SOME FACTORS AFFECTING THE KEEPING QUALITY OF GHEE.

by

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CHAPTER I.

INTRODUCTION.

Ghee (clarified butter; butterfat; butter oil, etc.) has been manufactured and used in India and some of the countries in the Middle East, from time immemorial. Vedic hymns of Indians written about 2000 to 3000 B.C. give frequent references to ghee as "Ghrat" used for diet and religious ceremonies.

At present it is the most important dairy product of India from several aspects. Out of total estimated milk production of 690 million Mds. in India (a Maund being 80 lbs.) nearly 52% is converted into ghee, which works out to be 76% of the total manufactured milk products. In the monetary value of £293.4 million for milk and milk products; ghee contributes £100 million, that is about one third of the total value and half of the value of the manufactured milk products. (Appendix I, Wright 1937). In a typical vegetarian diet of India (the majority of Indians are vegetarians); it is a very valuable source of Vitamin A, D, E and possibly K, and used in a variety of ways, i.e. for cooking, confectionary, religious rites, medicinal and ointment purposes (Appendix II). The annual per capita consumption of ghee for all above purposes is only 2½ lbs. (1.4 seer) which does not compare favourably with New Zealand or any other advanced countries

where butter is used to spread on the bread only. Of late, India has been exporting increasing quantities of ghee (about 80,000 mds. annually) to South, East and West Africa, Burma, Malaya, Ceylon etc. (Appendix III). Thus there existsa big demand for both home and export markets. Quantity of ghee produced in India is about 14 million mds. or half a million ton , which is nearly three times the production of butter in New Zealand, Denmark, Canada and Australia; equal to that of Germany and three quarters that of U.S.A. Ghee production in India is however, not even and varies greatly from place to place depending on many factors. It is mostly localized in five provinces and States (Punjab, United Provinces, Rajputana, Madras and Bihar) which together produce about 60%; the rest (40%) being distributed in 17 states and provinces. (Appendix IV). Likewise the consumption and percent marketed also varies depending upon quantity produced; dietary habits of people, monetary position of the producer etc. (Appendix V; Ghee report from II to V Appendix).

In view of the above facts, it is but natural that ghee out of all other dairy products; has received preference for research work in India. Both Indian and foreign workers have contributed to investigate various technical and scientific problems connected with production, adulteration, storage life, out-turn and nutrition. Out of all, the two problems most investigated by Indian workers

for the last two or three decades have been the question how to detect adulteration in ghee and the Vitamin A assey in ghee. For adulteration four methods have been tried, viz. chemical and physical constants method, fluorescent method, enzyme hydrolysis method and miscellaneous methods like tile test, Bellier-Kreis reaction etc., but so far the problem remains unsolved. Various workers have been connected with this work (e.g Godbole and Sadgopal (1930), Hawley (1933), Sunawala and Kothavalla (1935), Hawley (1936), Venkata-Giri and Bhargava (1937), Daroga and Sidheva (1938), Jha (1939), Muthanna and Mukherjee (1940), Doctor, Banerjee and Kothavalla (1940) Narsimhamurty and Suryanarayanamurty (1940)). Dealing with the problem of Vitamin A, an interesting series of articles have been contributed by Banerjee and co-workers. (e.g. Banerjee and Sunawala (1935 and 1936), Banerjee (1936), Banerjee and Dastur (1936; 1937 I and 1937 II), Banerjee and Doctor (1938), Banerjee (1938), Govindarajan and Banerjee (1940).) Various factors affecting Vitamin A in ghee like effect of heat, light, air, acidity etc., pro and antibodies for it and how to recover oxidised Vitamin A by treatment with hydrogen under pressure have been studied by them. No work seems to be done on Vitamin, D, E, K and carotene to any great extent.

More recently - Rangappa and Banerjee (1946 I), Srinivasan and Banerjee (1946 II), Rangappa, Srinivasan and Banerjee (1946 III), and Rangappa, Srinivasan and Banerjee (1946 I

have started another series of articles dealing with the studies on the methods of preparation of ghee from various angles ie. acidity, souring, indigenous method of manufacture and relation between method of preparation and property of butter and ghee etc.

However, problems dealing with keeping quality of ghee have not been investigated to any great extent; the literature dealing with this subject being very meagre and the evidence presented in it is confusing and does not clarify the position eg. for the effect of heat used in the manufacture of ghee various workers findings are as follows:-

Godbole and Sadgopal (1936) point out that sterilization of ghee by heat increases the subsequent tendency to become rancid possibly because natural anti-catalysts (sic) are thus destroyed. Patil and Hammer (1928) compared the keeping quality of butter, butter fat (heated not above 55°C) and ghee heated from 110° to 140°C by boiling off moisture and concluded that butterfat and ghee kept better than butter but between themselves there is not much difference. The superiority of ghee and butterfat over butter was attributed by them to the elimination of water and curd from the former and not due to heat used in the manufacture. (Ritter (1937), on the other hand after a similar comparison, said that butter oil (ghee) prepared by boiling off method is more resistant to the development of oxidised

flavour than filtered fat and in the same connection later on, Ritter and Nussbaumer (1939) observed that butter oil is less susceptible to oxidation when filtered at 100°C rather than 42°C. Mohr (1939) also after comparing butter oils filtered at 50° and 90°C, and ghee prepared by boiling off at 110°C, found that after 7 months the ghee was superior but adds that the difference had disappeared by the end of 11 months. From their findings Ewbank and Gould (1943) concluded that heating butter or butter oil to 127°C for 30 minutes hastens the oxidation, while Rafey, Richardson and Henderson (1944) observe that butter oil made by the process in which butter is heated to 110°C, to drive off moisture, the residue being removed by centrifuging, has been found to be more resistant to oxidative rancidity than when lower temperatures of isolation of the oil are used. More recently similar observations have been made by Josephson and Dahle (1946) when comparing keeping quality of butter heated to higher temperatures of 300 to 400°F for 10 minutes and that of filtered fat raised to the same temperatures. They also compared high temperature melted butter with filtered butter fat heated with lactose, dried skim milk, phospholipids etc., and in every case they conclude that butter melted at high temperatures by boiling off method was superior. Of course, Ritter and Rafey etal agree that improved keeping quality of ghee prepared by boiling off moisture at

higher temperatures is due to transfer of greater amounts of phospholipid material from the non-oil phase of butter to oil; but Josephson and Dahle did not find any effect of phospholipid in increasing the keeping quality. They found dried skim milk to be of better effect.

On the role of acidity, Banerjee and Doctor (1938) from their findings conclude that the best percentage of acidity for general purposes (for storage and nutrition) is considered to be 0.44% in the curd at churning and that ghee with 0.15% acidity kept satisfactorily during storage. They, therefore, state that the indigenous method of preparing ghee from curd which is thought to give higher acidity is not recommended. So also the process of melting butter into ghee by "boiling off" process (heating ghee over 100°C for driving off moisture); since it increases the acid value. On the other hand, in a later work, Rangappa and Banerjee (1946 I) conclude that curd acidity at churning of 1% to 1.5% giving ghee acidity of 0.1 to 0.15% lactic by "boiling off" process should result in a good quality butter and ghee, since the ratio of curd acidity at churning: ghee acidity, has been found by them as 10 to 12:1 in case of "Boiling off" method and 25 to 36 in case of "decantation method" (heating ghee below 100°C; decanting fat and then evaporation of moisture above 100°C). Such ghee is said not to give a rise in acidity exceeding 1.5% oleic during storage of one year and keep satisfact-

orily. But the same authors (1946 IV) at another place, find that two samples of village ghee -- Mandya in Mysore state and Hogenical in Madras province with ghee of similar low acidity of 0.1% and 0.05% respectively have given rise of, in the former case 0.5% in the first week and 6% in the second week and in the latter case 0.3% in the first week and 1.5% in the second week, thus not keeping well during storage and quickly deteriorating. They further state that deterioration of colour, flavour and texture is proportional to the development of acidity during storage, therefore, they recommend churning curd of low acidity and decantation method of ghee making to obtain low acidity in ghee for better storage life. Rise in acidity in ghee, to a varying degree, during storage has been observed by various workers (Godbole and Sadgopal (1936), Narsimhamurty (1941), Barnicoat (1945), Sengupta (1943) and Rangappa and Banerjee (1946IV). Barnicoat (1945) from his findings concludes that excess acidity is a powerful factor in catalyzing adverse changes both oxidative and hydrolytic, in the product during storage.

As regards other factors Godbole and Sadgopal (1936) mention that development of rancidity in ghee is stimulated in varying degrees by sunlight, light from quartz lamp, air, water, rancid ghee and metals like Fe, Cu and Zn etc. Patil and Hammer (1928) also found addition of water to ghee deteriorating the keeping quality, but Barnicoat

finds that presence of water by itself does not promote deterioration of the butterfat.

According to Davies (1940), deterioration of ghee in keeping quality is due to mainly oxidation of the fat; the other two types of spoilage ie. butyric* and ketonic** to which ordinary butterfat is subjected to in addition, have not been noticed so far to affect the storage life presumably due to the action of high temperatures used in its manufacture. Bacterial spoilage is rendered ineffective due to the same reason, and due to lack of adequate moisture in the fat, though there is a possibility of post heating contamination with bacteria due to unsterile containers. Ghee being mainly butterfat, the factors influencing oxidation of butterfat, ie. moisture, acidity, temperature, light, heavy metals, air etc., should affect the oxidation of ghee as well, but since the position and role of these various factors is not yet clear in the literature as pointed out above, an attempt is made in the present investigations to assess the proper role of some of the factors, viz. acidity, heat, bacterial culture used as starter, and moisture, as far as keeping quality of ghee is concerned.

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* Due to liberation of butyric acid mostly through the action of mono-and tributyrinases and acid hydrolysis.

** It is due to action of dry moulds or those which cannot hydrolyse fat so as to act on glycerol.

CHAPTER II.

MATERIAL AND METHODS.

(A) MATERIAL.

Milk:- In India, butter (Makhan) for melting into ghee is made from milk and not from cream as is done in New Zealand and other Western countries. Milk for the purpose of these experiments has been obtained from Massey College dairy farm. Fresh morning's milk has been mostly used without holding for any length of time and if holding was necessary it was put in the refrigerator. Invariably these milks have been made to correspond to buffalo milk of 8 to 10% fat by separating a portion of the same milk and adding the cream so obtained to the remaining milk.

Starter:- Ghee is made from sour milk and for souring in India, Dahi (traditional butter starter) cultures are used. For the purposes of these investigations three Dahi cultures have been obtained from India viz., Dahi No. 1, Dahi No. 2 and Dahi No. 3 through the courtesy of Dr. Zal. R. Kolthavalla; Dairy Development Advisor, Govt. of India. In addition two lactobacilli pure cultures ie. L.acidophilus and L.bulgaricus (Iowa) were obtained from D.R.I. Massey College through the valuable co-operation of Dr. H.R. Whitehead chief Basteriologist. According to requirements, one of them or all of them have been used for inocul-

ulating milk after boiling and cooling to body temperature.

For using these starters correctly and to identify them, a knowledge of their behaviour was thought necessary and, therefore, preliminary studies into some of their characteristics eg. morphology, staining behaviour and quantity and rate of acid production have been made. Briefly they are recorded as follows:-

Morphology and Staining:-

For these studies, inoculated 10cc. of sterilized skim milk and incubated at 37°C for 24 hours. Examined them under microscope with methylene blue stain or Gram stain, usually all cultures clotted at this stage, except No. 1. Table I summaries these characteristics.

TABLE I.

GENERAL CHARACTERSOF STARTER CULTURES.

Culture	Gram stain.	Sporing	Arrangement and Morphology of Cells.	Growth in Milk and General.
① Dahi No.1	+	-	Small slender rods arranged in chains or singly.	Grows slowly in milk and does not improve on sub-culturing. Had difficulty with M.B. * staining.
② Dahi No.2	+	-	Middle-sized rods very prominent metachromatic staining. Terminal knob. Occur singly.	Grows very well in milk and stains well with M.B. sta

Table I -- Continued.

Culture	Gram stain.	Sporing	Arrangement and Morphology of Cells.	Growth in Milk and General.
(3) Dahi No.3	+	-	Diplococci in pairs. Rarely in short or long chains.	Grows well in milk
(4) L.acidophilus	+	-	Middle-sized slender rods. Occuring in chains or singly.	Grows slowly at first but improves with sub-culturing.
(5) L.bulgaricus (Iowa).	+	-	Short thick rods. In chains long or short.	Grows very well at this temp.

* Methylene blue staining.

Acid Production Studies:-

Since acid production was the main property concerned in the present investigations, preliminary studies to give general idea about total acid production and rate of acid production by each culture have been undertaken.

For these studies, took 100 cc. of skim milk in each of the five flasks and sterilized by autoclaving at 15 lbs. sq.in. for 20 minutes. Inoculated with 10%, 5 days old cultures and incubated at 37°C. Then 9 grms. of sample were weighed from each lot at intervals of 18, 42, 66, 90 and 114 hours, and titrated with $\frac{N}{10}$ NaoH with phenolphthalein as indicator. From titration figures acidity calculated and expressed as percentage of lactic acid. Average of two experiments plotted in graph of Figure 1.

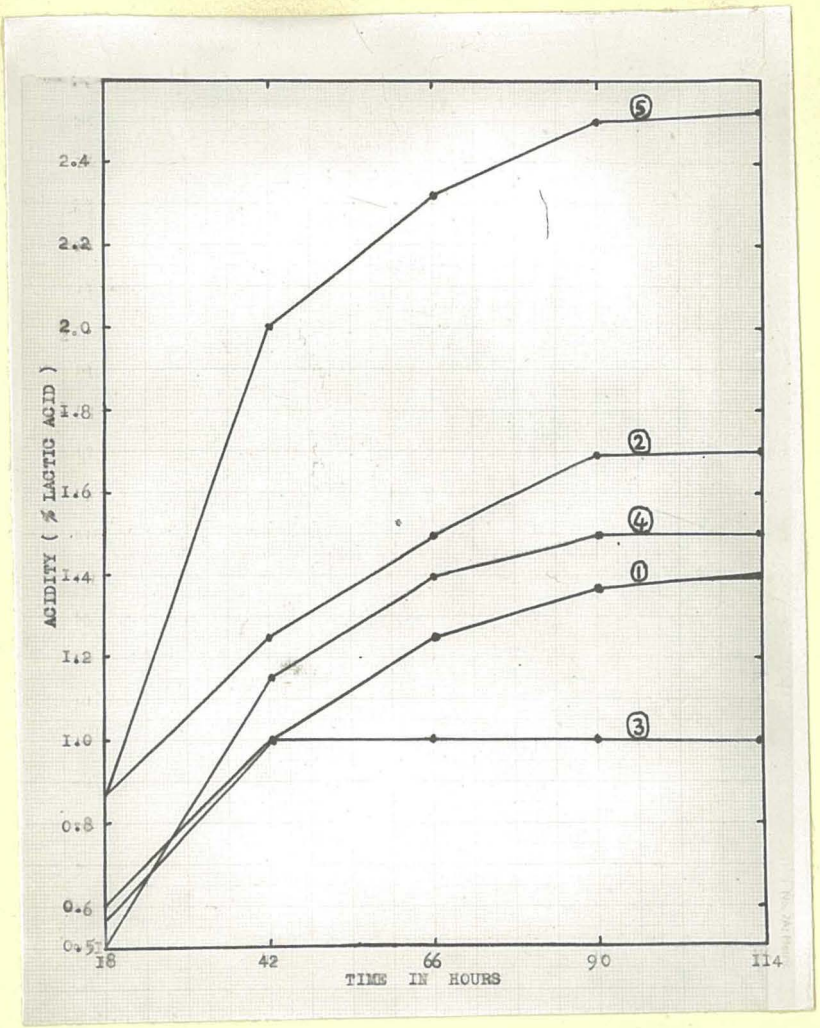


Fig. 1. Graph showing total acid production by different cultures. (For details see Table 1).

From the above graph (Fig. 1) it will be seen that L. bulgaricus (Iowa) produces highest acidity (2.52%), followed by Dahi No. 2 (1.7%), L. acidophilus (1.5%), Dahi No. 1 (1.4%), and Dahi No. 3 (1.0%) in order. The higher acid production is recorded for the first two days

ie. up to 42 hours, second day being most active, then it slows down till maximum is reached in about 90 hours. After this there is no acid production practically by any culture. The maximum acid production in case of Dahi No.3 (Diplococci) is reached within 42 hours after which it stops producing any more.

In order to gain a more exact idea of the rate and quantity of acid production by different cultures during the first 24 hours; since this is usually the period for souring milk for ghee making; another experiment was conducted. 100 cc. of milk for each culture was autoclaved as usual and inoculated with 10% 2 days old culture. Samples kept at $37^{\circ}C$ for incubation and 9 grms. from each was withdrawn at an interval of 4 hours. Titrated with $\frac{N}{10}$ NaoH and acidity calculated as percentage lactic. The results are plotted in Figure 2.

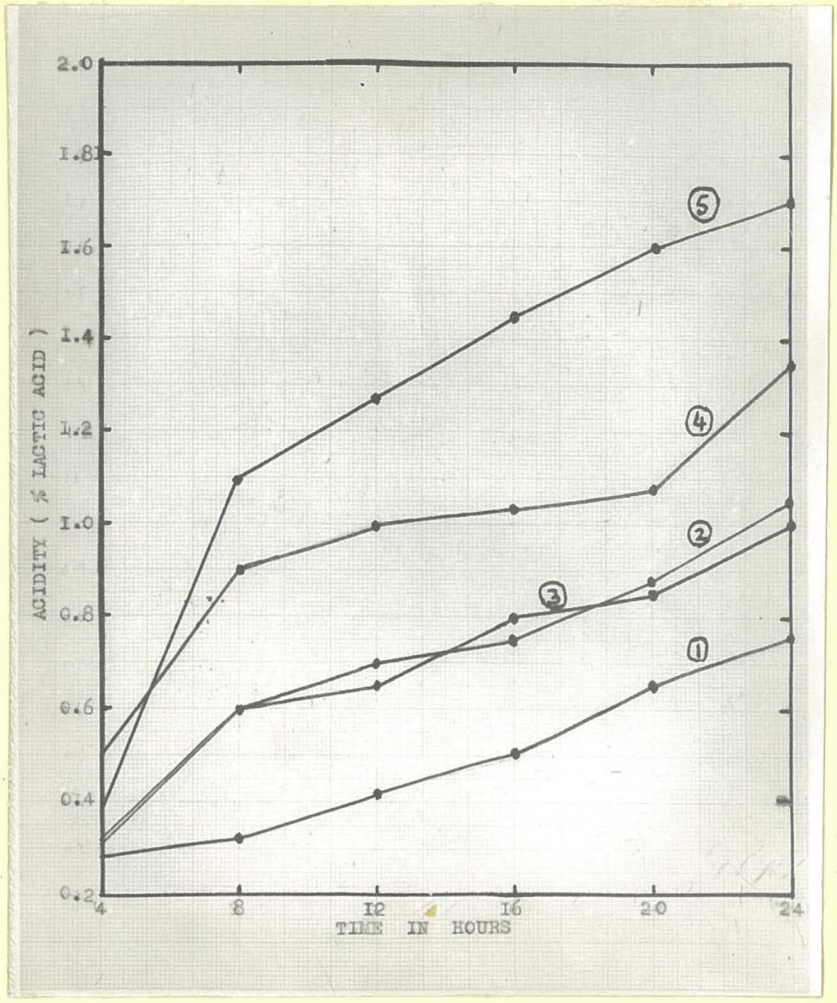


Fig. 2. Graph showing Quantity and Rate of Acid Production for the first 24 hours by different cultures. (For details see Table 1).

From the above graph (Fig. 2.) it will be seen that even within 24 hours there is no uniform rate of acid production from hour to hour. In two days old culture the lag period appears to be short and the cultures start growing within first 4 hours; in the second 4 hours i.e.

from 4 to 8 hours they show an enormous activity while after that the rate slows down. Cultures, L.bulgaricus and Dahi. No. 1 show more steady and gradual rise while the other three seem to behave irregularly. A little more acid has been recorded after 24 hours in this experiment by all cultures as compraed with the first graph and this may be probably due to younger culture used (2 days old as against 7 days old in the first case) and due to subculturing. However the trend is the same that L. bulgaricus (Iowa) is the fastest acid producer, while Dahi. No. 1, slowest; the rest are intermediate between the two. This trend is very well illustrated when we find that for production of 1% acid L. bulgaricus took about 8 hours, L. acidophilus 12 hours, Dahi No. 2 and No. 3., 24 hours and Dahi No. 1 more than 24 hours.

(B) METHODS.

(i) Preparation of Ghee.

The butter (Makhan) prepared in these experiments for conversion into ghee has been on the lines recommended by Indian Dairy Department in its report for the year 1940-41; which aims at standardisation of the deshi or indigenous method. Inter alia, it consists in boiling the milk for a while and then inoculating it with Dahi culture (5 to 10%) after cooling to 100°F and then incubating at room temperature. After 24 to 48 hours when the milk has clotted , it is churned at a temperature of 60 to 80°F,

depending upon the weather, winter or summer, the temperature of the curd being lowered or raised with addition of hot or cold water (5 to 10%). Incidentally water addition at this stage lowers the consistency of the clotted milk and hence facilitates the churning. When the grains start to form ie. froth appears at the surface, luke warm water (100°F) called breakwater to the extent of $\frac{1}{10}$ of the total bulk is added. Churning then continued for another 5 minutes. Next cold water is added (1/8 to 1/10th) to make the butter grains firm before collection and finally the butter is washed twice with cold water (50 to 52°F). The churnings have been done in the Massey College factory with 1 gallon experimental churn (Alfa-Laval). Variation to this technique according to the requirements of the experiment were made wherever needed.

For conversion of Makhan (butter) into ghee; samples were heated on a hot plate in a sauce-pan to various temperatures ie. 80°C, 110°C, 130°C and 150°C, according to experiment and for different times ie. momentarily, 10 minutes, 20 minutes, 30 minutes, etc., Generally at and above 110°C, the curd particles begin to turn brown due to humin formation and start circulating in the bottom of the pan in the form of convection currents in the fat and give a characteristic ghee aroma. Higher temperatures than 120°C give dark brown curd of different intensity and cooked flavour.

After the heating process was completed, the ghee samples were filtered with three-fold No. 1 (12.5c.m.Whatman) filter paper. In some cases where clear orange fat was not obtained they were re-filtered.

The above method was followed in all cases except Exp. No. 2 (preliminary) where direct cream evaporation method as suggested by French (1936) and modified by him in 1938 and with suggestions of Davies (1940) - has been used.

Plastic cream 70% was obtained by reseparating the 42% cream, washed or unwashed. This cream was in certain samples inoculated with starter cultures (L. acidophilus and L. bulgaricus (Iowa)) and incubated at 100°F for ripening. Sweet cream, however was held in the refrigerator overnight. Next day sweet cream and the acid cream both, were evaporated directly on the hot plate. The cream breaks in the process and the fat forms as a layer on the top; while the excess of watery layer settles at the bottom. Then fat is boiled free of moisture by heating to different temperatures ie. 110°C and 130°C and filtered.

