food consumption. However, it can be observed from this experiment that 20 per cent of ghee in the diet caused a higher cholesterol level in serum as compared to that caused by the same diet in which ghee was replaced with 20 per cent corn oil.

The results of the second animal experiment which was designed to study the rate of absorption of ghee are

presented in Table 9. In the group of rats which was used as a blank to determine the amount of endogeneous fat, the gastrointestinal tract was divided into three different parts, the stomach, small intestine and large intestine. The average fat content of each part of intestinal tract was determined for the whole group. It was found that the amount of the endogeneous fat present in stomach, small intestine, and large intestine were 4, 6, and 16 milligrams, respectively. The rate of absorption was observed to be higher in ghee than that obtained for corn oil when the duration of absorption time was three hours. However, this difference was not considerable when the absorption time was extended to six hours. This is in consistant with the findings of Thomasson (32). Applying the calculated time at which 50 per cent of the fat would be absorbed to a third group of rats, the values obtained were 53 per cent for corn oil and 49 per cent for ghee. The values for the rate of absorption from stomach, small and large intestine suggested that the percentage of fat in the stomach was the lowest at AT_{50} following by the highest value for the large intestine. However, the variation of values obtained from the small intestine was not as large as those of stomach and large intestine. Thus the distribution of the fat over stomach, small, and large intestine seems to be independent of the nature of the fat administered.

Table 9. Percentages of recovery of fats at different intervals in stomach, small intestine, and large intestine.

Duration of absorption time	Stomach	Small Intestine	Large intestine	Total recovery
			Corn oil	
3 hours	76	22	2	71
6 hours	78	19	3	34
4:28 hours (AT_{50})	73	22	5	53
			Butterfat	
3 hours	72	26	2	56
6 hours	75	22	3	32
4:36 hours (AT_{50})	67	26	7	49

Each figure in the table is the average of 5 determinations.

SUMMARY AND CONCLUSION

Ghee was prepared from yoghurt and from cream. Analysis of milk and yoghurt showed that yoghurt had a lower lactose content than milk. Comparison of the chemical and physical properties which were determined, revealed that the total acidity and biacetyl values were higher in the butter and ghee samples obtained from yoghurt than those samples prepared from cream. Gas-chromatographic analysis of long chain fatty acids showed lower amounts of unsaturated fatty acids in ghee as compared to milk. Color of the butter from cream was lighter (yellowish) than the color of butter from yoghurt (light yellow). Ghee from yoghurt had a greenish yellow color, while the ghee from cream was yellow. Organoleptic test run on rice cooked with different ghees, indicated a better flavor for ghee from cream than ghee from yoghurt. Shelf life of the samples of ghee with added two levels of moisture (5 and 20 per cent) was studied. It was found that the keeping quality of ghee from cream was generally superior to that of ghee from yoghurt.

Two animal experiments, one for the effect of ghee on the level of serum cholesterol, and another for the rate of absorption of ghee, were performed. Male albino rats were used as experimental animals. In the

first experiment serum cholesterol level was determined on three groups of six rats; one group was used for the initial level of serum cholesterol. Two groups received the same diet only different in the fat content, one had 20 per cent corn oil, and the other contained 20 per cent of ghee as the dietary fat. After a period of three weeks, animals receiving ghee in their diets developed a higher serum cholesterol level than those given corn oil in the diet. In the second experiment, seven groups of six rats were used. Animals were fasted for 48 hours and then a dosage of 250 milligrams of fat/100 square centimeters of body surface was orally administered to the animals. They were sacrificed three and six hours after administering the fat. Intact gastrointestinal tracts were removed and fat content of stomach, small and large intestine was separately measured for each animal. The time at which 50 per cent of the administered fat would be absorbed was calculated, on the basis of three and six hours absorption periods, and applied to a third group. Corn oil was administered to another set of animals to compare the rate of absorption of ghee with that of corn oil. It appeared that ghee was absorbed at a higher rate than corn oil when absorption time was three hours. However, this difference was not considerable when absorption time was six hours.

LITERATURE CITED

1. Association of Agricultural Chemists. "Official Methods of Analysis", 9th ed. Washington D.C. Association of Agricultural Chemists. 1960.

2. Bailey, A.E. "Industrial Oil and Fat Products" Interscience Publishers, Inc. New York. N.Y. 1957.

3. Carrol, K.K. Quantitative estimation of peak areas in gas-liquid chromatography. Nature 191:377. (1961).

4. Day, E.A. Autoxidation of milk lipid. J. Dairy Sci. 43:1360 (1960).

5. Deuel, H.J. "The Lipids", Vol:1. Interscience Publishers, Inc. New York. N.Y. 1951.

6. Deuel, H.J. "The Lipids", Vol:2. Interscience Publishers, Inc. New York. N.Y. 1951.

7. Deuel, H.J., J.R.L. Hallman and A. Leonard. The comparative rate of absorption of some natural fats. J. Nutr. 20:215 (1940).

8. Gill, M.S. "Fifth Year Research in Agronomy, Dairy, Poultry, Farming, Water Requirements of Crops, Mechanical Farming and Agricultural Extension". Department of Agriculture, West Pakistan. 1960.

