CHAPTER II

REVIEW OF LITERATURE
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Ghee is the Indian name for clarified butterfat. The importance of ghee as a dietary article is mainly ascribable to its mellow flavour and texture, its ability to supply the most important fat-soluble vitamins A and E and its remarkable resistance to microbial deterioration as compared to other milk products. These attractive features of quality are naturally governed by the conditions of manufacture and marketing. The keeping quality of ghee is dependent upon the type of raw materials used and also on the method of preparation.

1. Method of preparation of ghee

During the last many years considerable interest has been evinced by dairy scientists in tackling the problems associated with ghee manufacture both from the fundamental and applied points of view. Ghee is normally manufactured by i) desi (indigenous), ii) creamery-butter and iii) direct-cream methods. The method of preparation of the product by the indigenous method has been studied and also compared with the other newer and more economical methods of ghee manufacture by several investigators and the subject has been amply reviewed by Ray (1957), De and Srinivasan (1958), Rangappa and Achaya (1974) and Srinivasan (1976).
1) Desi method

The desi (indigenous) method consisted in curdling whole milk, churning the curd into butter and finally melting the butter into ghee. There are considerable variations in the technique followed in different regions for the preparation of ghee by desi-method. Both raw and processed milks and sometimes a mixture of these two, are used for curdling milk as a first step in the production of ghee. Storing curd for sometime before churning is also practised in some places.

Read (1938) suggested the use of a hay-box (packed inside with bhoosa) for the preservation of morning's processed milk in a good condition till the evening's milk was added preparatory to their curdling before churning. The use of hay-box prevented the absorption of a smoky flavour and also helped in more economical recovery of better quality of ghee and lassi (buttermilk). Srivastava et al. (1948) compared the quality of ghee prepared by hay-box and simmering processing of milk. They observed that the keeping quality of ghee prepared by hay-box process was inferior to that obtained by simmering method or the usual method of processing milk (just boiling the milk before making the curd).

Srinivasan and Banerjee (1946a) observed that boiling of buffalo milk for 10 minutes or until a 5% reduction in volume occurred was a satisfactory method of reducing its
bacterial count. Milk so processed soon after collection and seeded within 2-3 hours produced curd of firm texture and satisfactory flavour. The ghee prepared was acceptable, and the vitamin A and carotene contents were not affected by such treatment. Seed curd obtained from mixed flora was better than that produced from pure cultures of lactic acid bacteria. An acidity from 0.8 to 1.0% lactic acid was found to indicate optimum maturity of the curd. They observed that if the process was properly carried out, the final product did neither acquire any cooked flavour nor any significant loss in vitamin A or carotene. Rangappa and Banerjee (1946a) prepared ghee from butter by the decantation and boiling-off processes. Butter acidity was twice as that of ghee prepared by boiling and 5 to 6 times as that of ghee prepared by decantation. It was found that sodium hydroxide was the best alkali for neutralization of acidity in ghee. The same authors (1946b) also studied the effect of lipolytic enzymes, bacterial contamination and length of storage on the acidity of butter. They suggested that village butter should not be stored, but should be melted to ghee at once. They observed that lipolytic enzymes of the microflora of untreated milk or seed curd were responsible for the acidity development in butter on storage. The rate of development and peak of acidity were the highest at 19°-33°C. The mould *Penicillium* was found to be the causative organism in deterioration. Srinivasan and Banerjee (1946b) investigated the influence of acidity of the curd in the churning process as practised
They observed that acidity helped in arresting the foam formation. They also found that increase in acidity either by ripening to 0.7 – 1.0% with starter culture or by artificial increase to 0.4% by the addition of citric acid followed by souring of milk to 0.7% acidity with starter culture, helped the recovery of 90% of the butterfat. Artificial increase in acidity led to sudden coagulation of the curd resulting in fat losses as high as 30%. Rangappa et al. (1946) studied the preparation of ghee in a number of areas in India and observed that the general conditions for the preparation of ghee were unsatisfactory, the common faults being the use of raw milk for curdling, uncontrolled fermentation, and admixing of processed and unprocessed milk. Jani et al. (1947) in their attempts to standardise the desi curd process for the manufacture of butter observed that under controlled conditions it was possible to recover 90-92% of milkfat as ghee. The authors used an uni-directional gear churn provided with facilities for washing of butter. Paul and Shahani (1950) in a study on the effect of using different dahi cultures on the quality of butter and ghee found that different cultures produced different amounts of acidity in butter and ghee. They also found that though the cultures from different localities varied in their capacity to produce acidity in curd from raw milk, they did not make any difference with boiled milk. Rangappa and Banerjee (1950a) in their studies on the modifications of the indigenous process indicated the effectiveness
of a simple method of diluting the curd. The dilution of the curd with an equal volume of water was the best for churning. The authors also observed that the keeping quality of ghee was increased to some extent with an increase in the dilution of the curd.