(ii) Methods Used for Testing Keeping Quality.

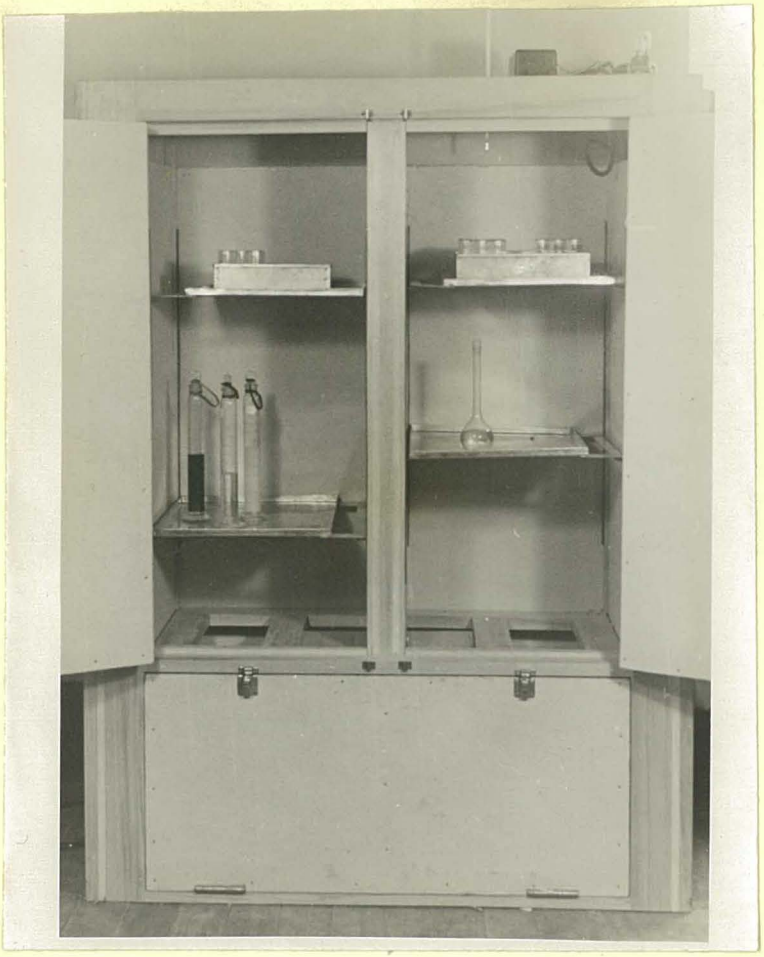
The duration of the induction period as a whole is of greatest practical importance, since it is upon this that storage life (from the point of view of oxidation) largely depends. The majority of natural oils and fats oxidise with a more or less well defined induction period during which absorption of oxygen and change in palatability either

cannot be detected or are relatively small. This is followed by a second period during which the velocity of the reaction increases in a logarithmic manner until it attains its maximal value. Once the period of rapid oxidation (logarithmic) has set in, a pronounced rancid odour and flavour quickly appear and it is almost impossible to save the fat from spoilage (Lea, 1938). Therefore, in these experiments, the end of induction period has been taken as an index of keeping quality. The rate of deterioration and end of induction period have been followed by chemical methods (described later) and organoleptic tests. The following are the two tests mainly used for following deterioration and to ascertain the keeping quality:-

(a) Incubation test at 35-40°C. (Indian summer temp.)

(b) Accelerated test at 100°C.

(a) Incubation method:-



Shows cabinet used for incubating ghee samples at 35 to 40°C. (Indian summer temperature.)

Fig. 3.

A special cabinet (4½' x 3½' x 1½') made locally of pine and insulated with asbestos as illustrated in Fig. 3 was regulated to give Indian summer temperature (35-40°C) though at a later date it had been thermostatically controlled to 100°F. Heating of this cabinet was done by four bulbs of about 300W capacity set at the

bottom. Air generated through a fan fixed at the back of the bulbs was passed over them and heated air thus obtained was circulated through the entire cabinet by means of a double jacket on its two sides. The bulbs were painted black or grey to exclude light and to take extra precaution for shutting of light, they were covered under wooden partition. Thus the cabinet was fitted to exclude light, an important factor for oxidation and maintained as uniform a temperature as possible.

Each treatment of various experiments was put in small tubes of equal size 3" x 1" and filled to the same level, thus keeping the volume and surface exposed to air, uniform. Each treatment was replicated four to five times. After the end of each period, ie. 15 days or 1 month according to need, a sample from each treatment was withdrawn and tested organoleptically and by three chemical methods, details of which are given below (ie. Peroxide Value, fat aldehyde value and carotenoid pigment value (yellow units)). End of the induction period was judged by the rapid absorption of oxygen and complete or partial bleaching of natural yellow colour into yellowish white or white. Chemical methods helped to give graphs and curves of proceeding of oxidation of the sample.

Besides these tests, acidities of many samples have been determined at the beginning (fresh) and at the end of induction period. Moisture content, however, was deter-

mined on the fresh samples only. Details of the various chemical methods used are as follows:-

(i) Peroxide value:-

The method used for determining peroxide value has been that given by Lea (1938) as 'simplified method' and modified by him in details only from time to time afterwards (Lea (1945) and (1946) etc.) The following procedure was followed:-

1 ml. of fat is measured into a test tube; not smaller than 7" x 7/8" and 22 ml. of a 3:2 mixture of glacial acetic acid and chloroform added. Then 0.5grm. of the powdered potassium iodide added and contents of the tube treated over a naked flame to boiling and boil for 30 seconds. Corked the tubes immediately, and cooled under a jet of running water. Poured off the contents of the tubes into 30 ml. of distilled water, added starch solution and titrated with 0.002 N Sodium thiosulphate. Duplicates were occasionally tested to keep a check on the method which agreed within 0.0, to 0.1 ml.

The quantity of Sodium thiosulphate used divided by 0.9 gives the peroxide value per gram of fat.

(2) Fat Aldehyde Value:- *

The methodfollowed for this value is that of Schibsted (1932) and modified by Mummery (1947). The procedure is

* Readings of fat aldehyde and carotenoid values have been taken in D.R.I. Massey College (Chemistry Lab.) with the kind permission of Dr. F.H. McDowall, Chief Chemist.

as follows:-

Pipette into a test tube (6" x 11/16") without lip from 0.5 to 2.0 ml of melted fat according to the freshness or otherwise of fat. Dissolve it in 12.5 ml of purified petroleum ether and add 2.5 ml of the rosaniline reagent. The tube is closed with a rubber stopper covered with tin foil and is then shaken at 30 R.P.M. for four minutes. Decant off 10 ml. of the petroleum ether layer and match the colour in a leitz calorimeter in comparison with a standard colour of cresol red of pH 7.8.

Schibsted originally proposed pH of Cresol red of 8.3 but since for butterfat more yellow is required in the standard solution, it has been modified to pH 7.8 to give desired tint. For taking readings on the calorimeter, in case of high colour, standard is set at 15 while in case of low colour, test solution is set at 15. In case of very high colour the solution is diluted before taking readings. From the readings so obtained, the fat aldehyde value is calculated as follows:-

$$\text{F.A Value} = \frac{100 \times Rs \times 0.1}{Rt \times X}$$

Where Rs = standard colour

Rt = test solution

X = wt. of fat in 100 ml. of petroleum ether.

(3) Acidity:-

The following procedure was adopted;-

6 ml (5 gm) fat pipetted into 250 ml flask with addition of 25 ml. rectified spirit that has been just neutralized to phenolphthalein. The whole thing is brought to boil and boiled gently with shaking for three to five minutes. Titrate at once with $\frac{N}{10}$ NaoH. Have a control. From the reading so obtained, the acidity is calculated by the formula:-

$$\text{Acidity as percentage of Oleic} = \frac{X \times 2.82}{R}$$

where X = cc. of $\frac{N}{10}$ alkali used

R = weight of fat

(Note:- In the original official method 10 gms. of fat taken but due to shortage of sample, only half taken here. Rectified spirit adjusted accordingly.)

(4) Carotenoid Value (or Yellow units).

For estimating carotenoid value (not carotene value) a quick routine method suggested by Mr. W.A. McGillivray, Biochemistry Dept., Massey College, on the basis of results obtained by Gillam (1934) has been followed in these investigations. It gave a little higher value than the usual saponification method possibly because of solvent-effect or because of a tendency for carotene to be destroyed or isomerised during the saponification process. For comparative results for effect of oxidation on colour, it has given a very useful data. The following pro-

cedure has been adopted:-

Took 4½ cc. (4 gms) of melted ghee or butterfat warmed slightly and then shaken for a minute or two with 16 ml. of petroleum ether to extract carotenoid pigments. In case, 4 gms. sample was not available, 2 gms was taken and petroleum ether adjusted accordingly. Took readings on Spekker using O.B.I. filters. The readings so obtained were converted into carotenoid value per gram.

(5) Moisture Content:-

Moisture content in these investigations were determined on the lines of the rapid method followed for estimation of moisture in dry butterfat in New Zealand. The procedure is as follows:-

About 5 gms. (6 cc.) of each sample was heated in a porcelain dish for 3 minutes on a very gentle flame, stirring with a rod in the process. Then cooled in the dessicator, weighed quickly and find out the loss in weight. Duplicates determined to check results occasionally.

Estimation of Moisture in Ghee.

Sunawala and Kothavalla (1935) have determined moisture content of ghee by heating 2 gms. of ghee in an oven at 100-110°C for three hours and finding the loss in weight of samples. Following this procedure (except 5 gms. of ghee taken instead of 2 gms), the following results have been recorded from three hours to 180 hours heating at

the same temperature. (Exp. IV).

TABLE 2.

Weight losses (-) and gains (+). (Exp. IV)
in grms. in 5 grms of fat (nearly).

Treatment No.	3 hours	6 hours	36 hours	60 hours	84 hours	180 hou
No. 1	+ 0.004	+ 0.011	+ 0.002	- 0.049	- 0.062	- 0.176
No. 2	+ 0.003	+ 0.009	+ 0.012	- 0.048	- 0.067	- 0.183
No. 3	- 0.006	- 0.001	- 0.011	- 0.057	- 0.067	- 0.183
No. 4	- 0.004	- 0.000	+ 0.007	- 0.062	- 0.067	- 0.212
No. 5	- 0.004	+ 0.003	- 0.008	- 0.055	- 0.071	- 0.221
No. 6	- 0.002	+ 0.002	- 0.009	- 0.046	- 0.057	- 0.174
No. 7	- 0.004	+0.003	- 0.019	- 0.061	- 0.071	- 0.252
No. 8	- 0.009	- 0.005	- 0.011	- 0.067	- 0.079	- 0.180
No. 9	- 0.000	+ 0.008	- 0.005	- 0.053	- 0.059	- 0.164
No.10	- 0.004	- 0.000	- 0.011	- 0.055	- 0.061	- 0.248

TABLE 3.

Drying losses percentage on heating.
(Calculated from Table 2, for various hours).
(+ represents gain in weight).

Treatment No.	3 hours	6 hours	36 hours	60 hours	84 hours	180 hou
No. 1	+ 0.080	+ 0.220	+ 0.040	0.980	1,240	3.520
No. 2	+ 0.060	+ 0.180	+ 0.240	0.960	1.340	3.660
No. 3	0.120	0.200	0.220	1.140	1.340	3.660
No. 4	0.080	0.000	+ 0.140	1.240	1.340	4.240
No. 5	0.080	+ 0.060	0.160	1.100	1.420	4.420
No. 6	0.040	+ 0.040	0.180	0.920	1.140	3.480
No. 7	0.080	+ 0.060	0.380	1.220	1.420	5.040

(Table contd. on page 26.)

Table 3 continued.

Treatment No.	3 hours	6 hours	36 hours	60 hours	84 hours	180 hours
No. 8	0.180	+ 0.100	0.220	1.340	1.580	3.600
No. 9	0.000	+ 0.160	0.100	1.060	1.180	3.280
No.10	0.080	0.000	0.220	1.100	1.220	4.960

From the above results, it will be seen that moisture determination by this method presents a complicated problem. Even in a few hours time some samples start gaining in weight through oxidation. So depending on the number of hours heated, one can get any result. Another thing clear from these reults is that when samples are heated for moisture determination in an oven at 100 to 110°C, the following changes in the ghee appear to be taking place ie. first the small percentage of moisture in the sample gets driven off (gives loss in weight), then the oxidation sets in (gives gain in weight) and lastly the fat breaks into component parts, volatalizing some of the volatile acids liberated which give the loss in weight. Different treatments attain these three stages at different hours of heating and it is really a problem to draw a line between loss due to true moisture evaporation or due to fatty acids volatalizing. Probably for these difficulties Ritter (1937) used Vacuum drying and Patil and Hammer (1928) used Mojonnier tester for determination of moisture content in ghee. In view of above facts, it appears the method advocated by Sunawala and Kothavalla, require further investigations and more exact def-

ining of conditions to suit all kinds of ghee treatments. For want of time, however, no further work on this method and comparative work on other methods used by Ritter and Patil and Hammer, could be undertaken. In the present investigations, solely on the ground of convenience, a very simple and quick method was followed for estimation of moisture in dry butterfat in factory, has been employed. It has given fairly constant figures, duplicates varying between 0.010 to 0.020% which should not materially affect the general trend of results. Of course, average of two has been taken in such cases.

(b) Accelerated Test Method:-

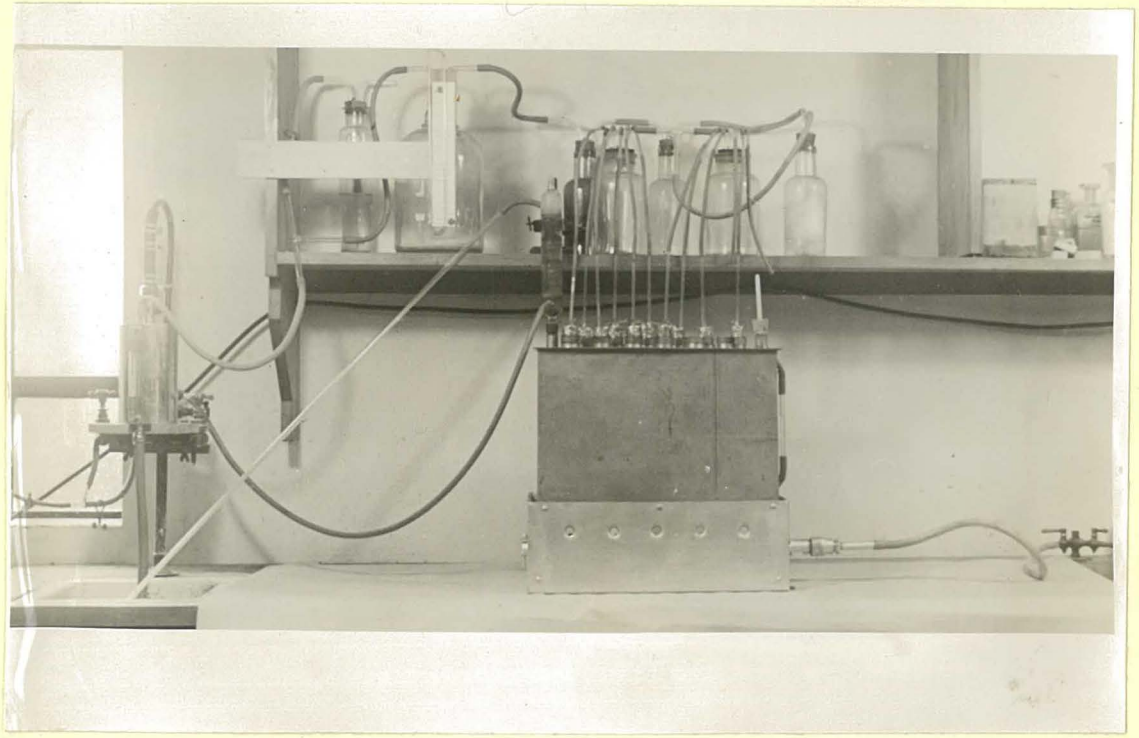


Fig. 4. Shows "Swfit Stability Tester" for testing oxidation of ghee samples by accelerated test at 100°C.

In order to cut down the time of test in the cabinet which takes from 4 to 8 months or more to show results, an accelerated tester commonly known as "Swift stability tester" has been used in these experiments. The tester used (Fig. 4) has been designed locally on the lines and principle of Wheeler (1932) and subsequently modified by King et al (1933), Freyer (1936) and others. 40 cc of the test fats or ghees have been filled in 8" x 1" tube with a lip and inserted in the water container at top in special made grooves. The water-bath is brought to boil with gas (100°C) and then dry air bubbled through the fat at a regular rate. Peroxide Value by the method of Lea, (cited previously), is determined initially, and at a regular interval of four to six hours, to give an idea of progress of deterioration in keeping quality. Bleaching of colour was marked at an hour's interval. The end of the induction period was judged as in other method by the rapid absorption of oxygen shown by sudden jump up in the peroxide value and the bleaching of colour. In case colour has not bleached completely before rapid absorption of oxygen takes place, the start of rapid oxygen absorption has been taken as the end of induction period. At the time of withdrawal of sample for testing at four or six hours interval, organoleptic tests have been noted.

Except Experiment I and II, this test has been used in all cases. For the three experiments, ie. Experiment III,

IV and V (B), both tests ie. incubation test and accelerated test - have been used.

CHAPTER III.

EXPERIMENTAL DATA.

Section (a):- Experiments to find out role of various factors like acidity; cooking process; bacterial culture and moisture.

In order to gain some knowledge as regards the possible factor (mentioned in Chapter I by various Workers) affecting the keeping quality of ghee; two preliminary experiments, (Experiments No. I and No. II) one of each method of making ghee (i.e. (i) from sour milk and (ii) from direct cream evaporation) were arranged. Three variables were included i.e. different bacterial cultures; different temperatures of cooking and different acidities of manufacture. After that in Experiments III and IV proceeded further to find out the effect of some of these factors under more controlled conditions to clarify the ideas and assumptions formed as a result of preliminary experiments.

Experiment I (Preliminary):-

Makhan (butter) for boiling into ghee was obtained in this experiment by inoculating two lots of one gallon each fresh milk with L.acidophilus and L.bulgaricus (Iowa) cultures and churning them at acidities of 0.6 and 1.75% lactic respectively. Each lot of butter obtained thus was heated to 110°C and 130°C . New Zealand butter made from (Massey College factory sample No. B.F.199, I-27-I) unneutralised cream of 0.11% acidity and vacreated at 200°F; was used as control. This butter was graded 93 points at Auckland and Wellington and it

was heated to 80°C and 130°C for the purpose.

These ghee samples were then incubated at 37°C for 11 weeks. Peroxide, Fat aldehyde and carotenoid value have been determined initially and at intervals of a week during the course of the induction period. Free fatty acid content could be estimated at the end of the induction period only. Organoleptic tests were also made which agreed with the chemical methods. Values obtained for Peroxide, Fat aldehyde and Carotenoids - have been plotted in graphs of Figures 5, 6 and 7 respectively and particulars of treatments, induction periods*(as indicated by peroxide value) and acidities are given in Table 4.

TABLE 4.

Particulars of Treatments, Induction Periods and acidities of Experiment I.

No.	Treatment.	Induction Period in weeks.	Acidity % Oleic (at end of induction).
No.1	Control-heated to 80°C	7	0.451
No.2	" " to 130°C	6	0.400
No.3	L.acidophilus butter heated to 110°C (churning acidity 0.6%).	8	0.730
No.4	- ditto - heated to 130°C	10	0.400
No.5	L.bulgaricus (Iowa) butter heated to 110°C (churning acidity 1.75%).	6	1.015
No.6	- ditto - heated to 130°C	7	1.071

*For purposes of these investigations; induction period as shown by Peroxide method has been arbitrarily taken as standard and all other methods compared with it.

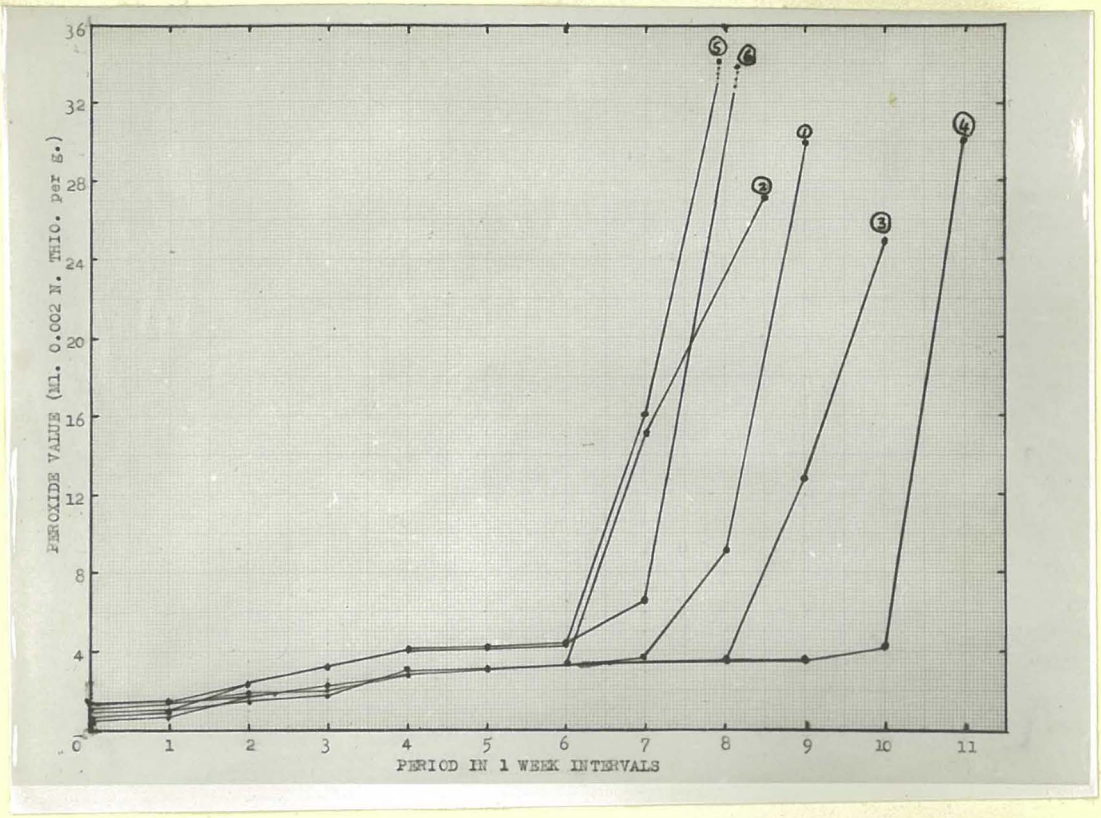


Fig. 5. Graph showing oxidation and induction periods by Peroxide method (details given in Table 4, page 31).