9. Glick, D. "Method of Biochemical Analysis", Vol:8. Interscience Publishers, Inc. New York. N.Y. (1960).

10. Gunstone, F.D. "An Introduction to the Chemistry of Fats and Fatty Acids". John Wiley and Sons. Inc. 440 Fourth Avenue. New York. N.Y. 1958.

11. Hegsted, D.M., S.B. Andrus., A. Godsis and O.W. Portan. The quantitative effects of cholesterol, cholic acid and type of fat on serum cholesterol and vascular sudanophilla in the rat. J. Nutr. 63:273 (1957).

12. Hilditch, T.P. "Industrial Chemistry of Fats and Waxes". D. Van Nostrand Company, Inc. New York. N.Y. 1949.

13. Irwin, M.H., H. Steenbock and V.M. Templin. A technic for determining the rate of absorption of fats. J. Nutr. 12:85 (1936).

14. Jacobs, M.B. "Chemical Analysis of Foods and Food Products". D. Van Nostrand Company, Inc. Princeton. New Jersey. 1962.

15. James, A.T. and A.J.P. Martin. Gas-liquid chromatography: the separation and identification of the methyl esters of saturated and unsaturated acids from formic acid to n-octadecanoic acid. Biochem. J. 63:144 (1956).

16. Lalitha, K.R. and N.N. Dastur. Color development of desi ghee. Indian J. Dairy Sci. 9:143 (1956).

17. Lee, M.O. Determination of the surface area of the white rat with its application to the expression of metabolic results. Amer. J. Physiol. 89:24 (1929).

18. Mallette, M.F., P.M. Althouse and C.O. Clagett. "Biochemistry of Plants and Animals". John Wiley and Sons, Inc. New York. N.Y. 1960.

19. McMullen, J.J. and L.V. Thomas. The absorbability of natural and modified fats. Food Res. 21:621 (1956).

20. Moore, J.H. and D.L. William. The effect of diet on the level of plasma cholesterol and the degree of atherosclerosis degeneration in the rabit. Brit. J. Nutr. 18:253 (1964).

21. Olson, R.E. Nutrition. Ann. Rev. Biochem. 28:467 (1959).

22. Olson, R.E., J.R. Jablonski and E. Taylor. The effect of dietary protein, fat, and choline upon the serum lipids and lipoproteins of the rat. Amer. J. Clin. Nutr. 6:111 (1958).

23. Orr, C.H. and J.E. Callen. Recent advances in the gas chromatographic separation of methyl esters of fatty acids.
Ann. N.Y. Acad. Sci. 72:649 (1959).

24. Patton, S., R.D. McCarthy., L. Evans and T.R. Lynn. Structure and synthesis of fat: 1-Gas chromatographic analysis.
J. Dairy Sci. 43:1187 (1960).

25. Rademeyer, L.J. and J. Booyens. The effects of variations in the fat and carbohydrate content of the diet on the levels of magnesium and cholesterol in the serum of white rats.
Brit. J. Nutr. 19:153 (1965).

26. Schultz, H.W., E.A. Day and R.O. Sinnhuber. "Symposium on Foods: Lipids and Their Oxidation", The Avi Publishing Company, Inc. 1962.

27. Sebrell, W.H. An over-all look at atherosclerosis.
J. Amer. Diet. Assoc. 40:403 (1962).

28. Singh, K.P. and S.N. Singh. Variations in the physico-chemical constants of ghee.
Indian. J. Dairy Sci. 13:143 (1960).

29. Sinnhuber, R.O., T.C. Yu and Te Chang Yu. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity.
Food Res. 23:626 (1958).

30. Soundararajan, R. Studies on the keeping quality of ghee.
Indian. J. Dairy Sci. 11:96 (1958).

31. Steenbock, H., H. Irwin and J. Weber. The comparative rate of absorption of different fats.
J. Nutr. 12:103 (1936).

32. Thomasson, H.J. The biological value of oils and fats.
J. Nutr. 59:343 (1956).

33. Triebold, H.O. and L.W. Aurand. "Food Composition and Analysis", D. Van Nostrand Company, Inc. Princeton. New Jersey. 1963.

34. Vachha, S.L., V.K. Leley, N. Narayana and J.A. Daji. Studies in the autoxidation of ghee (butterfat). Indian. J. Dairy Sci. 10:6 (1957).

35. Vachha, S.M., S.A. Vasavada, V.K. Letey, N. Narayana and J.A. Daji. Studies in the autoxidation of ghee (butterfat). Indian. J. Dairy Sci. 11:91 (1958).

36. Wilster, G.H. "Practical Buttermaking". O.S.C. Cooperative Association. Carvallis, Oregan. 1957.

37. Wiseman, G. "Absorption from the Intestine". Academic Press. Inc. 111 Fifth Avenue. New York. N.Y. 10003. 1964.

38. Yu, T.C. and R.O. Sinnhuber. Removal of interfering pigments in determining malonaldehyde by the 2-thiobarbituric acid reaction. Food Tech. 16:115 (1962).

39. Zlatkis, A., B. Zak and A.J. Boyle. A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 41:486 (1953).