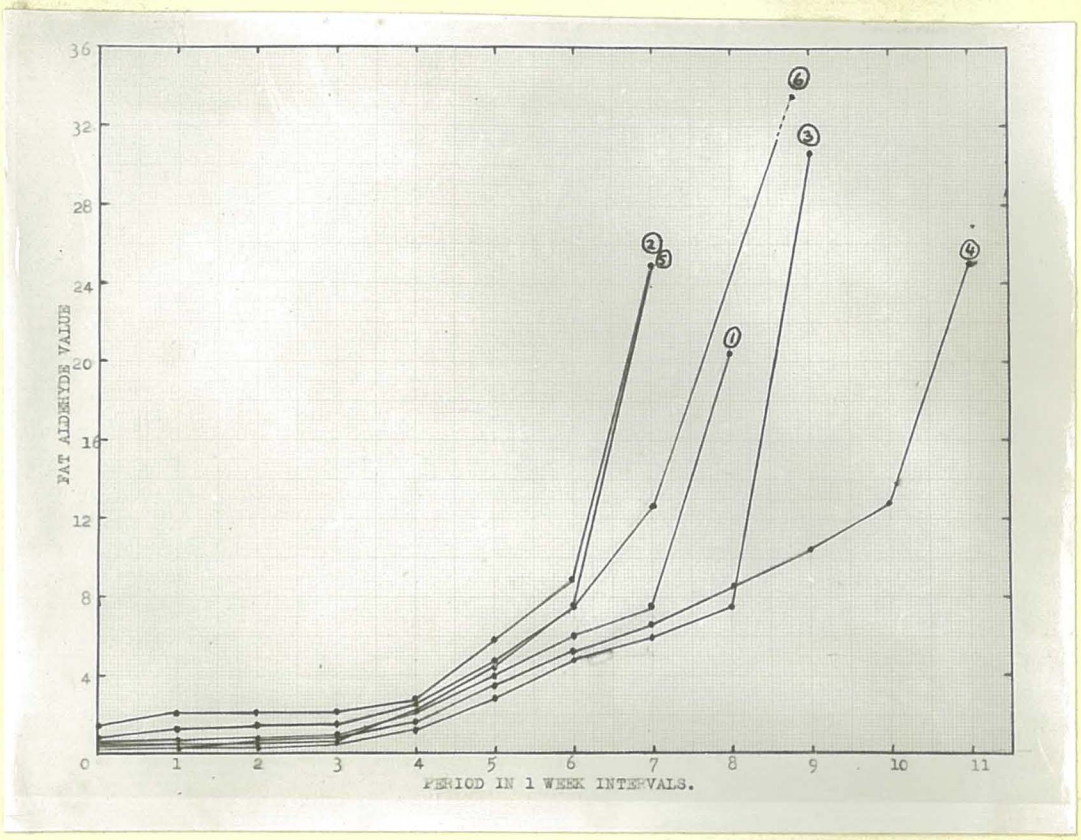


Fig. 6. Graph showing oxidation and induction periods by Fat aldehyde method (Curves 1 - 6 correspond with treatments listed in Table 4, page 31).

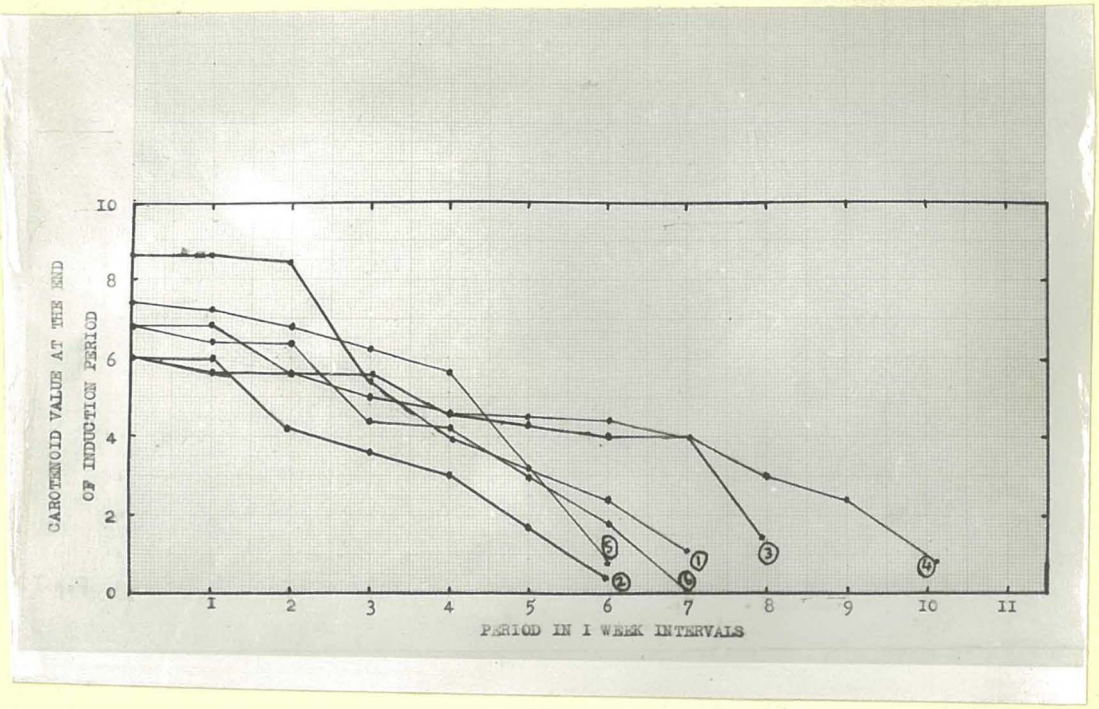


Fig. 7. Graph showing bleaching of colour (Carotenoid pigments) by oxidation (Curves 1 - 6 correspond with treatments listed in Table 4, page 31).

The above results show that:-

(i) L. acidophilus cultured ghee heated to 110°C and 130°C is superior to control and L. bulgaricus ghee similarly heated by 1 to 4 weeks and that L. bulgaricus sample has

nearly the same keeping quality as control.

(ii) That higher (130°C) and lower (110°C) temperatures differ in their effect on keeping quality; higher temperature heating being superior to lower one. This is true in both types of ghee, ie. obtained either by souring with L. acidophilus or L. bulgaricus cultures. However, in the case of control the effect is just the opposite of those observed in case of soured milk samples, i.e. lower temperature giving higher induction period and vice versa.

(iii) That both methods i.e. Peroxide and Fat aldehyde have given identical induction periods except in sample No. 6 where, by Peroxide method 7 weeks are recorded against 6 of aldehyde method. Thus there is a good correlation between these two methods.

(iv) All samples show bleaching at the end of induction period but to a varying degree, e.g. samples 2, 4, 5, and 6 giving practically complete bleaching (arbitrarily carotenoid value of 1 and below 1 is treated for this purpose as fully bleached since in ghee perfect white is difficult to obtain due to changes brought about by heating at higher temperatures), while in case of samples No. 1 and 3, partial bleaching has been recorded giving a value of above 1. However, 1 or 2 weeks after this period, all became fully bleached.

Experiment II (Preliminary):-

Since the cream evaporation method of French (1936) is likely to have great possibilities in simplifying the ghee

manufacturing process, this experiment was arranged to find out the storage life of the product so obtained. For this purpose, 4 gallons of 42% fresh cream was procured and divided into two equal lots. One lot was diluted with warm water (100 to 110°C) so as to wash the casein out of cream to reduce curd content, and reseparated to give 70% plastic cream; while the other lot was reseparated as such, for getting plastic cream, without washing. The first lot was further subdivided into two equal halves; one half kept for evaporating sweet while the other half was soured with L. acidophilus culture. The second lot likewise was divided into two and soured with L. acidophilus and L. bulgaricus cultures separately. All these four lots were then evaporated directly on the hot plate at 110°C and 130°C except sweet cream lot which by chance got heated to 115°C and 130°C instead. Massey College butter from project No. B.F. 203, 1-5-3 made from cream with 0.14% initial acidity neutralised to 0.07% and vacreated at 202°F was used as control after heating to 80° C and 130°C. It was graded 92 points at Wellington and Auckland. The samples so obtained were incubated in the cabinet (35°C to 40°C) and withdrawn for all the chemical tests mentioned in Experiment I at intervals of 2 weeks for a period of 22 weeks. Results are tabulated and plotted in Table 5 and graphs of Figures 8, 9 and 10 respectively.

TABLE 5.

Particulars of Treatments, Induction Periods and Acidities of Experiment II.

No.	Treatments.	Induction Periods in weeks.	Acidity % Oleic	
			Initial	Last
No.1	Control heated to 80°C	10		
No.2	Control " " 130°C	6	0.169	0.33
No.3	Sweet cream (washed) heated to 115°C	16	0.169	0.36
No.4	Sweet cream (washed) heated to 130°C	18	0.169	0.34
No.5	Sour cream (washed) heated to 110°C (L.acidophilus culture).	10		
No.6	Sour cream (washed) heated to 130°C.(do.culture).	10	0.282	0.44
No.7	Sour cream (unwashed) heated to 110°C (L.acidophilus culture)	10	0.169	0.28
No.8	Sour cream (unwashed) heated to 130°C (do.culture).	10		
No.9	Sour cream (unwashed) heated to 110°C (L. bulgaricus culture)	14		
No.10	Sour cream (unwashed) heated to 130°C (do.culture).	10	0.169	0.30

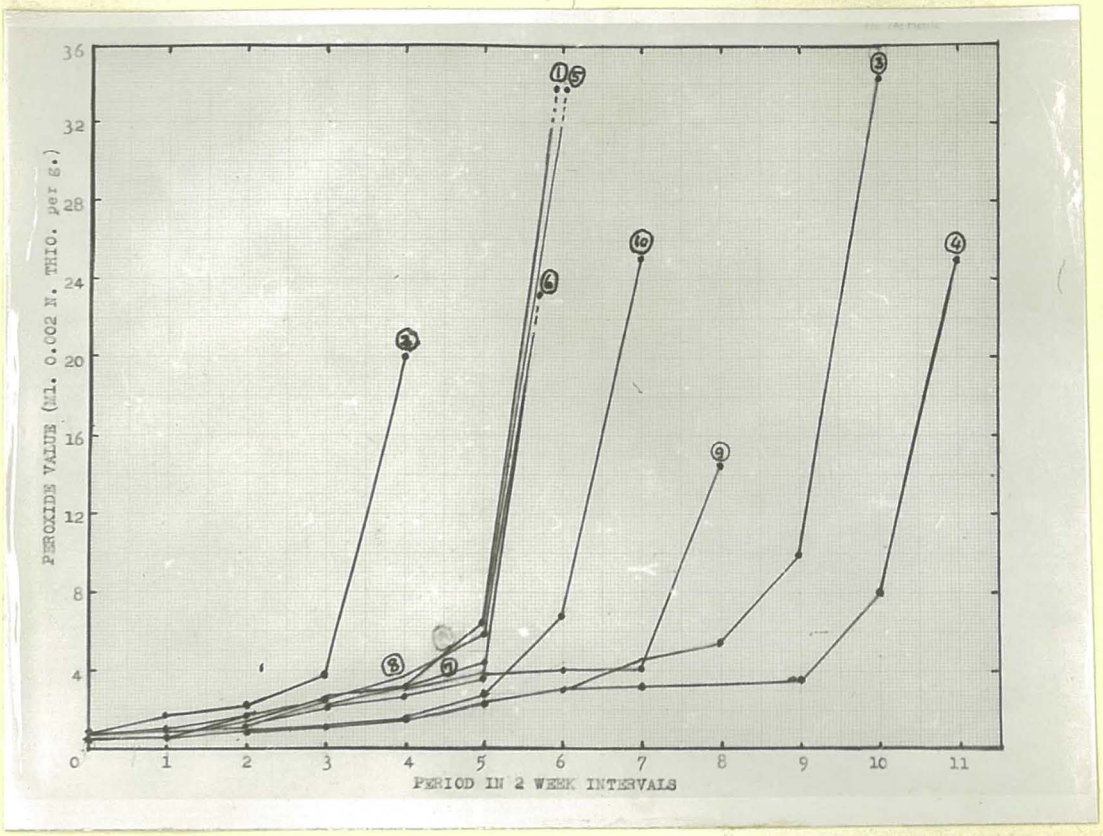


Fig. 8. Graph showing oxidation and induction periods by Peroxide method (details given in Table 5, page 37).

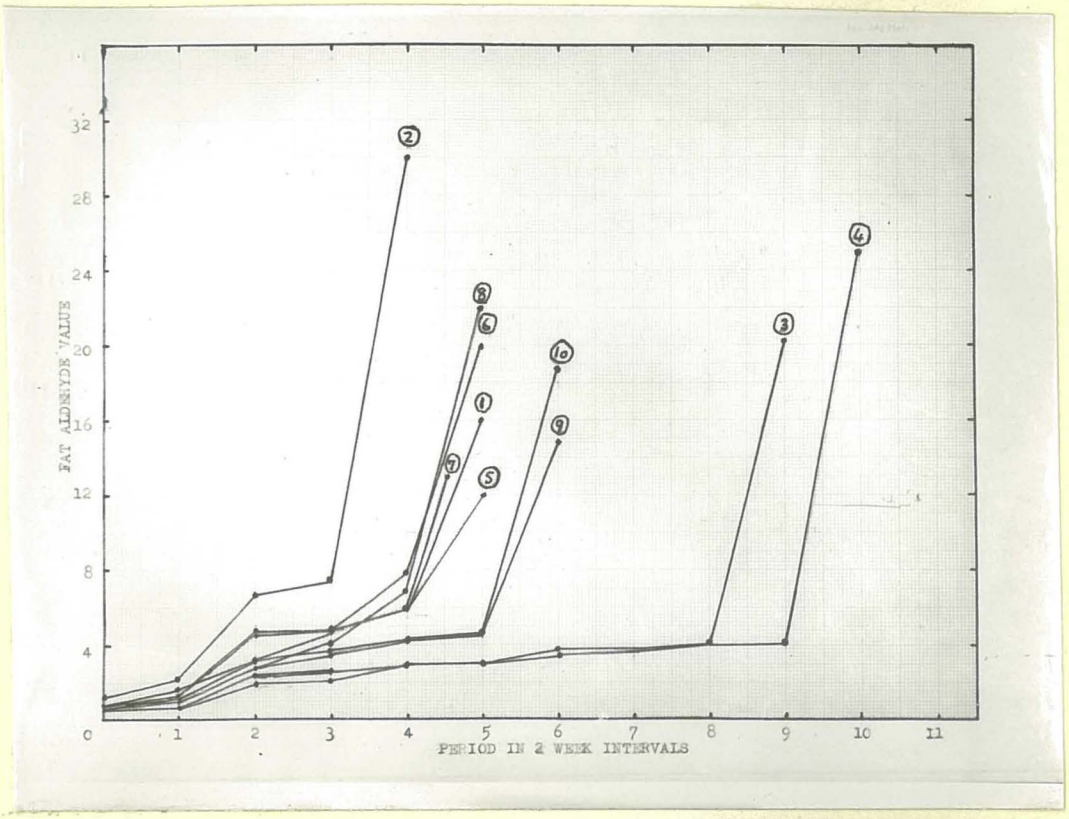


Fig. 9. Graph showing oxidation and induction periods by Fat aldehyde method (curves 1 - 6 correspond with treatments listed in Table 5, page 37).

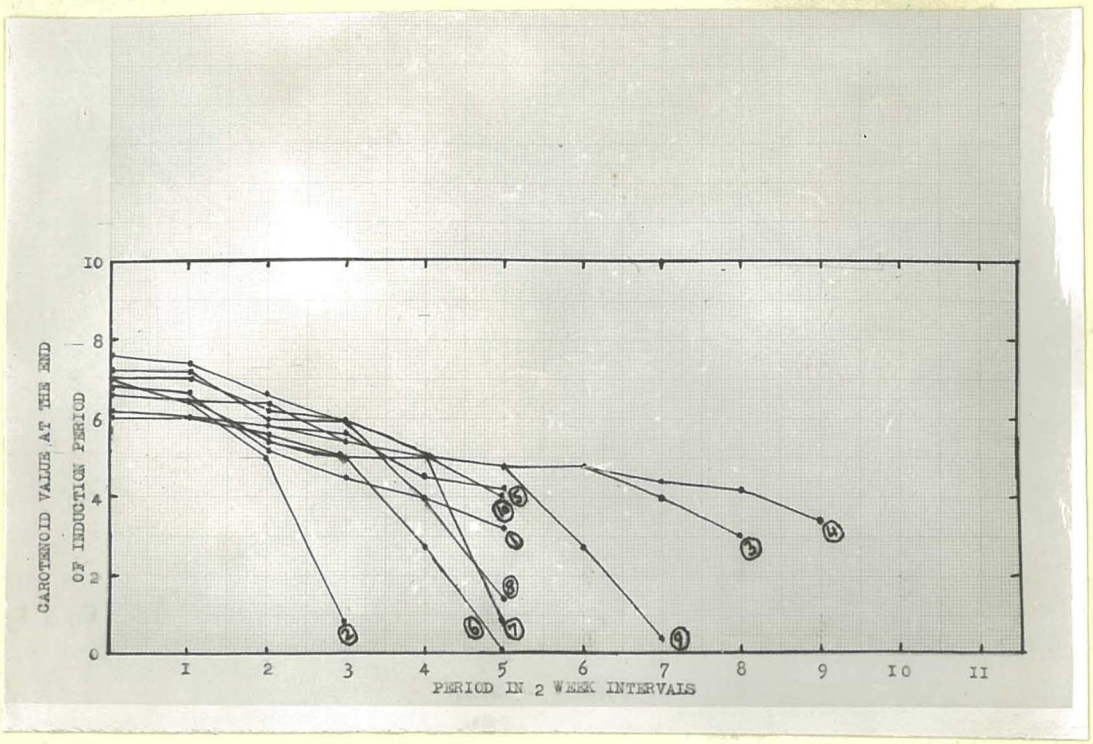


Fig. 10. Graph showing bleaching of colour (Carotenoid pigments) by oxidation. (Curves 1 - 6 correspond with treatments listed in Table 5, page 37).

From the above data it will be seen,

(i) That there is difference in keeping quality when the cream treatment is varied before evaporation (i.e. sweet or sour). Sweet cream on the whole, in both temperatures

keeping better than sour cream and control. In case of sour cream (with L. acidophilus) there do not appear to be any effect of washing and not washing cream before separation into plastic cream as is evident from samples, 5, 6, 7 and 8. The effect of washing and not washing on sweet cream has not been investigated.

(ii) That contrary to Experiment I, L. acidophilus ghee has not shown any superior keeping quality over L. bulgaricus and control.

(iii) That identical induction periods have been given by both method i.e. Peroxide and fat aldehyde in case of samples Nos, 2, 3, 4 and 10; while in case of samples Nos. 1, 5, 6, 7, 8, and 9 there is a difference; the range of difference being 2 to 4 weeks. However, the trend of results in both methods is the same i.e. sample 4 has longest induction period followed by 3, while sample 2 has the shortest.

(iv) Like Experiment I, all samples show bleaching at the end of induction period but to varying degrees; samples Nos. 2, 6, 7 and 9 showed complete bleaching while samples Nos. 1, 3, 4, 5, 8 and 10 show incomplete bleaching. However, after one or two weeks after this, they were all completely bleached.

Further work on this method of manufacture of ghee was discontinued for want of time.

Experiment III:- Variation in Keeping Quality Due to Difference in Starter Cultures.

Dahi cultures (mixed or pure bactobacilli) are used throughout India for souring milk in ghee manufacture. However, like cheese there are certain disadvantages in using mixed

cultures i.e. uniformity in quality cannot be maintained from day to day and therefore, it is likely that in future, pure cultures or single strains of pure cultures may be isolated and employed for ghee manufacture. The question will therefore arise as to which pure or single strain culture gives better keeping quality and other qualities. Experiment I (preliminary suggested that L. acidophilus is superior from keeping quality point of view than L. bulgaricus (Iowa). This was thought feasible on the ground that perhaps, there might be some difference between the metabolic end products of these two bacteria which may exert some influence on the keeping quality. To test this assumption, one gallon milk each was soured with three Dahi cultures (No. 1, 2 and 3) obtained from India, and two pure cultures (i.e. L. acidophilus and L. bulgaricus (Iowa) got from D.R.I. Massey College. So that the effect due to bacterial cultures may not be affected by different acidities produced by them during the course of souring for certain number of hours, they were all taken to the same acidity i.e. 0.90% lactic. Different cultures took varying number of hours for reaching this acidity as will be apparent from graph in Figure 2 (page 14). Churnings were done as usual and butter from each culture was heated to 110°C and 130°C. Samples were tested for induction periods by usual method of incubating them in the cabinet and testing at intervals of four weeks for a period of 36 weeks. In addition an accelerated test by the "Swift Stability Tester" was run on the samples. Results obtained are tabulated and plotted in Table 6 and graphs of

Figures 11, 12, 13 and 14 respectively.

TABLE 6.

Particulars of treatments, induction periods and acidities of Experiment III.

No.	Treatments.	Induction Period		Acidity % oleic	
		In hours by accelerated test.	In weeks by Incubation. test.	Initial	last
No.1	Dahi No.1 heated to 110°C	18	20	0.676	0.90
No.2	Dahi No.1 heated to 130°C	30	24	0.676	0.91
No.3	Dahi No.2 heated to 110°C	18	20	0.733	0.84
No.4	Dahi No.2 heated to 130°C	44	28	0.733	0.88
No.5	Dahi No.3 heated to 110°C	22	24	0.676	0.73
No.6	Dahi No.3 heated to 130°C	46	28	0.676	0.75
No.7	L.acidophilus heated to 110°C	26	24	0.676	0.84
No.8	L. acidophilus heated to 130°C	30	24	0.733	0.84
No. 9	L. bulgaricus(Iowa) heated to 110°C	18	20	0.733	0.84
No.10	L. bulgaricus (Iowa) heated to 130°C	56	32	0.733	0.75

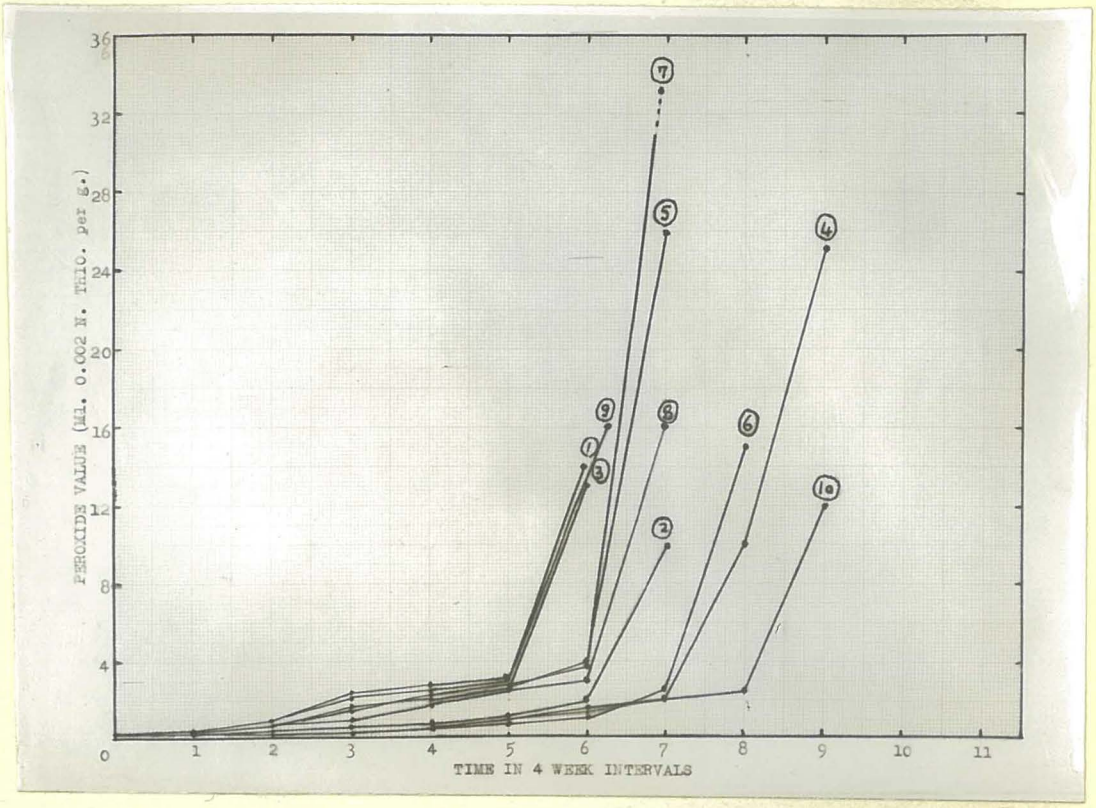


Fig. 11. Graphs showing oxidation and induction periods by Peroxide method. (Details given in Table 6, page 43).

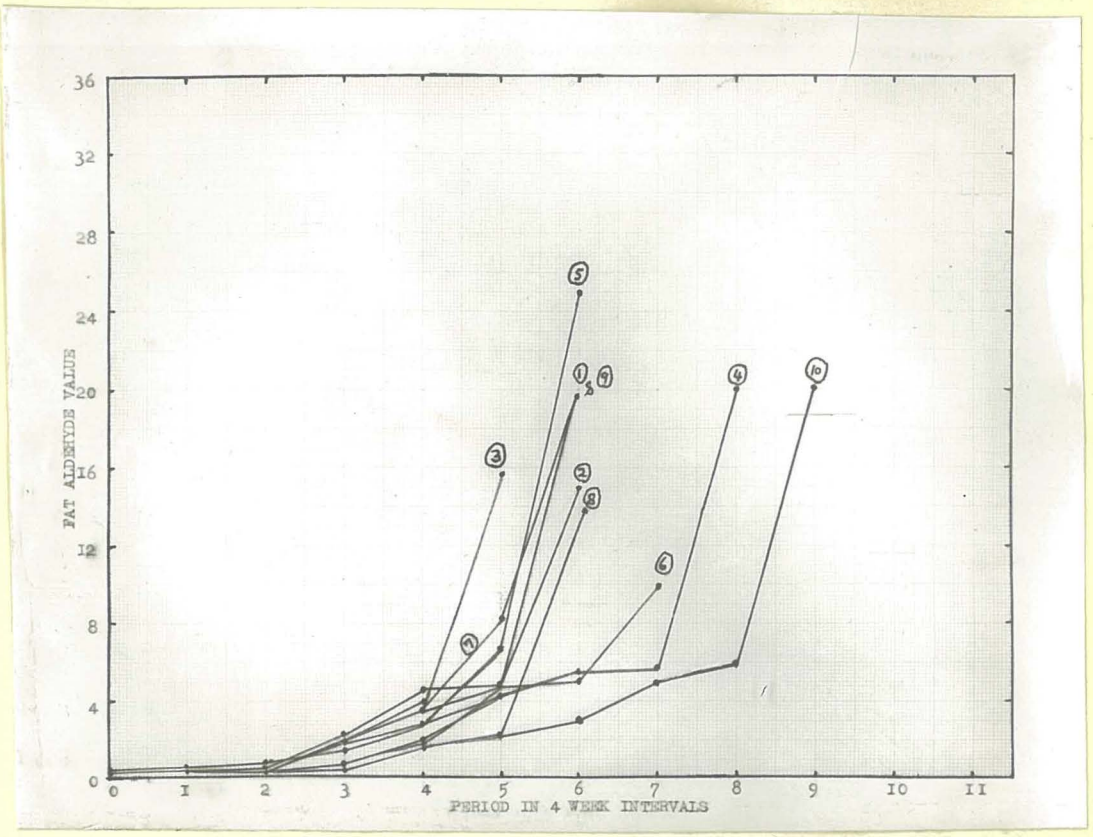


Fig. 12. Graph showing oxidation and induction periods by Fat aldehyde method. (Curves 1 - 6 correspond with treatments listed in Table 6, page 43).

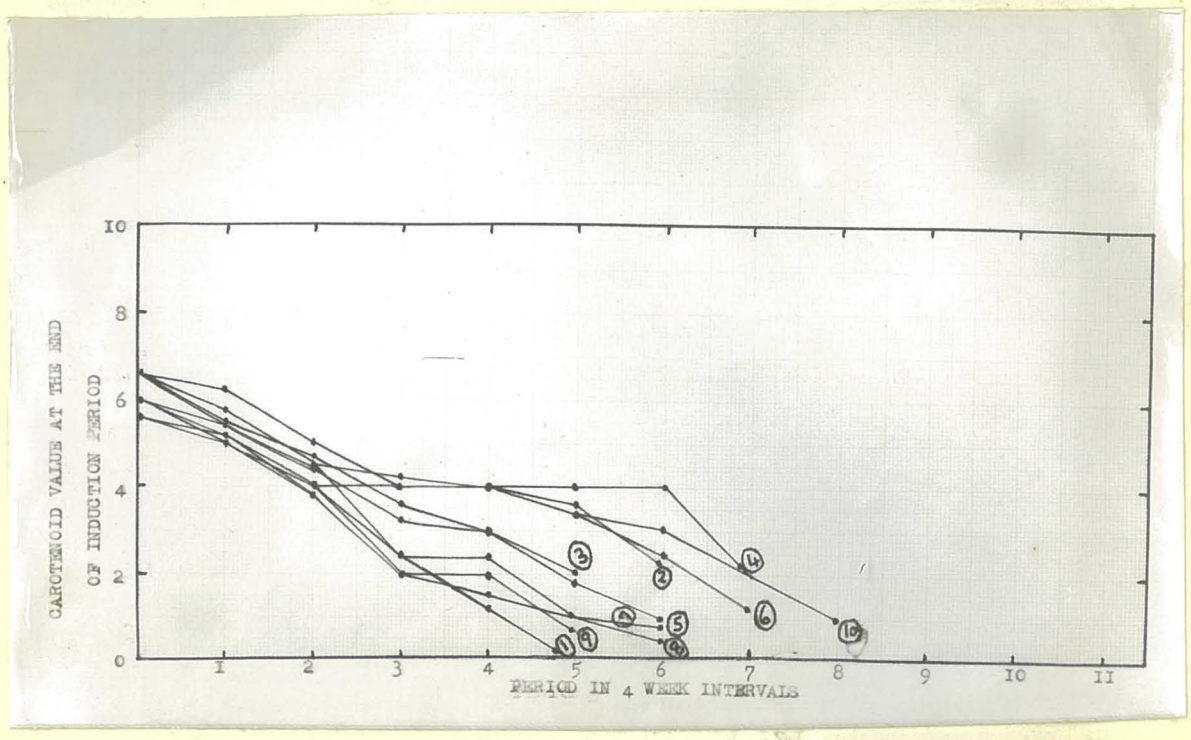


Fig. 13. Graph showing bleaching of colour (Carotenoid pigments) by oxidation. (Curves 1 - 6 correspond with treatments listed in Table 6, page 43).

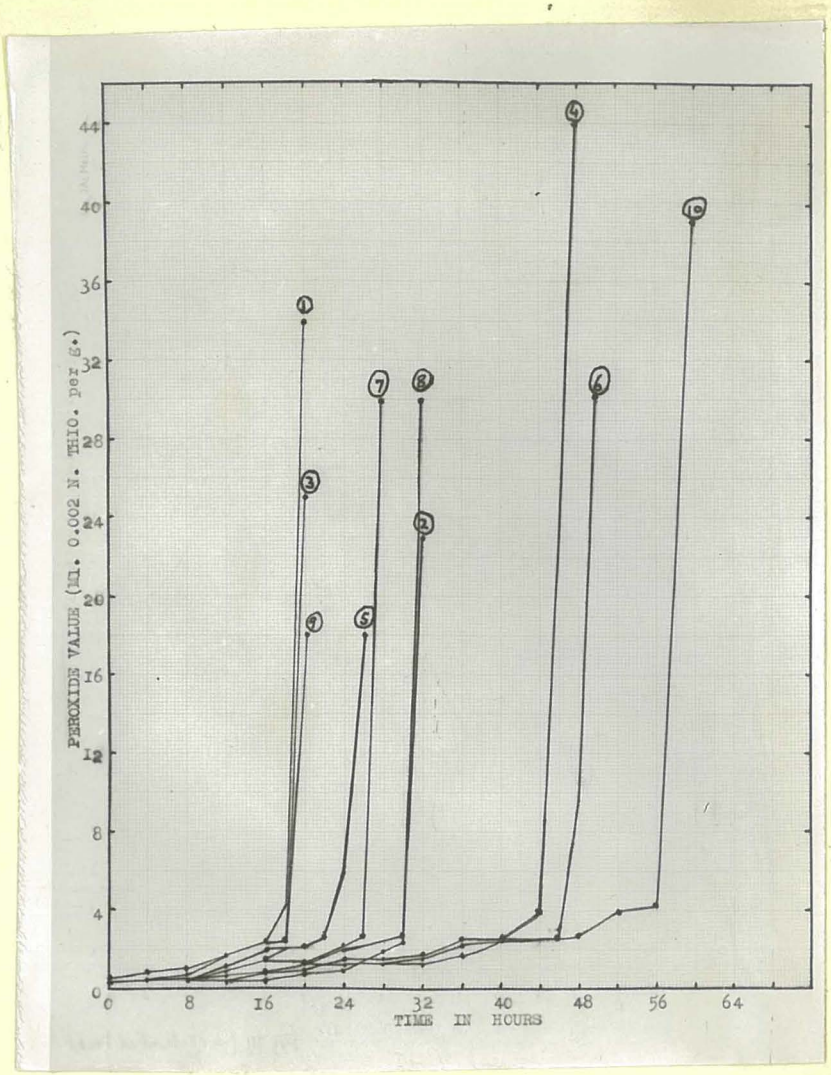


Fig. 14. Graph showing oxidation and induction periods by accelerated test at 100°C. (Curves 1 - 6 correspond with treatments listed in Table 6, page 43).

From the above data, it will be seen

(i) That there is a variation in induction period noticed in

different cultures and more in higher temperatures (130°C) and less in lower temperatures (110°C) but this does not appear to be due to difference in bacteria since the same culture under identical conditions has given two different induction periods when heated to 110°C and 130°C, (as will be apparent from all samples except 7 and 8 (only in incubation test)). Also both temperature treatments of one culture is not clearly superior to other cultures. Thus the variation in this experiment appears to be due to temperature effect rather than due to different bacteria. So also L. acidophilus has not behaved similarly to Experiment I showing its superiority over L. bulgaricus and therefore, it is thought likely that difference in Experiment I between these cultures may be due to different acidity produced by them during an incubation period of 18 hours.

(ii) There appears to be a general (not absolute) correlation between the induction period observed by accelerated test at 100°C and incubation test at 35 to 40°C; the correlation trend being maintained in both 110°C and 130°C heated samples. By both tests No. 10 sample has given the longest induction period followed by No. 6 and No. 4 while shortest is given by No. 1 and 3. However, in certain cases, the difference of 2 to 8 hours in the accelerated test did not give any significant difference in the incubation test, e.g. in samples, 2, 5, 7 and 8.

(iii) Like Experiment II, identical induction periods have been given by both Peroxide and Aldehyde methods in case of samples 1, 4, 9

and 10, while unidentical periods have been recorded in case of samples 2, 3, 5, 6, 7 and 8 the range of difference being four weeks in each case. However, the trend of results in both cases is the same i.e. sample No. 10 giving the longest induction period followed by No. 4 and No. 6, whilst No. 3 gave the minimum induction period.

(iv) All samples show bleaching at the end of the induction period but to a varying degree, e.g. sample Nos. 1, 5, 7, 8, 9 and 10 show complete bleaching, while samples 2, 3, 4 and 6 show partial bleaching. Complete bleaching however in them was obtained after 3 to 4 weeks.

(v) All samples show rise in acidity during storage of 32 weeks but range of rise is small i.e. from 0.022 to 0.236%.

Experiment IV:- Variation in Keeping Quality Due to Different Acidity. (same Culture).

As mentioned in Chapter I, many workers found rise in acidity during storage of butterfat or ghee, and they have linked it to the keeping quality, higher acid ghee supposed to give shorter storage life and vice versa. In order to test this assumption, 4 gallons of milk was inoculated after usual processing with 10% L. bulgaricus culture and the lot divided into four tinned cans, each with a gallon. They were incubated for different hours so as to give acidities of 1.1%, 1.7% 2.2% and 2.5% at churning. Butter so obtained was melted to 110°C and 130°C from each acidity. Massey College butter made from unneutralised cream having an acidity of 0.08% and made in the usual way was heated to 80°C and 130°C to serve as control.

Oxidation studies, as usual were carried on by incubating them in the cabinet for 36 weeks and withdrawing samples at intervals of four weeks for testing. An accelerated test was also run. Result obtained are tabulated and plotted in table 7 and graphs 15, 16, 17 and 18 respectively.

TABLE 7.

Particulars of treatments, induction periods, acidities and Moisture percentage of Experiment IV.

No.	Treatment	Induction Periods		Acidity % oleic		Moistur
		in hours by accelerated test.	in weeks by incubation test.	Initial	Last	%
No.1	Control heated to 80°C	12	16	0.1692	0.4512	0.04
No.2	Control heated to 130°C	30	20	0.1692	0.3384	0.19
No.3	1.1% heated to 110°C	12	16	0.3384	0.5640	0.46
No.4	1.1% heated to 130°C	42	28	0.5076	0.6076	0.27
No.5	1.7% heated to 110°C	18	16	0.6768	0.8640	0.23
No.6	1.7% heated to 130°C	30	24	0.6768	0.7768	0.19
No.7	2.2% heated to 110°C	18	16	0.9024	1.2092	0.24
No.8	2.2% heated to 130°C	42	28	0.9024	1.350	0.53
No.9	2.5% heated to 110°C	18	16	0.5012	0.5640	0.19
No.10	2.5% heated to 130°C	18	20	0.5076	0.5640	0.29

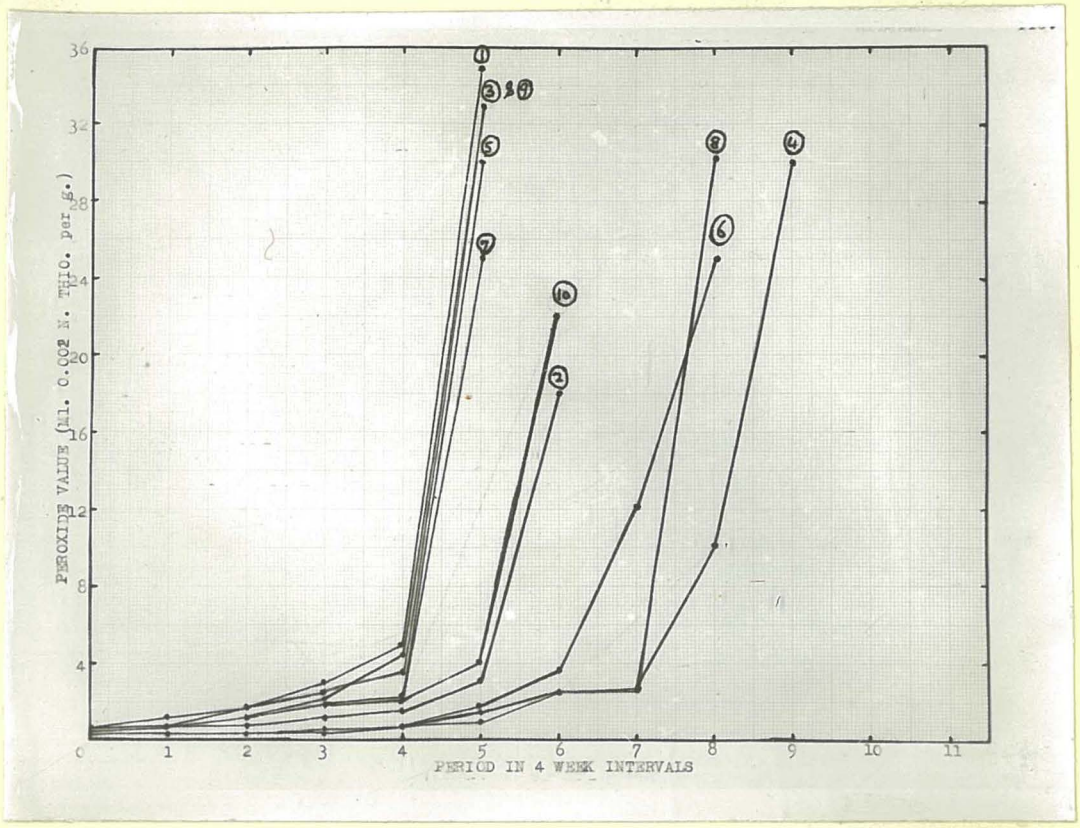


Fig. 15. Graph showing oxidation and induction periods by Peroxide method. (Details given in Table 7, page 50).

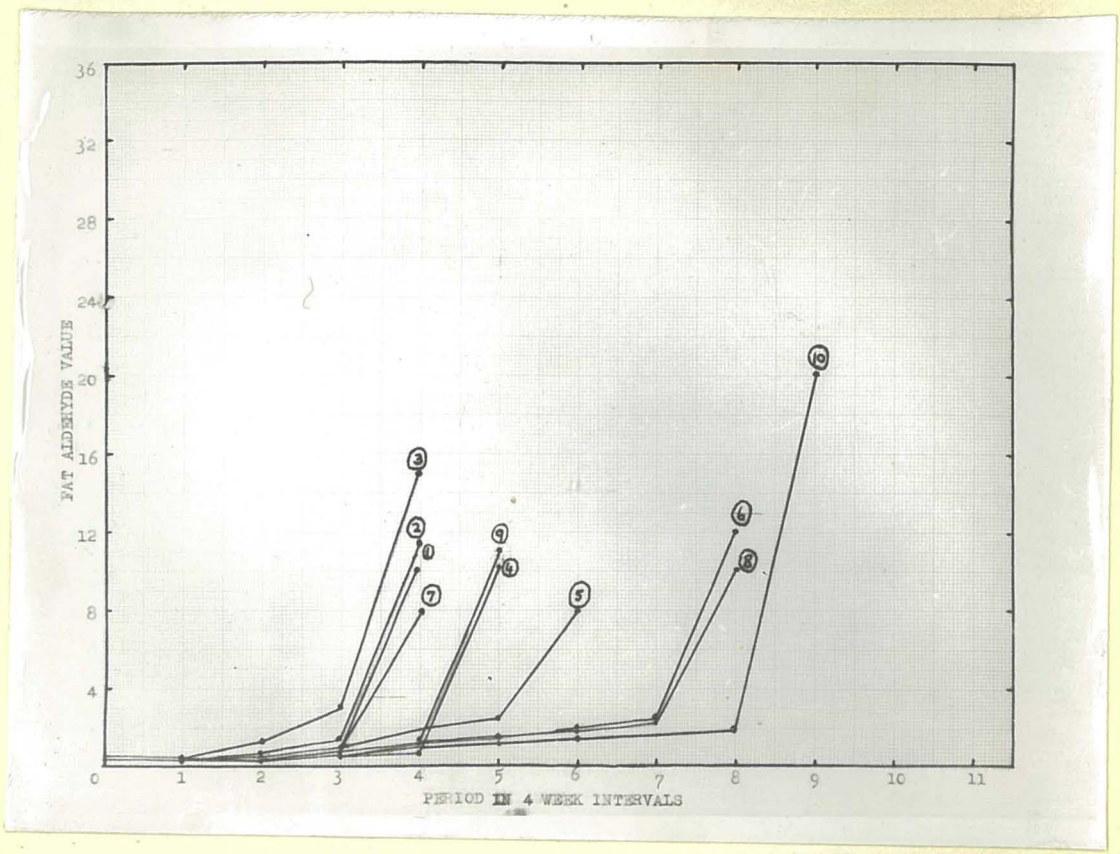


Fig. 16. Graph showing oxidation and induction period by fat aldehyde method. (Curves 1 - 6 correspond with treatments listed in Table 7, page 50).

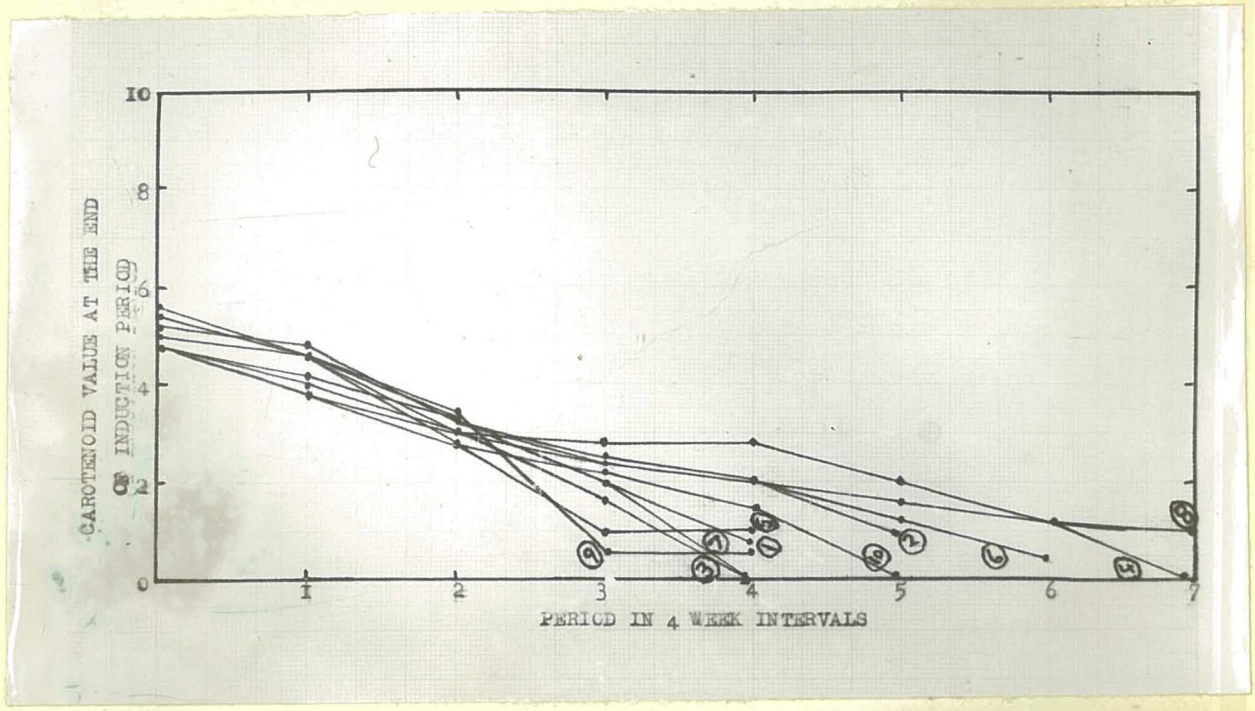


Fig. 17. Graph showing bleaching of colour (Carotenoid pigments) by oxidation. (Curves 1 - 6 correspond with treatments listed in Table 7, page 50).

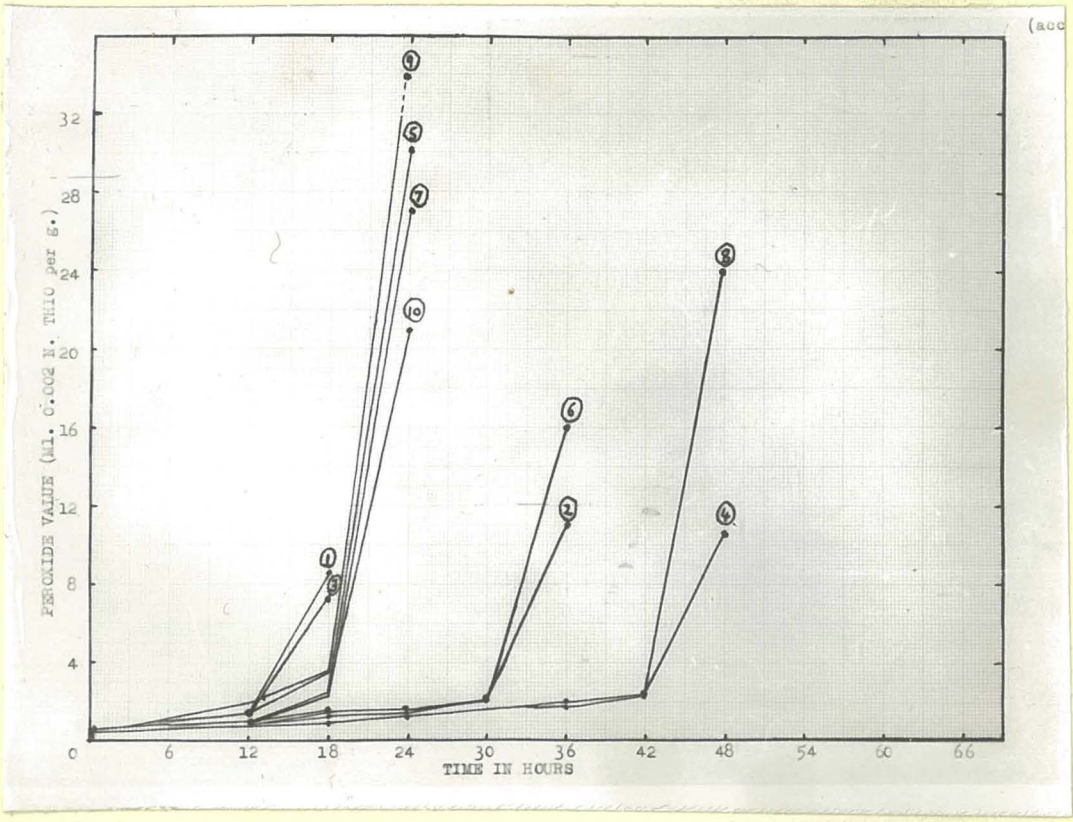


Fig. 18. Graph showing oxidation and induction periods by accelerated test at 100°C. (Details given in Table 7, page 50).

From the data presented above, it will be seen

(i) That there do not seem to be any relation between ghee acidity and keeping quality, e.g. sample 8 having the highest

acidity of 0.9024% has given the same induction period (i.e. 28 weeks by incubation test) as sample 4 having an acidity of 0.5076%. Not only that, but even the same acidity in two samples has not given the same induction period e.g. sample 5 and 6 and 7 and 8. Had acidity as such, any effect on keeping quality, there would have been regular graduation in storage life based on acidity and accordingly sample Nos 1 and 2 should have given longest induction period, while sample Nos. 7 and 8, the shortest, but this has not been obtained in these experiments.

(ii) Also there do not seem to be any correlation between curd acidity at churning and the keeping quality as had that been the case, then Sample No. 1 and No. 2 with 0.08% acidity should have given greatest storage life, and it being cut short progressively as the acidity rose to 1.1, 1.7, 2.2, and 2.5%. But again this is not the case.

(iii) Not whole acidity recorded at churning is transferred to ghee. The ratio of curd acidity at churning; ghee acidity in these experiments is about 7 to 12:1.

(iv) Like Experiment III, all samples show rise in acidity during storage of 28 weeks, the range of rise being 0.0564 to 0.4476% oleic but this rise has also no correlation with the keeping quality, e.g. rise of 0.4476% highest in sample No. 8, has not affected its induction period being longest in the incubation test too.(It was already longest in the accelerated test).

(v) As in Experiment III, there is a general and not absolute correlation between the induction period recorded by accelerated

test and that obtained by the incubation test; the trend being (in both tests) that Sample No. 4 and No. 8 gave the longest induction period, while No. 1 and No. 3 the shortest. One exception to this is sample No. 2 which behaved peculiarly in the accelerated test.

(vi) In this experiment, there is no correlation between the results of Peroxide method and Fat aldehyde method; both showing different trends in the gradation of samples for induction periods and except samples 8 and 9, there is no identical induction periods observed.

(vii) At the end of induction period, all samples show complete bleaching in this experiment as judged by the previous procedure.

(viii) Also there appears to be no correlation between moisture content of ghee and keeping quality (under the conditions of this experiment) as sample No. 8 having highest moisture content of 0.53% has given the longest induction period (42 hours) while sample No. 1 with lowest moisture content of 0.04% has given shortest induction period (i.e. 12 hours). Not only that, but even samples with different moisture content e.g. sample No. 4 with 0.27% and No. 8 with 0.53% -- both have given the same induction period of 42 hours and conversely, samples with same moisture content (0.19%) have given different induction periods, e.g. sample No. 9 -- 18 hours while sample No. 6 -- 30 hours. (Results by accelerated test).

Section (b). Experiments to Demonstrate the Importance of Cooking Process and Various Factors Affecting it.

From Experiments I, III and IV, it appeared that there do not seem to be any relation between keeping quality and starter culture; acidity and moisture content as found normally in ghee. But in all these, one thing was very clear, that is, lower temperatures (80°C and 110°C) giving lower keeping quality as compared to higher temperature (130°C) which gave better keeping quality persistently. From this it was assumed that perhaps out of all factors investigated; the process of cooking, seems to be most vitally concerned with keeping quality and that it might be controlled by some factors. To verify this hypothesis, the following well planned experiments (Experiments V (A. & B) and Experiment VI) were conducted.

Experiment V (A) :- Variation in Keeping Quality Due to Difference in Cooking or Heating Technique.

For the purpose of this experiment, a sample of ghee prepared by the usual process (with 1.4% churning acidity and using L. bulgaricus (Iowa) as starter) was divided into three lots. First lot was heated to 80°C for half an hour to precipitate curd and filtered. Then a portion of it was heated to 110°C and 150°C, momentarily (i.e. heated without contact with curd). Second lot of similar amount was heated to 80°C for half an hour but not filtered and after taking a sample for this temperature, proceeded to heat to 110°C and 150°C moment-

arily (i.e. heated in contact with curd). The third lot was heated to 110°C in contact with curd for various times, i.e. 10 minutes, 20 minutes and 30 minutes, to find out if time and temperature has any effect. For oxidation and induction period studies the samples were tested by accelerated test. The results are summarised in Table 8.

TABLE 8.

Particulars of Treatments, Induction Periods and Acidities of Experiment V (A).

No.	Treatment.	Induction Period in hours by accelerated test.	Acidity % olei (Initial)
No.1	Control, heated 80°C for ½ an hour	8	0.3384
No.2	Heated to 110°C momentarily without contact with curd	4	0.3384
No.3	Heated to 150°C - do -	0	0.4512
No.4	" to 80°C for ½ an hour	8	0.3384
No.5	" to 110°C momentarily in contact with curd	12	0.3948
No.6	Heated to 150°C - do -	4	0.3948
No.7	" to 110°C for 10 minutes in contact with curd	12	0.4512
No.8	Heated to 110°C for 20 minutes in contact with curd	16	0.4512
No.9	Heated to 110°C for 30 minutes in contact with curd	12	0.4512

From this experiment it would appear that

(i) Heating to higher temperature in contact with curd, (i.e. unfiltered fat) enhances the storage life of ghee while heating without contact with curd (i.e. filtered fat) reduces it.

(ii) Also it appears that while heating in contact with curd, holding time at particular temperature has some effect on the keeping quality, i.e. at 110°C holding for momentarily, 10 minutes and 30 minutes - all gave an induction period of 12 hours showing no difference; while holding for 20 minutes at the same temperature, enhanced the induction period by 4 hours

The above observations suggest that cooking process in ghee manufacture is very important from keeping quality point of view, and it is affected by time, temperature treatment, so also heating in contact with curd or otherwise; the former having beneficial effect while the latter having adverse effect on the keeping quality when heated above 100°C.

Experiment V (B):-

The previous experiment (i.e. V (A)), gave some broad hints as regards the importance of cooking process, therefore in order to get more precise idea of this process and factors governing it, it was repeated with some minor changes to obtain results under well regulated conditions.

For this purpose, 4 lbs. of butter soured with L. bulgaricu (Iowa) culture and churned at 1.2% acidity, was obtained and divided into two lots. The first lot was heated to 80°C for half an hour and filtered. After taking a sample for this temperature, it was heated to 110°C, 130°C and 150°C momentarily (i.e. without contact with curd). The second lot was heated to similar temperatures and time, but without previous filtering i.e. in contact with curd and then filtered. Uniform rate of heating applied to all samples; the flow of current

being controlled by Sunvic thermostat (50 on Survic and Medium Switch on hot plate). For oxidation studies and induction periods, the samples were put through both, i.e. incubation and accelerated tests. The results are tabulated and plotted in Table 9 and graphs of Figs. 19, 20 and 21 respectively.

TABLE 9.

Particulars of treatments, induction periods, acidities and Moisture percentage of Experiment V (B).

No.	Treatments	Induction Period		Acidity % oleic.		Moistu %
		In hours by accelerated test.	In weeks by incubation test.	Initial	last	
No.1	Control heated to 80°C for ½ an hr.	18	12	0.113	0.2256	0.2
No.2	Heated to 110°C momentarily without contact with curd	16	12	0.113	0.282	0.3
No.3	Heated to 130°C momentarily without contact with curd	12	8	0.169	0.282	0.1
No.4	Heated to 150°C momentarily without contact with curd	8	8	0.169	0.282	0.2
No.5	Heated to 80°C for ½ an hour (Control)	16	12	0.169	0.2256	0.2
No.6	Heated to 110°C momentarily in contact with curd	20	14	0.169	0.2256	0.1
No.7	Heated to 130°C momentarily in contact with curd	24	16	0.226	0.3346	0.3
No.8	Heated to 150°C - do -	36	more than 20 weeks	0.226		0.3

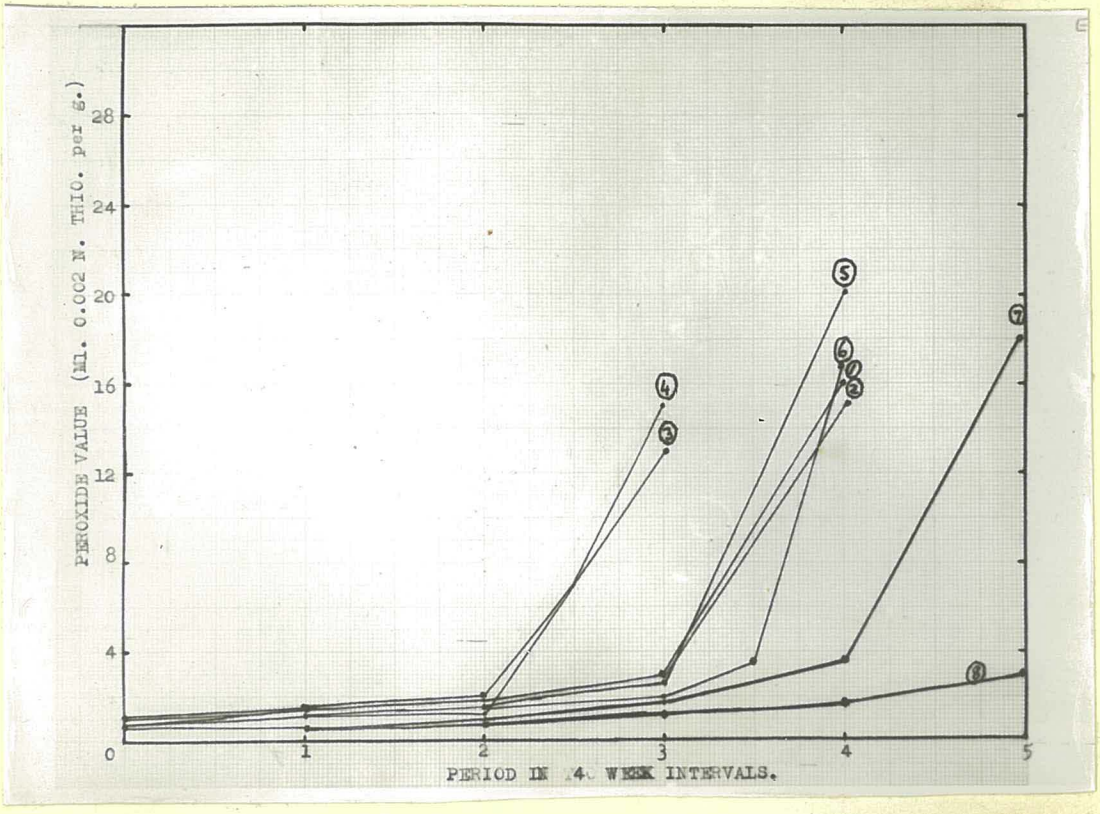


Fig. 19. Graph showing oxidation and induction periods by peroxide method. (Details given in Table 9, page 60).

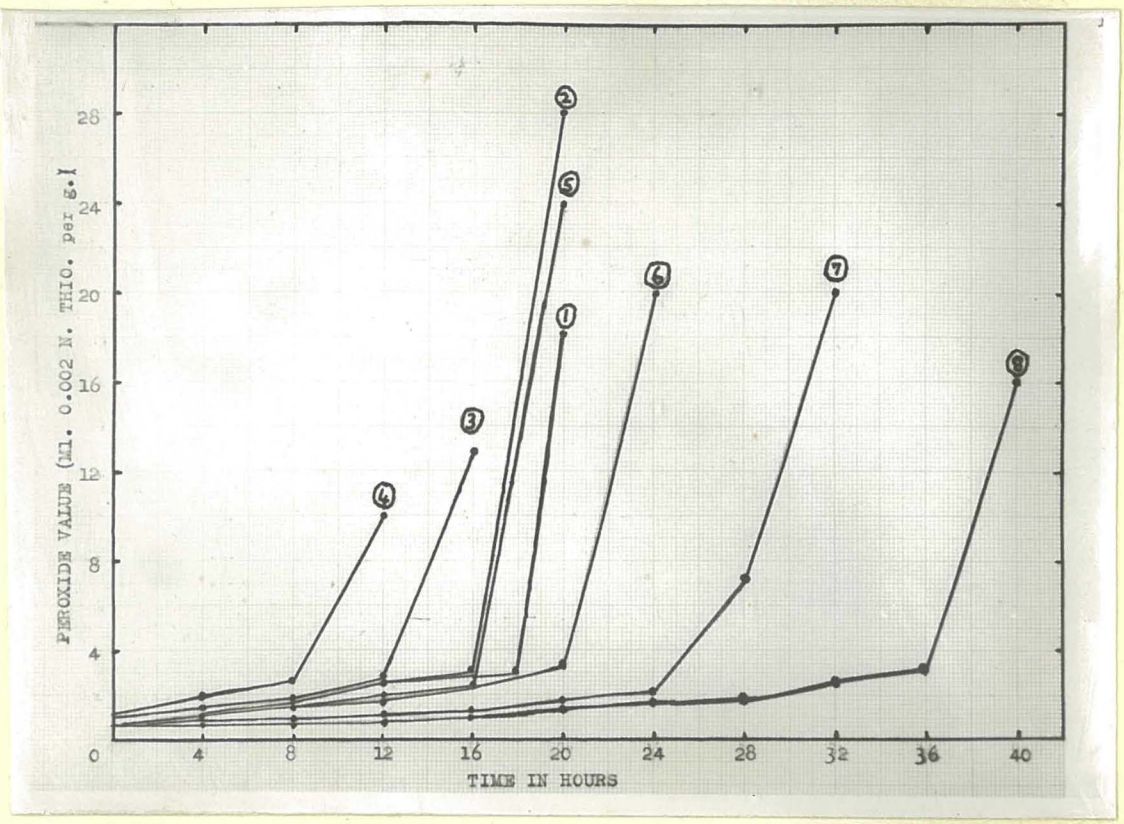


Fig. 20. Graph showing oxidation and induction periods by accelerated test at 100°C. (Details given in table 9, page 60).

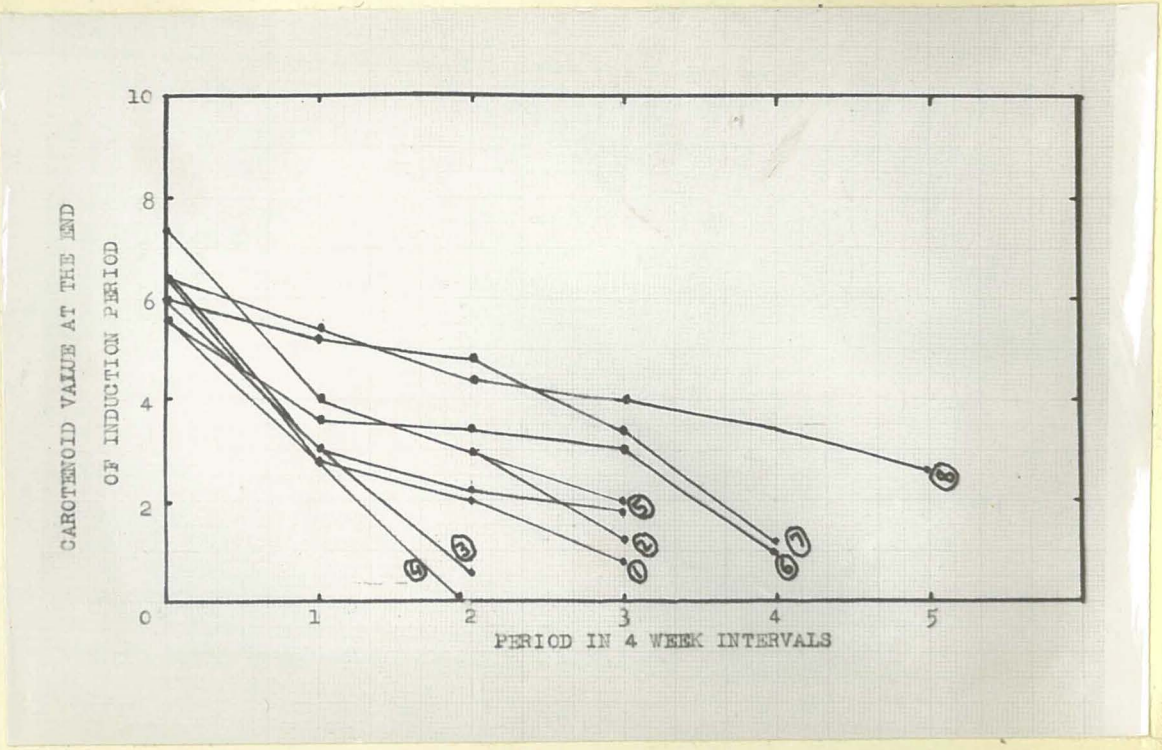


Fig. 21. Graph showing bleaching of colour (Carotenoid pigments) by oxidation. (Curves 1 - 6 correspond with treatments listed in Table 9, page 60).

From the data presented in this experiment, it would be seen:-

(i) That there is enough evidence to confirm the results of

previous experiment (i.e. Experiment V (A)) that in ghee manufacture cooking process is very important from keeping quality point of view, and that heating in contact with curd gives better keeping quality, the storage life being in proportion to the temperature of cooking or heating, higher temperatures giving better results than lower ones. On the other hand, heating ghee without contact with curd to higher temperatures reduces the life of ghee in the proportion of the temperature applied for cooking.

(ii) There is good correlation between the results of the incubation test and accelerated test, it being more clear cut in the samples heated in contact with curd. In samples heated without contact with curd, difference of 2 to 4 hours in the accelerated test has not given any significant difference in the incubation test.

(iii) All samples show bleaching at the end of induction period but to a varying degree, e.g. samples 1, 3 and 4 show complete bleaching, while others show partial bleaching.

(iv) As regards moisture, like Experiment IV, there do not seem to be any correlation between moisture content and keeping quality, e.g. samples No. 7 and 8 having highest moisture content, i.e. 0.37 and 0.35% respectively have given longest induction periods while lower ones like No. 3 and No. 4 with 0.17 and 0.22% moisture respectively have given shorter induction periods.

(v) The range of rise in acidity during storage in this Experiment is 0.0566 to 0.169% oleic.

Experiment VI:- To find out effect of rate of cooking and stirring on the Keeping Quality of Ghee.

Some of the results in Experiment V (A) suggested that effectiveness of cooking process is controlled by time and temperature factors, e.g. at 110°C, 20 minutes heating gave better keeping quality than heating for momentarily, 10 minutes and 30 minutes. This meant indirectly that perhaps rate of cooking may be concerned with this phenomenon since different times are needed to reach the same temperature when heated by slow or rapid rate of cooking, the length of time and temperature regulating the incorporation of the antioxidants or start of oxidation in the fats, thus affecting the keeping quality ultimately. This action was thought to be aided by stirring continuous or otherwise.

To test above assumption, sour milk was churned at 0.5% acidity by the usual process and butter so obtained was divided into two lots. First lot was heated to 110°C and 130°C, by slow and rapid rate of heating with stirring three to four times as the routine procedure is, while the second lot was heated exactly the same way and to same temperatures, but with continuous stirring. Of course the samples were filtered after heating as usual, and tested by the accelerated test for oxidation and induction periods. The slow or rapid rate of heating was controlled by change of switch on the hot plate, i.e. keeping on Low or Medium and regulated the even flow of current by Survic thermostat. A record of temperature rise every three minutes was kept (Figures 22 and 23) and it was

observed that to raise temperature from 60°C to 130°C by rapid rate of heating, required only 24 minutes while by slow rate of heating to reach to same temperature 102 minutes were needed. The results are summarised in Table 10 and plotted in graph of Fig. 24.

TABLE 10.

Particulars of Treatments; Induction Periods and Acidities of Experiment VI.

No.	Treatments.	Induction Period in hours by accelerated test.	Acidity % ole	
			Initial	La
No.1	Heated to 110°C by rapid rate with routine stirring	12	0.6	0.
No.2	Heated to 130°C by rapid rate with routine stirring	30	0.6	1.
No.3	Heated to 110°C by slow rate with routine stirring	12	0.5	0.
No.4	Heated to 130°C by slow rate with routine stirring	18	0.5	0.
No.5	Heated to 110°C by rapid rate with continuous stirring	12	0.6	0.
No.6	Heated to 130°C by rapid rate with continuous stirring	18	0.6	0.
No.7	Heated to 110°C by slow rate with continuous stirring	12	0.9	0.
No.8	Heated to 130°C by slow rate with continuous stirring	6	1.0	1.

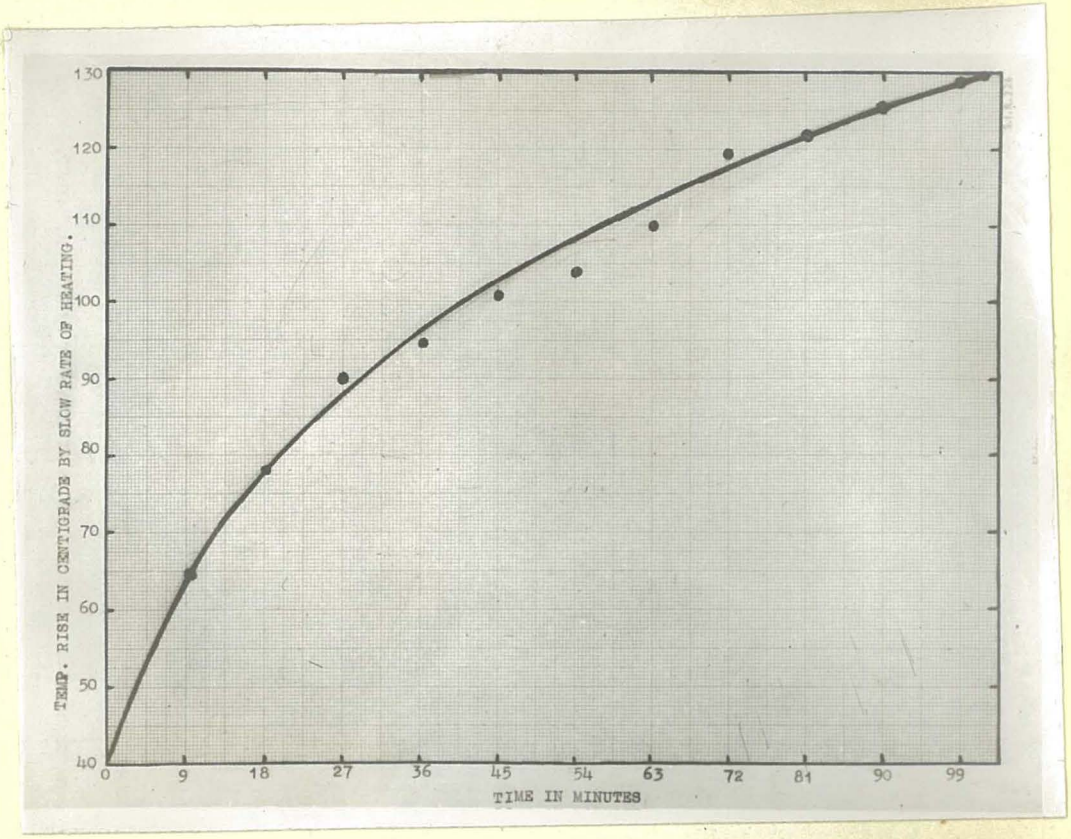


Fig. 22. Graph showing rate (time and temperature) of slow heating.

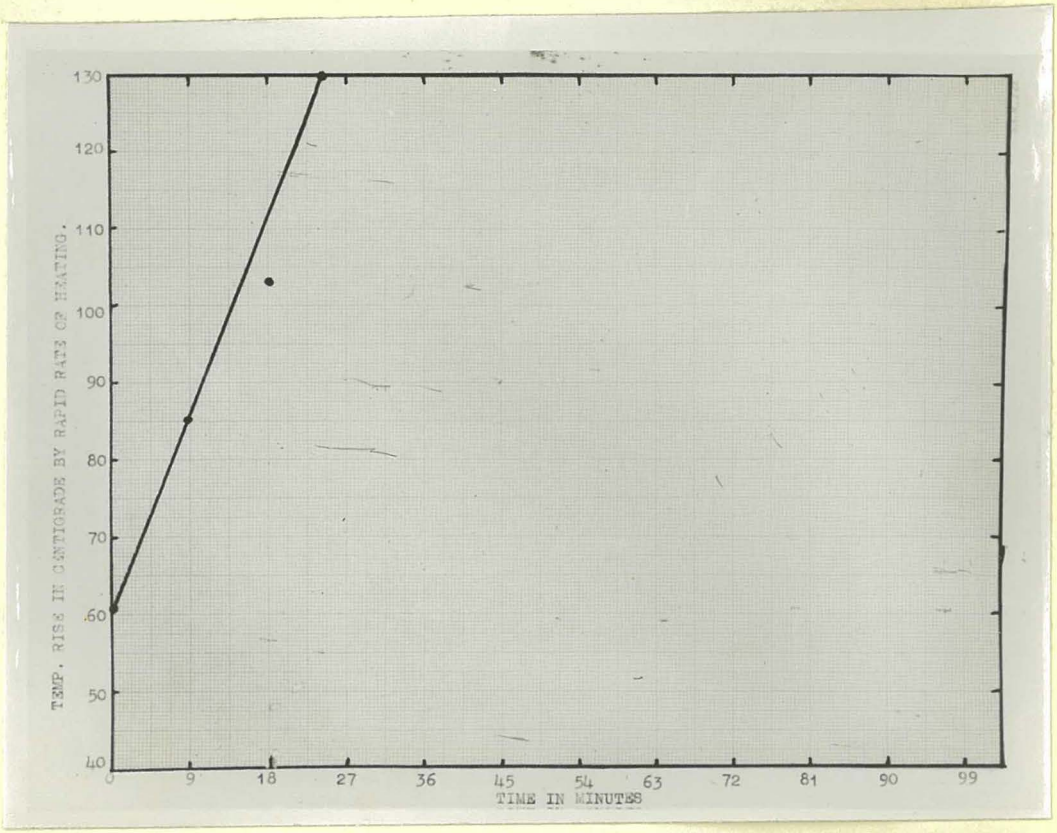


Fig. 23. Graph showing rate (time and temperature) of rapid heating.

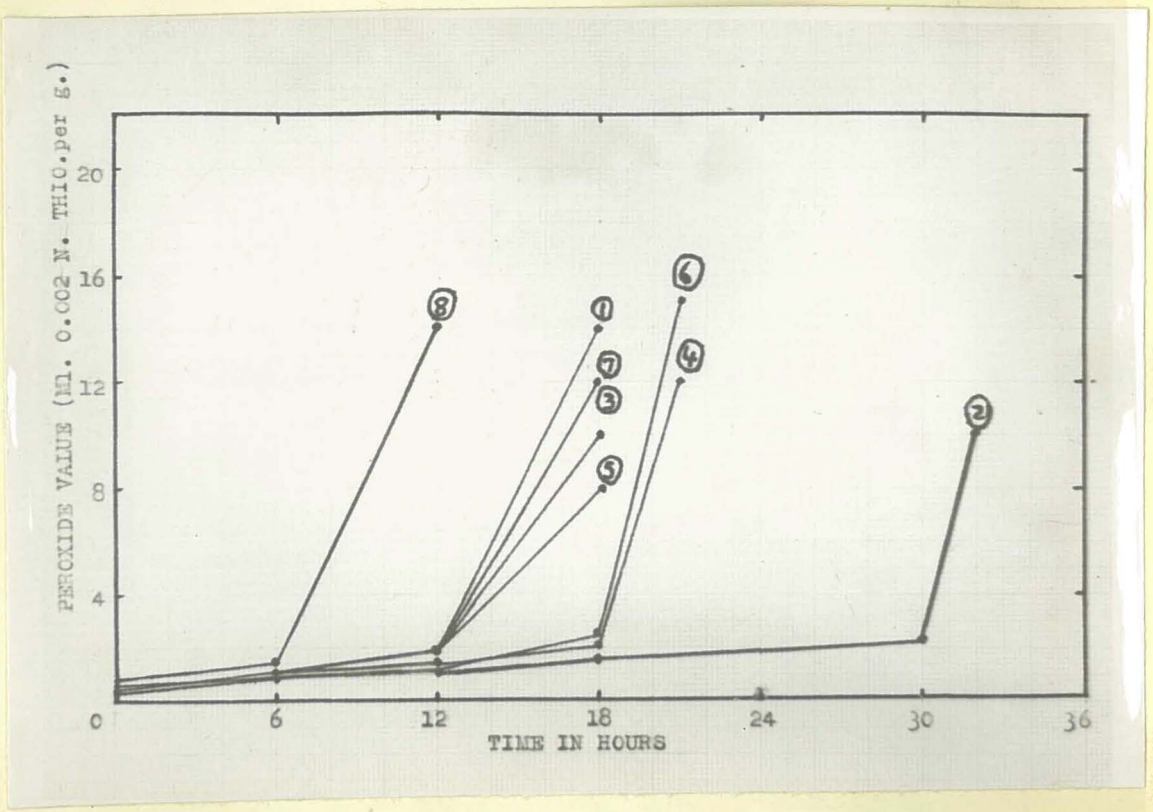


Fig. 24. Graph showing oxidation and induction periods by accelerated test at 100°C. (Details given in Table 10, page 66).

From the data presented above, it will be seen that:-

(i) The rate of heating affects the keeping quality in the higher temperature, i.e. 130°C while in the lower one i.e. 110°C, it

remains unaffected (whether the stirring is continuous or otherwise), the rapid rate of heating being better than slow rate, from keeping quality point of view (as shown by samples Nos. 2 and 4 and 6 and 8.

(ii) That continuous stirring has no advantage over the usual method of stirring three or four times during the process, as practised at present, rather, it has shown effect of shortening the induction period in the higher temperatures in both rapid and slow rate of heating, the lower temperature being unaffected.

(iii) The range of rise in acidity in this experiment after the oxidation by accelerated test is from 0.00 to 0.2% in all samples except sample No. 2 (0.5%) and No. 8 (0.3%). This is a significant fact from the point of view of supposed rise in acidity for oxidation of ghee or butterfat.

Section (C). Experiments to Locate the Ingredient or Ingredients Responsible for Antioxidant Action in Curd.

From the data presented in Experiments V (A and B), the importance of heating in contact with curd is evident. It was thought therefore, that there might be some ingredient or ingredients of curd exerting the antioxidant action and that by separately analysing the action of each ingredient, the one or more than one ingredient responsible for this effect could be located. With this point in view Experiments from VII to X were arranged.

Experiment VII:-

In this experiment, effect of lactose, casein, skim milk powder and lecithin is analysed. For that, fresh Massey College factory butter was melted at 80°C, for half an hour and filtered. Then 40 cc. sample each of this was heated separately with 1% lactose, 1% casein (lactic), 1% skim milk powder and 1% egg yolk to 110°C and 130°C for 10 minutes on hot plate. Filtered them after treatment and then tested for oxidation and induction periods by accelerated test at 100°C. Butterfat melted as such at 80°C was used as overall control to compare effect, while this fat heated to 110°C and 130°C without anything, served as controls for their series. The details of treatments together with the induction periods are given in Table 11 and plotted in Graph of Fig. 25.

TABLE 11,

Particulars of Treatment and Induction Periods of Experiment VII (A).

No.	Treatment.	Induction Period in hours by accelerated test.
No.1	Control heated to 110°C.	12
No.2	Butterfat + 1% lactose heated to 110°C	12
No.3	Butterfat +1% casein (lactic) - do -	12
No.4	Butterfat +1% skim milk powder- do -	12
No.5	Butterfat +1% egg yolk - do -	18
No.6	Control Butterfat heated to 130°C	6
No.7	Butterfat + 1% lactose heated to 130°C	4
No.8	Butterfat +1% casein (lactic) - do -	6
No.9	Butterfat +1% skim milk powder- do -	6
No.10	Butterfat +1% egg yolk - do -	12
No.11	Control of all Butterfat heated to 80°C	12

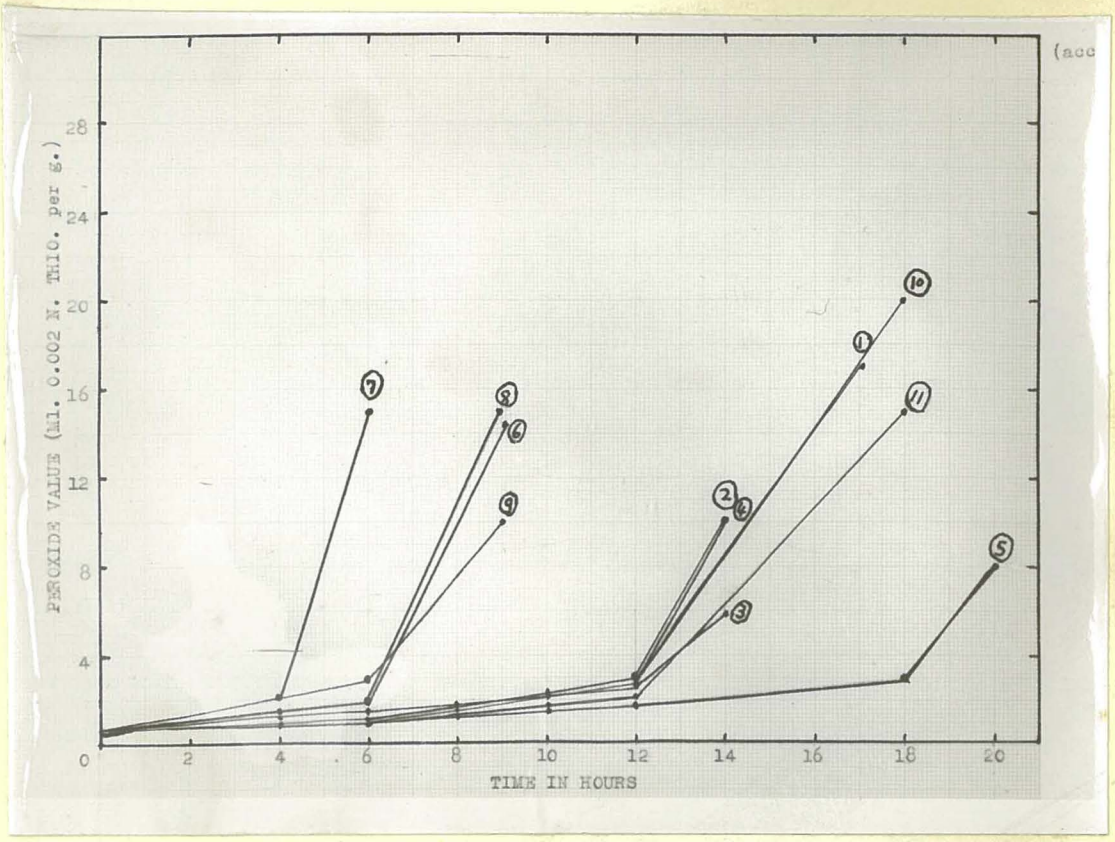


Fig. 25. Graph showing oxidation and induction period by accelerated test at 100°C. (Details given in Table 11, page 72).

From the above experimental data it will be seen that, only egg yolk (representing lecithin) heated to 110°C had given better keeping quality over all other treatments and

control (80°C). Egg yolk heated to 130°C has given longer induction period when compared with butterfat heated alone to 130°C but only equal to that of butterfat heated alone to 80°C (control of all). Lactose, skim milk powder and casein in the higher temperature have given shorter induction periods than those in the lower temperature and control.

This experiment was repeated on the above lines with the only change that instead of heating to 10 minutes, in this case it was heated momentarily. The results showed once again the superiority of egg yolk in giving longer induction period than control and others. Rather in this experiment, both temperatures i.e. 110°C and 130°C (egg yolk treated) gave better result than control and others.

Experiment VIII:- Effect of Egg Yolk Heated to Different Time and Temperature on the Keeping Quality.

From Experiment VII, it is observed that heating butterfat with egg yolk had the effect of prolonging the storage life. Hence further interest in this connection was to find out if the action of egg yolk was controlled by the time and temperature factor which may be concerned in the incorporation of required amount of lecithin in the butterfat.

For this purpose previously melted (80°C) and filtered butterfat kept in the ice chest, was used. 40 cc each of this was heated with 1% egg yolk for different times (i.e. momentarily, 10 minutes, 20 minutes and 30 minutes) and different

temperatures (i.e. 80°C, 110°C and 130°C). Samples were filtered after treatment and tested for oxidation and keeping quality by accelerated test. The details of the treatments together with the induction periods are given in Table 12 and plotted in graph of Fig. 26.

TABLE 12.

Particulars of Treatments and Induction Periods of Experiment VIII.

No.	Treatment.	Induction period in hours by accelerated Test.
No.1	Butterfat + 1% egg yolk heated to 80°C momentarily (Control).	6
No.2	Butterfat + 1% egg yolk heated to 80°C for 10 minutes	6
No.3	Butterfat + 1% egg yolk heated to 80°C for 20 minutes	6
No.4	Butterfat + 1% egg yolk heated to 80°C for 30 minutes	6
No.5	Butterfat + 1% egg yolk heated to 110°C momentarily	6
No.6	Butterfat + 1% egg yolk heated to 110°C for 10 minutes	6
No.7	Butterfat + 1% eggyolk heated to 110°C for 20 minutes	6
No.8	Butterfat + 1% egg yolk heated to 110°C for 30 minutes	12
No.9	Butterfat + 1% egg yolk heated to 130°C momentarily	6
No.10	Butterfat + 1% egg yolk heated to 130°C for 10 minutes	6
No.11	Butterfat + 1% egg yolk heated to 130°C for 20 minutes	4
No.12	Butterfat + 1% egg yolk heated to 130°C for 30 minutes	2

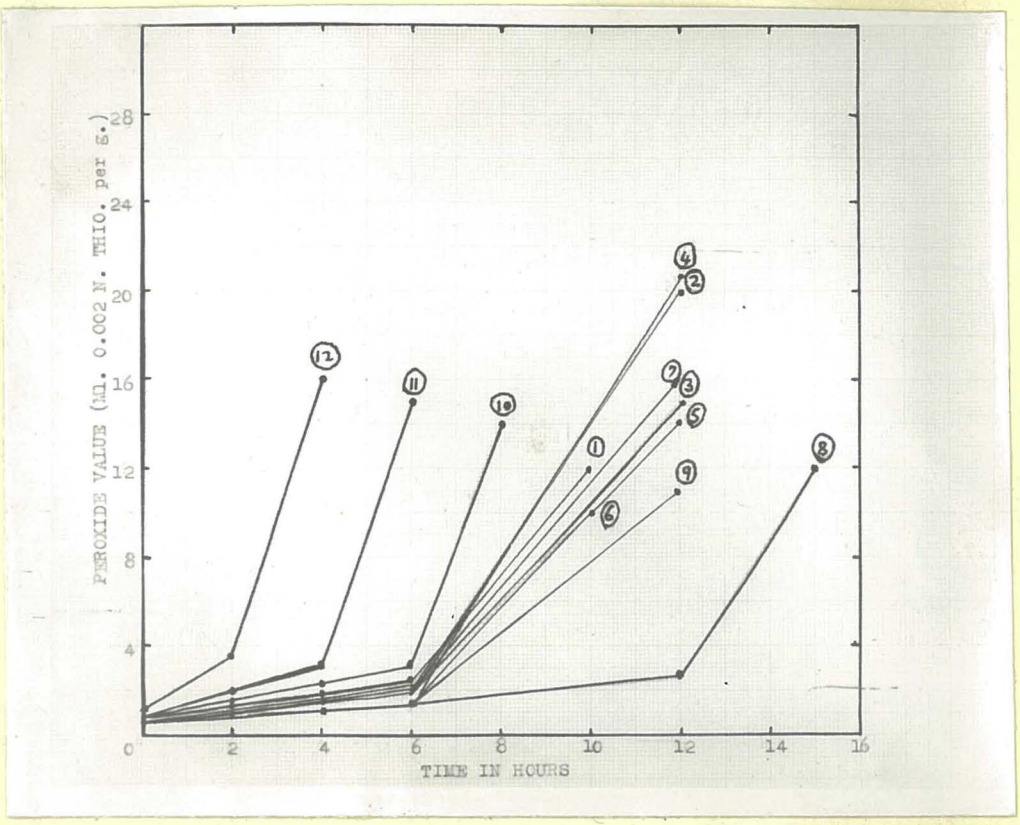


Fig. 26. Graph showing oxidation and induction periods by accelerated test at 100°C. (Details given in Table 12, page 75).

From the above data, it will be observed that there is no effect of egg yolk heated to 80°C for momentarily, 10, 20 and 30 minutes, but at 110°C and 130°C (higher temper-

atures) the effect is well marked in showing good or adverse effect on keeping quality e.g. heating butterfat with 1% egg yolk at 100°C for 30 minutes gave double the induction period than that of control (No. 1) and other treatments in the same series (i.e. 100°C heated for momentarily, 10 minutes and 20 minutes) but in case of 130°C, there is no difference in the treatments heated for momentarily and 10 minutes, while when heated for 20 and 30 minutes there is adverse effect of shortening the induction period.

Therefore, it appears that incorporation of required amount of lecithin of egg to give butter keeping quality is controlled by time and temperature factors, less time and temperature giving no effect while more time and temperature giving harmful effect by probably starting oxidation in the fat during the process of heating used for incorporation.

Experiment IX:- Effect of S.H. (Sulphydryl compounds) produced or Destroyed by Heating s.n.f. on Keeping Quality.

Lea (1946) in a note on the presence of heat labile sulphur in milk powder concludes that the efficiency of high temperature preheated milk powder against development of tallowy flavours is due to the production of antioxidant active sulphydryl compounds by the action of heat on the protein. In order to find out if same effect is produced when butter is heated to ghee; following experiments were arranged.

Experiment IX (A):-

For this experiment, 400 cc of fresh skim milk was obtained from D.R.I. Massey College and heated at 70 to 80°C

for 24 hours under vacuum to drive off moisture and get s.n.f. at low temperature. To release antioxidants (if any), 4 gms. each of s.n.f. (to give 10%) was weighed in eight beakers and heated to different temperatures (i.e. about 80°C, 100°C, 110°C and 130°C temperatures) and at each temperature the holding time was 20 and 30 minutes. Then added 40 cc. of fresh butterfat (melted at 80°C and filtered) and heated for 30 minutes at 80°C, shaking frequently and evenly to give fat of each sample a chance to take up antioxygens (if any) formed from heated skim milk and then lastly they were filtered.

In addition, 4 gms. (10%) of freeze dried milk* was also shaken with 40 c.c. fat at 80°C for 30 minutes to release and incorporate antioxygens and filtered. Butterfat melted at 80°C and shaken evenly as in other treatments was included as control. Oxidation and induction periods studies were made by putting the samples through the accelerated test. Details of the treatments together with induction periods are recorded in Table 13.

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* Freeze drying of this milk was done in the Plant Chemistry Laboratory with the valuable co-operation of Dr. J. Melville and N.O. Bathurst.

TABLE 13.

Particulars of Treatments and Induction Periods of Experiment IX (A).

No.	Treatments.	Induction periods in hours by accelerate test.
No.1	Butterfat heated to 80°C (Control.	6 hou
No.2	10% Freeze dried s.n.f. + butterfat	less than hou
No.3	10% s.n.f. (lab.dried) heated to 76 to 80°C for 20 minutes + butterfat	- do -
No. 4	10% s.n.f. (lab.dried) heated to 76 to 80°C for 30 minutes + butterfat	- do -
No.5	10% s.n.f. (lab-dried) heated to 100°C for 20 minutes + butterfat	- do -
No.6	10% s.n.f. (lab.dried) heated to 100°C for 30 minutes + butterfat	- do -
No.7	10% s.n.f. (lab.dried) heated to 108 to 115°C for 20 minutes + butterfat	- do -
No.8	10% s.n.f. (lab.dried) heated to 108 to 115°C for 30 minutes + butterfat	- do -
No.9	10% s.n.f. (lab.dried) heated to 130°C for 20 minutes + butterfat	6 hour
No.10	10% s.n.f. (lab.dried) heated to 130°C for 30 minutes + butterfat	6 "

From the above data it appears that under the conditions of this experiment, there do not seem to be any antioxidant

released at lower temperatures i.e. from 76 to 115°C. However at higher temperature i.e. 130°C for both timings, i.e. 20 and 30 minutes, there is an indication that antioxidants are released since these two treatments have given induction periods equal to control. Ordinarily, had there been no effect of antioxidant released, at this temperature and time, (i.e. 130°C for 20 and 30 minutes), the samples must have gone off before control since heating to 130°C without contact with curd gives reduced induction period when compared with 80°C heated butterfat (Control).

Experiment IX (B):-

The above being an important experiment, it was repeated with some alterations thought necessary in technique, time of heating and holding e.g. instead of heating for 20 and 30 minutes, heated for momentarily and 10 minutes. Dropped 100°C and included 150°C instead, and dropped freeze dried milk, since it could not be obtained in time. The method of releasing antioxidants was also modified a little. Instead of heating dried skim milk to various temperatures and time for releasing antioxidants and then adding fat to take up these antioxidants - in this case 10% lab. dried skim milk is mixed with fat and then heated to different temperatures and time mentioned above and filtered. As usual, the samples were tested by the accelerated test and the results are given in Table 14 and plotted in graph of Figure 27.

TABLE 14.

Particulars of Treatments and Induction Periods of Experiment IX (B).

No.	Treatments.	Induction periods in hours by acceleratio test.
No.1	Control butterfat heated to 80°C momentarily	6
No.2	Control butterfat heated to 80°C for 10 minutes	6
No.3	10% (lab.dried) s.n.f. + butterfat heated to 80°C momentarily	6
No.4	10% (lab.dried) s.n.f. + butterfat heated to 80°C for 10 minutes	6
No.5	10% (lab.dried) s.n.f. + butterfat heated to 110°C momentarily	6
No.6	10% (lab.dried) s.n.f. + butterfat heated to 110°C for 10 minutes	6
No.7	10% (lab.dried) s.n.f. + butterfat heated to 130°C momentarily	12
No.8	10% (lab.dried) s.n.f. + butterfat heated to 130°C for 10 minutes	12
No.9	10% (lab.dried) s.n.f. + butterfat heated to 150°C momentarily	12
No.10	10% (lab.dried) s.n.f. + butterfat heated to 150°C for 10 minutes	12

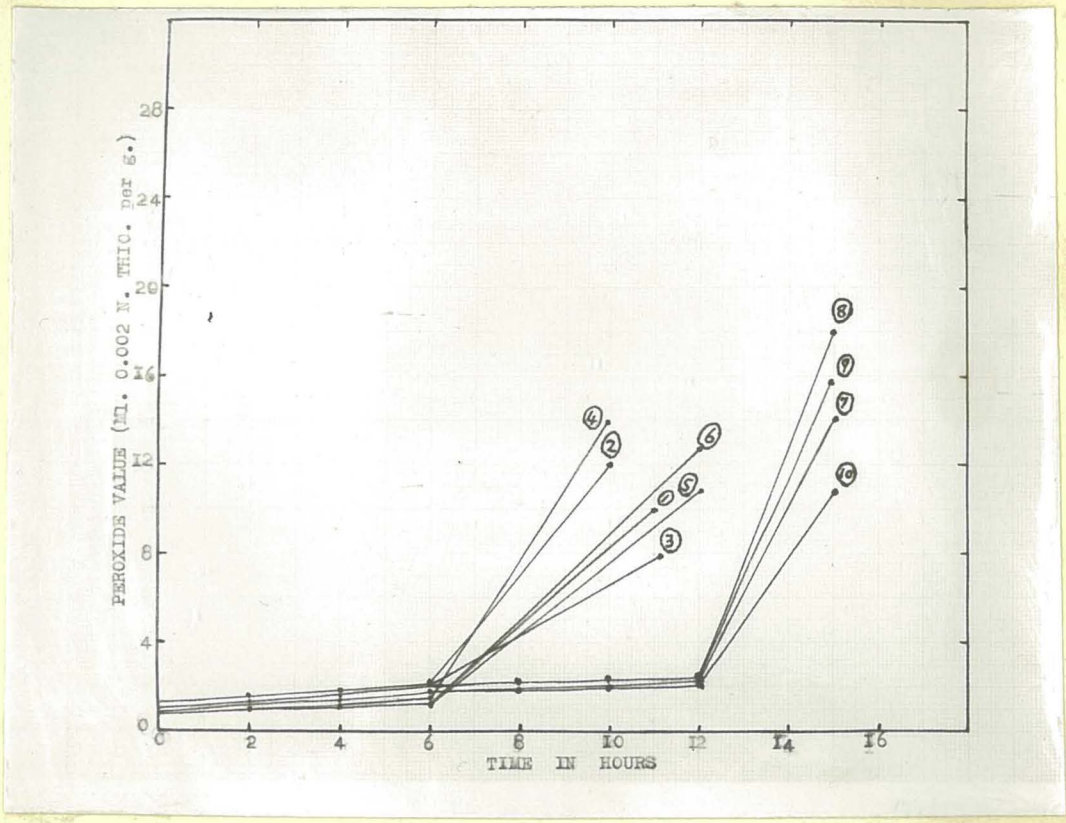

Fig. 27. Graph showing oxidation and induction periods by accelerated test at 100°C. (Details given in Table 14, page 80).

From the above data, it will be observed that under the conditions of this experiment, at higher temperatures i.e. 130°C and 150°C antioxidants are released which is

indicated by giving an induction period of 12 hours as against 6 of control. However, heating to lower temperatures i.e. 80°C and 110°C do not seem to give advantage over control. This confirms the previous observations more clearly. Probably these conditions are better suited for releasing and incorporating antioxidants than those in Experiment IX (A).

Experiment X:-

In this experiment the effect of S.H. compounds produced or destroyed has been investigated by another technique, ie. instead of first drying the skim milk under vacuum or in freeze and then heating for various temperatures and time to release and incorporate antioxidants - in this, skim milk as such has been added and heated with fat to different temperatures, filtered and tested by the accelerated test as usual. The following procedure was followed:-

Took 40 c.c. each of fat (melted at 80°C for half an hour and filtered) and heated with 4 c.c. (10%) of skim milk to different temperatures (i.e. 80°C, 110°C, 130°C and 150°C) and for different timings, (i.e. for 10 and 30 minutes). During the process stirred evenly and then filtered. Control heated in similar way but skim milk not added, was included also. Table 15 shows the details of treatments, with result.

TABLE 15.

Particulars of Treatments and Induction Periods of Experiment X.

No.	Treatments.	Induction periods in hours by accelerate test.
No.1	Control butterfat heated to 80°C for 10 minutes.	6
No.2	Control butterfat heated to 80°C for 30 minutes	6
No.3	10% skim milk + butterfat heated to 80°C for 10 minutes.	less than 6 hours.
No.4	10% skim milk + butterfat heated to 80°C for 30 minutes	- do -
No.5	10% skim milk + butterfat heated to 110°C for 10 minutes	- do -
No.6	10% skim milk + butterfat heated to 110°C for 30 minutes	- do -
No.7	10% skim milk + butterfat heated to 130°C for 10 minutes	- do -
No.8	10% skim milk + butterfat heated to 130°C for 30 minutes	- do -
No.9	10% skim milk + butterfat heated to 150°C for 10 minutes	- do -
No.10	10% skim milk + butterfat heated to 150°C for 30 minutes	- do -

From the data presented above, it would appear that under the conditions of this experiment, there is no indication of any antioxidants released by treating with skim milk as such.

Further investigations are, however, needed to find out if the same results are given under different set of conditions, i.e. different percentage of skim milk, different time of heating and holding etc., to conclude definitely on the merits and demerits of this procedure for releasing and incorporating antioxidants in butterfat. However, for want of time this could not be done.

CHAPTER IV.

DISCUSSION AND CONCLUSIONS.

Butterfat because of its unique composition is subject to three types of rancidity (or deterioration) viz. (i) butyric (due to liberation of butyric acid through the action of mono and tri-butyrinases and acid hydrolysis) (ii) ketonic(due to action of dry moulds on glycerol) and oxidative (due to action of air). Out of these, the only rancidity to which ghee is susceptible, in the normal course, is the oxidative rancidity (Davies, 1940). Lea (1938) too emphasizing the importance of oxidative rancidity in fats said: "The most important and from a scientific point of view, the most interesting form of rancidity is that produced by the action of oxygen of the air on the fat". Various workers in the past (cited previously) have contributed to assign functions to different factors like moisture, acidity, heat etc., on the deterioration of ghee or butteroil due to oxidative rancidity but the evidence presented in them is conflicting to a great extent and does not clarify the position. Therefore, in these investigations an attempt has been made to clarify the position as far as possible as regards proper role of various factors especially moisture, acidity, heat and bacterial culture, on the deterioration of ghee due to atmospheric air which ultimately affects itskeeping quality.

Out of the above four factors considered viz, moisture, acidity, heat and bacterial culture, the evidence presented in

Chapter III suggests that action of heat in preparation of ghee is most important from keeping quality point of view. The data presented in Experiments I, III, IV and VI clearly and consistently show that heating ghee to different temperatures ie.. 80°C, 110°C and 130°C, result in different keeping quality, the trend being (in almost all cases) that higher temperatures keep better than lower ones and that in order of storage life they will be 80°C, 110°C and 130°C by common Indian method of making ghee from sour milk. Somewhat similar observations have been recorded by Ritter (1937) and Rafey et al (1944) which are contrary to the observations made by Godbole and Sadgopal (1936), that sterilization by heat in ghee increases the subsequent tendency to become rancid. (However, it is possible to explain results of these authors on the basis of material cited in the subsequent paragraph.) Also there is no evidence in the present investigations to support the view held by Patil and Hammer (1928) that there is not much difference between the keeping quality of butterfat heated not above 55°C and ghee heated to 110 - 140°C, and so also their contention that superiority of ghee and butterfat over butter can be attributed to the elimination of water and curd from the former and not due to heat used in the manufacture.

The increase in keeping quality with rising temperature seems to be governed by three main factors viz; (i) heating in contact with curd (ii) time and temperature relationship and

(iii) the rate of heating or cooking. In Experiment V (B) makhan (butter) is heated to 80°C for half an hour to settle curd and then treated in two ways, viz; one lot being filtered to get rid of curd, while the other lot not filtered i.e. retaining curd. Both lots are then heated to 110°C, 130°C, and 150°C momentarily and filtered and tested in cabinet for incubation test and in the "Swift Stability Tester" for accelerated test. Both results agree in the fact that heating of ghee in contact with curd increases the induction period from 16 hours to 36 hours (accelerated test) and 12 to more than 20 weeks (incubation test) for 80°C, and 150°C respectively. The increase in induction period follows the same trend as before i.e. least for lower temperature 80°C and increasing with rising temperatures i.e. 110°C, 130°C and 150°C in regular order. But the results for the lot heated without curd is just the reverse, i.e. with rising temperatures (from 80°C to 150°C) the induction period shortens from 18 hours to 8 hours (accelerated test) and 12 weeks to 8 weeks (incubation test) respectively. Ritter and Nussbaumer (1939) observe that unfiltered boiled down butter is more stable than filtered butter and that filterate obtained at 100°C is more stable than that obtained at 42°C, but they do not correlate it to the importance and presence of curd. On the basis of these observations, it seems likely that the results obtained by Godbole and Sadgopal (cited above) i.e. increase of tendency to become rancid by sterilization with heat - may be due to their heating ghee to high temperatures without contact with

curd. The second factor important in the increase of keeping quality with rising temperature appears to be the effect of time and temperation operation. In experiment V (A), a sample of ghee is heated to 110°C (of course in contact with curd) for 10, 20 and 30 minutes, out of which that heated for 20 minutes gave an increase in the induction period of four hours over the ones heated to 10 and 30 minutes, there being no difference between those heated to 10 and 30 minutes and heated momentarily. From this it appears probable that heating for momentarily and 10 minutes did not incorporate enough antioxidants while heating for 30 minutes overcomes the effect of antioxidants incorporated and starts oxidation in the fat so that the net effect given was the same. On the basis of these results it can easily be guessed why Ewbank and Gould (1943) obtained the hastening of oxidation by heating butter or butter-oil to 127°C for 30 minutes, since it is more than likely that by heating to this temperature and time, there is possibility of start of oxidation (besides incorporating antioxidants) which will result in quick oxidation ultimately.

Besides these two, there is the third factor influencing the cooking process, i.e. the rate of heating or cooking. In Experiment VI, the same lot of ghee is heated to 110°C and 130°C by rapid and slow rate of heating and continuous and occasional stirring (3 to 4 times as routine). It is found that rapid rate of cooking has better keeping quality in the higher temperature i.e. 130°C, while lower temperature (110°C)

gave no difference. It appears that oxidation sets in during slow heating to high temperature (since it takes a lot of time to reach that) which decreases the life of slow cooked ghee. Stirring (which might help to incorporate anti-oxidants of curd into ghee) whether continuous or occasional (as is the routine) does not seem to effect keeping quality materially.

From the above observations, a question naturally arises as to what is there in the curd taken up by heated ghee which results in better keeping quality. An attempt has been made in the subsequent experiments from VII to X to find out an answer to this. Of course the investigations are by no means complete on this chapter but some interesting light is thrown on the possible causes of this phenomenon. Butter-fat curd contains s.n.f. of milk, i.e. (lactose, protein, minerals etc) and associated with these is the lecithin in form of lecithoprotein. Besides these there may be some other chemical groups released in small quantities yet unidentified properly, e.g. sulphydryl group and other reducing substances, which might be having an influence on the keeping quality of ghee. Out of four ingredients tried separately in Experiment VII, i.e. lactose, casein (lactic), skim milk powder and egg yolk (to give lecithin), egg yolk has given more induction period than all the treatments and control. This indicates that lecithin is concerned with this phenomenon; lactose, casein and skim milk powder giving the same induction

periods as control at lower temperature, while at higher temperature (130°C) they exert an influence in shortening the life of ghee. Ritter (1937) assumed that lecithin contained in small amounts in cooked butter gives increased storage life, while Rafey, et al (1944) found phosphorous content (indicating lecithin presence and incorporation), correlated with increased stability of cooked butterfat. The latter workers also found increased induction periods with purified Soyabean phospholipid. But Josephson and Dahle (1946) did not find increased stability by heating butterfat with milk phospholipids or membrane protein. This may be probably due to the fact that not required amount of lecithin might have been incorporated in the fat under the conditions set out in their experiments. The incorporation of lecithin seems to be controlled by time and temperature operation(Experiment VIII); the keeping quality depending on the incorporation of required amount of lecithin into the fat without setting in oxidation, in the incorporation process. In this experiment 110°C heated for 30 minutes only (and not for 0 to 20 minutes) seems to give appropriate incorporation but higher temperature i.e. 130°C (from 0 to 30 minutes) appear to start oxidation and especially in 20 to 30 minutes while lower temperature i.e. 80°C (time from 0. to 30 minutes) does not seem to release and incorporate the requisite amount of lecithin into the fat to influence the storage life for better.

However, lecithin alone does not appear to be the sole factor, responsible for increase in keeping quality. From

Experiment IX and X it would appear that sulphydral compounds released during heating of s.n.f. of milk might have also the effect of prolonging the storage life of butterfat when heated to appropriate time and temperature. Gould and Sommer (1939) and Josephson and Doan (1939) have found that S.H. compounds act as antioxidants. It is known that sulphydral group of compounds are known to be released from the protein surrounding the fat globules in milk by the effect of heat. Liberation of heat volatile sulphides of milk is of great current interest and Lea (1946) has devised methods both qualitative and rough quantitative for estimation of heat labile sulphur in fresh milk andmilk powders. Due to want of time, however, direct estimation on ghee samples could not be made in the present investigations, though there is indirect evidence of their presence and influence from Experiments IX and X cited above. Josephson and Dahle (1946) found increased induction periods by heating butterfat with dried skim milk and this may possibly be again due to the same phenomenon i.e. liberation of S.H. compounds. Further evidence of their presence is afforded by the cooked flavour they impart to ghee on heating to higher temperatures in presence of butter serum. Other reducing groups produced by the heating of proteins belong to tyrosine and tryptophane type (Mirsky and Anson, 1936). Marvel (1936) having patented the use of tyrosine and its ethers as antioxidants in edible fats and oils.

Barnicoat and Palmer (1939) observes that most of the anti-oxygenic effect of milk plasma would appear to be due to the

presence of soluble phosphates and citrates. They further state that addition of even 0.05% soluble phosphates and 0.1% citrate to creams had surprising effect in acting as antioxidants. Also Barnicoat (1947) found promising result by adding citrates to butter for prolonging storage life. It is probable that besides phosphate in form of phosphorous incorporated in ghee as pointed out above, citrate and citric acid or their derivatives might be present there too, and exerting some beneficial effect on the keeping quality. Butter for boiling into ghee is obtained from milk soured by addition of Dahi cultures and it is likely that during souring apart from lactic fermentation, citric acid fermentation takes place giving that flavourful aroma to ghee. This flavourful aroma is as the result of production of volatile acid and neutral compounds (diacetyl, acetyl methyl-carbinol and 2,3 butylene glycol) from citric acid and citrates of milk by certain types of bacteria in Dahi culture. There appears to be some difference of opinion between workers as regards the proper action of diacetyl on butterfat, e.g. Ritter and Nussbaumer (1939 VI) think that diacetyl acts as a pro-oxidant in butter oil, so that when this is removed by steam distillation in boiling process, the stability of fat increases. King (1931) and Hammer (1933) support this view. On the other hand Barnicoat (1937), Wiley (1939) and Rafey et al (1944) did not find any adverse affect of diacetyl on oxidation. In view of above, it is worthwhile to investigate the role of citric acid,

citrates and their derivatives to find out if they have any influence on the keeping quality of ghee.

Influence of acidity on keeping quality of ghee is the second factor investigated. From observations in Experiments III, IV, V (B) and VI, it would appear that not only there is no relation between ghee acidity and keeping quality but also there is no correlation between acidity of milk (curd) at churning and rise of acidity during storage. Because had there been anything of this sort, the lowest acid ghee in Experiment IV (sample No. 1 with 0.1692%) should have given longest induction period, while highest acid ghee (i.e. 0.9024% sample No. 8) would have given shortest induction period, but the result recorded is that sample No. 1 has given an induction period of 12 hours, while that of sample No. 8 is 42 hours (accelerated test). Even there are instances where two samples having the same acidity did not give the same induction period e.g. sample No. 7 and 8 in Experiment IV with an acidity of 0.9024% in each case, have given induction periods of 18 and 42 hours respectively (accelerated test). This is because the former had been heated to 110°C while the latter had been heated to 130°C. And so also is the case with sample No. 5 and No. 6 with same acidity, i.e. 0.6768%.

Likewise there is no relation between the curd acidity at churning and storage life, e.g. taking the instance in Experiment IV again, the lowest acid ghee sample of 1.1% at churning in Sample No. 3 and No. 4 have not given longest induction

period as against higher acidity of 1.7%, 2.2%, and 2.5%, rather the induction periods of all of these have been independent of acidity content at churning as the case with acidity content of ghee.

Apart from the above two facts, rise in acidity during storage was observed in these investigations, like other workers (cited in Chapter I). But the rise is small as compared with some of the other workers and is not uniform in all samples and experiments. Considering the lowest and highest values different experiments have given different ranges, e.g. in Experiment III, it is from 0.022 to 0.236%; in Experiment IV it is 0.0564 to 0.4476%; in Experiment V(B) it is 0.0566 to 0.169% while in Experiment VI it is 0.00 to 0.5% . These acidities have been obtained in different periods, i.e. from 6 to 8 months in Experiment III, 4 to 7 months in Experiment IV and 2 to 5 months in Experiment V(B) when stored at 35 to 40°C in the cabinet. But from data obtained in the above experiments, there is nothing to suggest that rise in acidity during storage has any relation with the keeping quality, e.g. if there had been any correlation with rise in acidity and keeping quality, then sample No. 8 in Experiment IV (having highest acidity in the series) and with a rise of maximum acidity in the series i.e. 0.4476%, should have given least induction period, while sample No.10 with 0.0564% rise (lowest in the series,) should have given the highest induction period, but they have given induction periods of 28 weeks (maximum) and 20 weeks (last but one) respectively in the incubation test.

Other samples in this experiment and in Experiment III and V(B) too show the same trend. The fact that rise in acidity has nothing to do with the deterioration of ghee by air - can be further illustrated from Experiment VI where oxidation has taken place in samples Nos. 1, 6 and 7 without any change in acidity while in few others, like 3, 4, and 5 there is rise of 0.1 and 0.2% only after oxidation (accelerated test).

These results agree with those of Lea (1931) where-in he states that he could not find marked change in susceptibility as a result of increasing the free fatty acid content of mutton fat from 0.24 to 0.52%. But they are in contrast to those obtained by Holm etal (1927) and Greenbank (1936) wherein the former states that addition of quantities of free acid, barely perceptible by titration, materially increases susceptibility. Also there is nothing to support the view held by Rangappa and Banerjee (1946I) wherein they state that colour, flavour and texture of ghee deteriorate on storage and are fairly proportional to the development of acidity. Observations similar to the last authors have been recorded by other Indian workers also.

In India, great emphasis is laid on the acid content of ghee, the value and quality of it being judged mainly on its acid content. This is because acidity has been linked up with keeping quality, flavour and aroma. For this reason, statutory standards for acidity have been defined by various provincial and Central Governments and Army Authorities, e.g.

for Army requirements, a typical ghee of good quality should possess acidity of not more than 9 points*(2.5% oleic), acidity between 9 to 11 points being accepted for blending and ghee having an acidity over 11 points being rejected (Wright 1937), on the ground of likely deterioration in keeping quality on storage. Agmark ghee (Government graded) should possess not more than 1.5% oleic (5 points nearly) for pure cow or buffalo ghee while for mixed one, it is not more than 2.5% oleic (9 points). However, from the results obtained in the present investigations, it is doubtful if acidity as such derived from souring of milk can be linked up with deterioration due to oxidation and if acidity of ghee can serve as an index of keeping quality.

Moreover, the acid content of Indian ghees as seen by the above standards is very high as compared with the acid content of ghee that can be derived by souring of milk since the ratio of acidity at churning; acidity in ghee, obtained in these investigations is 7 to 12:1. Rangappa and Banerjee (1946 I) also found similar ratio i.e. 10 to 12:1 (by boiling off process). Assuming that highest acidity of 3% lactic is developed (though in general it is less) by certain lactobacilli or mixed culture at churning - it will give acidity of about 0.8 to 1.3% oleic only in ghee. Hence the high acidity of 1.5 to 2.5% prescribed for even the best quality ghee, does not appear

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* A point of acidity means the amount of acid reckoned as oleic acid, contained in 10 grams of a sample of ghee which is exactly neutralised by tenth normal sodium hydroxide. This is 0.282%.

to be all derived from souring of milk or heat treatment of ghee but some other cause appears to be responsible for this excess acidity. Also the rise of acidity during storage noted by workers, e.g. 1.97% oleic within 24 months (Wright, 1937) - is very high. Many workers have obtained more than that too. It is probable that this excess acidity in fresh ghee and subsequent abnormal rise during storage - may be accounted for by the activity of lipolytic micro-flora since Rangappa and Banerjee (1946 IV) have noticed that under tropical conditions, these organisms grow at an alarming rate in unwashed butter stored from 1 to 7 days before melting into ghee as the practice is in Indian villages, and thereby the fatty glycerids are broken down into free fatty acids by them. Once the lipolysis has taken place in butter, the free fatty acids pass into ghee on clarification and it is very difficult to get rid of them. Further it is stated that under village conditions where loose butter is stored after churning, the rise in acidity is four times within a week and 25 times within a fortnight. The same authors point out that the free fatty acidity derived from lipolysis is auto-catalytic and is proportional to its strength. Therefore, it is more than likely that the free fatty acid content measured in ghee as acidity by various workers (which gave deterioration in proportion to their strength) is most probably derived from the activity of lipolytic micro-flora and has nothing to do with the acidity of ghee derived as a result of souring which is practised for the sake of aroma and flavour.

Another possible reason why acidity in Indian ghees seemed to correlate with keeping quality is the likely contamination with metals. Since ghee is boiled off in Indian village in open iron pans of varying size and shape, it is quite likely th a fair amount of iron is possibly passing into ghee through this source. Thus the acidity contained in ghee may help indirectly to accelerate the action if iron by ionising it to varying degrees for more effective action, and the ionization may be proportional to the acid content. Action of metals including iron in producing "tallowiness" have been studied by many workers (e.g. Golding and Feilman (1905); Hunziker and Hosman (1918); Hunziker, Cords and Nissen (1929); Emery and Henley (1922); Davies (1928); Davies (1932); King et al (1933); Barnicoat and Palmer (1939) and others). It has been found that in both metallic form and in solution, some of the metals are active pro-oxidants - in milk, milk products and other fats and oils. The list is headed by copper which has been found to be active even in 1:100 million parts (Lea, 1936). Effectiveness of iron has been found to vary but ordinarily it is regarded as one third to one tenth parts active than copper. In acid solution (Lea 1936) found that iron is approximately one twentieth as active as copper though in neutral and alkaline solution, i.e colloidal ferric hydroxide, the metal is inactive at a concentration of 5 p.p.m. Iron even in organic combination (i.e. haemoglobin, met-haemoglobin and haemin) has been shown to be effective in acceler-

ating the oxidation of linseed oil at 37°C and is not inhibited by cyanide in this state (Robinson, 1924). It is possible that some similar effect may be produced in ghee and therefore, the effect of iron in catalizing oxidation in presence of high acidity in ghee requires investigations. At present there do not seem to be any studies reported on these lines.

Yet another cause of rise of acidity and its subsequent effect on deterioration in Indian ghee may be the presence of lipolytic micro-flora and small quantity of oxidized ghee contaminating through the improperly-cleaned and sterilized earthenware containers in which ghee and butter for ghee is stored in villages before it is marketed. Observations on some of the above effects have been made by Rangappa and Banerjee (1946 IV).

In view of results obtained in the present investigations and the discussion in above paragraphs, it will be seen that souring of milk up to 2.5 to 3.0% lactic acid even (though generally it is less), to give desired aroma and flavour in ghee should not in any way effect the keeping quality in the normal way. This is of course different from the effects found in butter by various workers (e.g. Overman (1936); Davies (1936); White et al (1929, 1930) etc.); Where in they state that though ripening the cream increases the desirable flavour of the butter, keeping quality is also impaired, and butters of full flavour tend to deteriorate abnormally rapidly on storage. This is probably due to the difference in comp-

osition of the two products, i.e. ghee and butter, the former affording less favourable conditions for deterioration due to oxidation than the latter. Therefore, it seems unnecessary to lay emphasis on manufacture of ghee from curd having an acidity of 0.44% at churning or near abouts (Banerjee and Doctor 1938) which will not give proper aroma and flavour. Also it appears unnecessary to adopt "decantation method" of making ghee (i.e. melting butter at low temperature to settle curd, then decanting the clear fat and evaporation of moisture subsequently by heating above 100°C) in preference to "boiling off" method. (i.e. evaporating moisture by direct heating over 100°C), simply because the lower acidity so obtained is not of any advantage over the latter method. Rather there is disadvantage of lower keeping quality in "decantation method" due to heating ghee above 100°C, without contact with curd to drive off moisture. (Experiment V (Aand B)). The high initial acidity with abnormal rise on storage in Indian ghees ultimately resulting in quick deterioration - appears to be due to the lipolytic micro-flora, either acting on the fat during storage of butter before melting into ghee or due to infection through the improperly sterilized earthenware containers in which ghee is stored before marketing. In addition, the incorporation of "Fe" during heating in open iron pan seems to catalyse the subsequent acid hydrolysis of ghee at a very rapid rate. Traces of oxidized ghee left on the surface and in pores of earthen container may also help to-

wards the same end. Therefore, the best method to prevent deterioration of ghee during storage seems to be to prevent the action of lipolytic micro-flora before and after manufacture and also to prevent contamination with iron and oxidized ghee. Attempts to obtain small reduction in acidity of ghee by incomplete souring and "decantation method" of melting (as recommended by some workers) do not seem to be of any great avail for the purpose.

The third factor investigated is the influence of bacterial culture on the keeping quality of ghee. From the observations made in Experiment I, there were indications that there might be some effect on keeping quality due to bacterial culture as shown by superiority of L. acidophilus over L. bulgaricus (Iowa) but later Experiments, i.e. III and IV have not borne out this contention. Hence it is possible that difference observed in Experiment I might be due to different acidity, developed by two cultures during souring of 18 hours or so. Therefore, from the evidence obtained in these experiments, it is not possible to conclude that there is any direct relation of bacterial culture as such on keeping quality. Of course, there had been a marked difference observed in flavour and aroma, e.g. L. acidophilus gives better aroma and flavour than L. bulgaricus, Dahi No. 1 giving as L. acidophilus but Dahi No. 2 and No. 3 being intermediate. Also there is difference observed in the type of butter obtained, i.e. colour, moisture held and boiling characteristics. Therefore, the choice of one or the other culture may be more from these con-

siderations.

Influence of moisture is the last factor investigated. The moisture percentage determined in Experiments IV and V(B) range from 0.04 to 0.37% and only in two cases, these limits are exceeded, giving 0.46 and 0.53% respectively. Thus the moisture percentage obtained in them is well within the standard of not more than 0.5% prescribed for best quality Agmark ghee of India (Government graded). However, there is no correlation observed between moisture content and keeping quality in any of these experiments - so it appears that moisture as such has no effect on deterioration or otherwise of ghee. Similar observations have been made by Barnicoat (1945). These results support the view held by French et al (1935) that there is no influence of moisture on the induction period of lard at 50°C., but are contrary to the results obtained by Greenbank and Holm (1924) wherein they concluded that water increased the length of the induction period of butterfat at 95°C, and also to those of Lea (1936) wherein he found moisture frequently shortening the induction period of lard in glass at room temperature. In fact, it appears that the studies on moisture, as the probable cause of deterioration in ghee is of more academic interest since it is possible to manufacture ghee by the usual process, well below 0.5% prescribed for the best quality Agmark ghee. Further reduction in moisture content, if desired, can be obtained by centrifuging and vacuum drying (Mcdowall et al, (1942) and Barnicoat (1945)).

Throughout these investigations, the methods employed for detection of oxidation and end of induction periods have

been Peroxide Value (Lea), Fat aldehyde (Schibsted) and carotenoid value (measuring contents of carotenoid pigments; reduction and bleaching of which shows the progress of oxidation and end of induction period.) The main point of interest in them being not the actual oxidation values obtained by different methods but the difference between the test samples and the control and between test samples themselves, for grading the samples on the basis of induction periods in order of longer or shorter resistance or susceptibilities. As mentioned previously in either method, the end of the induction period has been judged by the rapid absorption of oxygen and bleaching of colour and in case colour has not bleached completely before rapid absorption sets in, the former has been taken as the end of induction. Further for these investigations, induction periods obtained by Peroxide method (Lea) have been taken arbitrarily as standard and other methods compared with it.

Comparing the results of induction period obtained by Peroxide and Fat aldehyde methods, it will be seen from Experiments I, II, III and IV (in which both of them determined) that except Experiment IV, there is a fair correlation (not absolute) between the trend of gradation obtained in the induction periods by both methods. In many cases in them, identical induction periods have been obtained and in case of unidentical periods, the difference recorded is from 2 to 4 weeks (incubation test). On the other hand in Ex-

periment IV, neither is there any correlation observed between the trend of induction period nor identical induction periods recorded except two samples. Since in the literature, both possibilities are found i.e. Wiley (1939) and Lea (1934), observed fair correlation between these two methods, while Barnicoat and Palmer (1939) did not find any correlation between them -- it is difficult to judge whether the difference observed in Experiment IV is due to methods themselves or due to experimental error and hence requires further investigations. But from the above results it will not be far from correct to assume that there is a fair correlation between the two methods and that both are capable of giving an index of oxidative susceptibility in ghee in majority of cases.

When the bleaching of colour (Carotenoid Value) is considered, it is observed that there is a fair correlation between the progress of oxidation as recorded by colour bleaching and Peroxide method too. However, (except in Experiment IV, in which there is complete bleaching of colour in all samples), there is not sharp bleaching of colour in all cases at the end of induction period as recorded by Lea (1934). Of course, bleaching is found in all samples, but to a varying degree i.e. some complete while others incomplete. Barnicoat and Palmer (1939) also record similar observations. In all these samples, it is observed that there is not pure white colour after bleaching and a faint brown tinge persists in some. This might be possibly due to the incorporation of

brownish colour of casein charred during the high temperature employed for ghee heating. Bleaching curve obtained too is gradual and not sharp and it appears there is resistance by the antioxidants incorporated in ghee heating at every stage. Banerjee and others (1936 and 1938) found similar trends but Lea (1934) records sharp bleaching curve in oxidation of butterfat in lamplight.

Comparing the results of accelerated test and incubation test in Experiments III, IV and V(B) it is observed that there is fair correlation (not absolute) between the two tests in arranging ghee samples in gradation of their susceptibilities. Temperature of heating i.e. to higher or lower -- does not seem to affect these results and so also the contact or otherwise of curd, though there is a tendency to show better correlation when the samples are heated in contact with curd than without, in which case 2 to 4 hours difference in the accelerated test is not perceptible in incubation test(Experiment V(B). Also in certain cases, difference of 2 to 8 hours in the accelerated test has not given any difference in the incubation test (e.g. Experiment III, samples 2, 5 7 and 8). Organoleptic tests generally agreed with the chemical methods. Except these few limitations both tests seem to answer very well the resistance and suceptibilities of different treatments in ghee. The results of accelerated test particularly are very interesting so far as that it can be usefully employed for getting quick results in ghee.

CHAPTER V.

SUMMARY.

Effect of four factors, viz., moisture, heat, acidity and bacterial culture on deterioration of ghee due to oxidation has been investigated. It has been found that of these four factors, heat employed in evaporation of moisture in ghee is most important from the point of view of keeping quality. It has been shown that heating ghee to various temperatures (i.e. 80°C to 150°C) results in different keeping quality, the trend being that ghee heated at higher temperatures keeps better than others. In addition it has been shown that increase in keeping quality with rising temperature; seems to be governed mainly by three factors, viz. (1) heating in contact with curd, (ii) time and temperature relationships and (iii) the rate of heating or cooking. Heating ghee in contact with curd to higher temperatures results in better keeping quality while heating without contact with curd, the storage life is reduced. Time and temperature relationships and rate of cooking are concerned with incorporation of requisite amount of antioxidants into ghee, less heat being incapable of incorporating proper amount, while excessive and continuous heat promotes oxidation in addition to incorporation of antioxidants.

On the question of the ingredient or ingredients in curd responsible for improved keeping quality there are strong indications that lecithin (as shown by the increased induction

period caused by heating butterfat with egg yolk) acts as antioxidant. Sulphydryl compounds also act as antioxidants appears likely from the data obtained in the present investigations.

In contrast to butter, acidity in ghee, derived from souring of milk as such does not seem to effect keeping quality as supposed by many workers. Also there is no correlation found between acid content of ghee, rise in acidity during storage and acidity of sour milk or curd at churning and the keeping quality. In light of these findings, the causes of high initial acidity in Indian ghees and subsequent abnormal rise during storage are discussed and it has been suggested that best method to avoid deterioration in Indian ghees due to this cause is to prevent the action of lipolytic microflora before and after ghee heating and also to prevent contamination with iron (incorporated through iron pans used for ghee boiling) and oxidized ghee. Attempts to attain small reduction in acidity of ghee by incomplete souring and "decantation method" of ghee melting (as recommended by some workers) do not seem to be of any great avail for the purpose; on the contrary the aroma and flavour may be adversely affected.

The moisture content of ghee as found in normally made samples (i.e. below 0.5%) and the type of bacterial culture used for souring in its preparation have not been found to affect its keeping quality.

Further, it has been found that there is fair (not

absolute) correlation between Peroxide Value (Lea) and Fat aldehyde value (Schibsted) in indicating induction periods which enables ghees to be graded according to their resistance and susceptibilities towards oxidation. Bleaching of colour has been also observed in all cases at the end of induction periods but to a varying degree, i.e. some complete while others partial.

Also fair (not absolute) correlation has been observed for stability test in ghee between the results of the incubation test (35 to 40°C; Indian summer temperature) and accelerated test at 100°C. The results of the accelerated test are interesting since it can be usefully employed for getting a quick estimate of the storage life of the product.

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APPENDIX I.

Utilization of Milk in India. (Wright, 1937).

No.	Particulars.	Quantity in Million Maunds.	As P.C. of Total Milk Production.	As P.C. of Total Milk Manufactured.	Value in Million Pounds. ***
I	Total Production of Milk Utilized	690	..	..	293.4
II	(a) as Liquid milk	215.0	31.2	..	107.5
	(b) for Manufacture	475.0	68.8	..	185.9
	Ghee	364.0	52.7	76.5	100.0
	Khoa	52.2	7.6	11.0	39.2
	* other indigenous milk products	16.7	2.4	3.5	22.3
	Dahi(curds)	26.2	3.8	5.6	19.7
	** Butter	10.3	1.5	2.2	3.0
	Cream	2.8	0.4	0.6)	1.7
	Ice Cream	2.8	0.4	0.6)	
	Cheese	negligible	..	..	..
	Total ..	690.0	..	..	293.4

* This includes products such as Mallai, rabree and khurchan which are obtained by evaporating the milk to a less degree than khoa.

** Includes both "country" and "creamery" butter.

*** Converted from Rupees.

APPENDIX II.

Utilization of ghee in India (1942).
(Report on marketing of ghee and other milk products in India).

Province/State.	Total Supplies.	Quantities used for different purposes.					
		Culinary Purposes.		Confectionary.		Religious purposes.	
		Quantity.	Percentage	Quantity.	Percentage.	Quantity.	Percentage
	(Thousand Mds).	(Thousand Mds.)	(%)	(Thousand Mds).	(%)	(Thousand Mds).	(%)
Ajmer	86	70	81	13	15	3	4
Assam	51	35	68	14	27	2	5
Bengal	879	571	65	299	34	9	1
Bihar	718	503	70	201	28	14	2
Bombay	613	521	85	80	13	12	2
Central Provinces	444	324	73	120	27	Neg.	Neg.
Kashmir	90	89	99*	1	1	Neg.	Neg.
Madras	1,259	995	79	252	20	12	1
Mysore	161	124	77	32	20	5	3
Nizam's Dominions	383	341	89	38	10	4	1
North West Frontier Province	163	139	85	24	15	Neg.	Neg.
Orissa	117	85	50	53	45	6	5
Punjab	2,138	2,031	95	86	4	21	1
Sind	500	420	84	70	14	10	2
United Provinces	1,758	1,231	70	492	28	35	2
Others	4,662	3,590	77	932	20	140	3
India ..	14,022	11,042	79	2,707	19	273	2

* Includes ghee used for dressing hair.
** Includes ghee used for snuff.

APPENDIX III.

Total exports of ghee from India. (Report, Marketing of ghee and other milk products in India.).

Year.	By Sea.										By land frontier routes.			
	British India Ports.						Kathiawar Ports.							
	Straits Settlements.	Burma	Federated Malaya States.	Hong Kong.	Others	Total.	Portugese East Africa.	Union of South Africa.	Others.	Total.	Nepal.	Afghanistan.	Total.	Total.
1935-36	17,076		3,354	2,021	10,229	32,680	2,951	2,343	2,488	7,782				42.8
1936-37	18,349		4,032	2,531	11,805	36,717	3,271	1,901	2,925	8,097				47. 1
1937-38	20,339	23,445	5,600	2,266	9,849	61,499	4,874	1,402	5,635	11,911	2250	53	2303	75.7
1938-39	21,372	19,036	5,221	3,229	11,605	60,463	4,024	3,384	5,542	12,950				75.7
1939-40	25,534	27,422	4,993	2,619	13,633	74,201	3,648	3,050	4,013	10,711				87.2
A verage.	20,534	13,981	4,640	2,533	11,424	53,112	3,754	4,416	4,120	10,290	225	53	23 3	65.7

APPENDIX IV.

Annual Production of Ghee in India, (1943).

(Report - Marketing of Ghee and Other Milk Products in India.)

Province/State.	Milk Production	Percentage of milk converted into Ghee.	Quantity of milk converted into Ghee.	Yield of Ghee per maund of milk.	Quantity of Ghee produced.	Percentage of Total Indian production.
	1	2	3	4	5	6
	Thousand Mds.	Per cent.	Thousand Mds.	Seers.	Thousand Mds.	Per cent.
Provinces -						
Assam	3,550	25.20	895	2.00	45	0.3
Bengal	41,264	31.50	12,998	1.75	569	4.1
Bihar	31,419 *	40.74 *	12,799	2.37	759	5.4
Bombay	17,457	53.7	9,374	2.18	511	3.6
Central Provinces	11,431 *	81.76 *	9,346	2.00	467	3.3
Madras	56,809	56.60	32,154	1.70	1,367	9.8
North-West Frontier Province	6,747	39.00	2,631	2.00	132	1.0
Orissa	5,061 *	39.20 *	1,984	1.91	95	0.7
Punjab	117,660 *	30.00 *	35,298	2.50	2,206	15.7
Sind	23,509	35.00	8,228	2.25	463	3.3
United Provinces	107,181 *	32.15 *	34,458	2.25	1,938	13.8
Others	3,676	42.90	1,577	2.16	85	0.6
Total	425,764	37.99	161,742	2.14	8,637	61.6
States -						
Baroda	8,666	46.80	4,056	2.25	228	1.6
Central India	6,898	81.76	5,640	2.00	282	2.0
Gujerat Agency	2,882	46.80	1,349	2.25	76	0.6
Gwalior	17,388	68.88	11,976	1.95	581	4.1
Kashmir	5,109	33.40	1.706	2.25	96	0.7
Mysore	8,833	36.41	3,216	1.91	154	1.1
Nizam's Dominions	9,435 *	66.45 *	6,270	2.39	375	2.7
Rajputana	55,445	70.0	38,811	2.25	2,183	15.6
Punjab	23,536 *	40.33	9,492	2.20	523	3.7
Western India	17,487	46.80	8,184	2.25	460	3.3
Others	19,685	42.86	8,437	2.02	427	3.0
Total	175,364	56.53	99,137	2.17	5,385	38.4
GRAND TOTAL ..	601,128	43.40	260,879	2.15	14, 22	100

*Figures have been revised in the light of more recent enquiries and differ from those already

APPENDIX V.

Quantities retained by producers for domestic consumption and the marketable surplus of ghee in India (1940).

(Report - Marketing of Ghee and Other Milk Products in India.)

	Ghee Production.	Retention by Producers.		Marketable surplus.	
		Quantity	Percentage	Quantity	Percentag
	Thousand Maunds.	Thousand Maunds.	(%)	Thousand Maunds.	(%)
)vinces --					
Assam	45	9	20.5	36	79.5
Bengal	569	114	2o.0	455	80.0
Bihar	759	229	30.1	530	69.9
Bombay	511	143	28.0	368	72.0
Central Provinces	467	74	15.9	393	84.1
Madras	1,367	137	10.0	1,230	90.0
North-West Frontier Province	132	77	58.0	55	42.0
Orissa	95	49	51.4	46	48.6
Punjab	2,206	1,721	78.0	485	22.0
Sind	463	9	2.0	454	98.0
United Provinces	1,938	485	25.0	1,453	75.0
Others	85	19	22.3	66	77.7
Total,	8,637	3,066	35.5	5,571	64.5
tes --					
Baroda	228	68	30.0	160	70.0
Central India	282	45	15.9	237	84.1
Gujerat Agency	76	23	30.0	53	70.0
Gwalior	581	116	20.0	465	80.0
Kashmir	96	5	5.0	91	95.0
Mysore	154	8	5.0	146	95.0
Nizam's Dominions	375	11	3.0	364	97.0
Rajputana	2,183	153	7.0	2,030	93.0
Punjab	523	422	80.7	101	19.3
Western India	460	138	30.0	322	70.0
Others	427	123	28.9	304	71.1
Total	5,385	1,112	20.6	4,273	79.4
GRAND TOTAL	14,022	4,178	29.8	9,844	70.2