ii) Creamery-butter method

In creamery-butter method, the cream obtained by mechanical separation of milk is churned to butter which is clarified into ghee thereafter. Generally, butter obtained from sour cream is used, since ghee from sweet cream-butter is flat and tasteless.

Kothavalla and Cox (1927) reported the preparation of good flavoured ghee with better yields by boiling butter made from soured cream. Khubchandani (1937) examined the effect of addition of citric acid and sodium citrate to cream on the resultant butter and ghee. The author observed that the addition of sodium citrate and citric acid to cream improved the aroma of butter but reduced the keeping quality; and that the ghee made from such butter had a more pleasant aroma than untreated creamery-butter ghee. Patel et al. (1949) studied the differences in yield and quality of ghee obtained from cow and buffalo milks by creamery-butter method.

iii) Direct-cream method

In the direct-cream method of ghee preparation, the cream either ripened or unripened is directly clarified into ghee.
Dave (1935) in his comparative studies on the two methods of manufacture of ghee from cream and creamery-butter, observed that the method of direct conversion of cream into ghee was an economic proposition. French (1936) suggested a new method of making ghee from washed cream. The washing of cream by dilution and reseparation, substantially increased the fat recovery by the reduction in the non-fatty milk solids of washed cream. Guriye et al. (1949) carried out systematic studies on the direct cream heating method of ghee manufacture. They observed 2-14% loss of fat in ghee-residue. Dilution of cream with water to the original volume of milk and re-separating removed considerable amount of milk serum. This process reduced the weight of ghee-residue and increased the net out-turn of ghee. Acidification of sweet unwashed cream to 0.16% by addition of lactic acid also had the same effect. Natural souring was found to help in the quicker clarification of cream. El-Sokkary and Ghoneim (1949) studied the effect of acidity, fat content and washing of cream on its direct cooking for the preparation of samna (ghee). They observed that ripening of cream to an acidity of 0.40% quickened the cooking of cream and reduced the fat loss in mourta (ghee-residue). Cream of higher fat content and washed cream also had the same effect. Rangappa and Banerjee (1950b) claimed that ghee made from cream containing 0.10% citric acid gave a high yield and good flavour. Mani et al. (1954) studied in detail the process of ghee making directly from cream. They
found that the net yield of ghee was slightly more from cream washed with saline or acidulated water (0.05% lactic acid) than cream washed with ordinary water.

iv) Temperature of clarification of ghee

In the method of preparation of ghee, the temperature used for clarification is found to vary from place to place. Normally it is heated until the moisture is almost completely removed and light to dark-brown coloured residue starts settling at the bottom. The intensity of the development of aroma is largely dependent on the temperature/time used towards the end of clarification. In this boiling-off or direct-heating process of ghee manufacture, the clarification temperature may be allowed to rise to 110°-120°C and in some cases even beyond that. For markets demanding a raw curdy aroma the clarification is effected at a temperature not exceeding 100°C. In Andhra Pradesh, the ghee is generally raised to a temperature of 150°C, yielding a markedly burnt flavour. But in Gujarat, the temperature used is very low (below 100°C).

Sethna and Bhat (1950) observed that as the temperature increased from 100° to 130°C, there was a commensurate decrease in the percentage of moisture. Ghee prepared at 120° and 130°C were of good quality. A temperature above 130°C imparted a heated flavour on the ghee produced. Apart from this 'boiling-off' process, 'decantation' method or 'pre-stratification' method is also used for the manufacture of ghee from butter.
Bangappa and Banerjee (1946a) found that when butter was maintained for about an hour at 100°C in an electric oven, the scum, fat and butter-milk were separated into three clear strata. The fat layer was separated out and heated to 110°C for 5 minutes to help in the removal of the adhering moisture and to precipitate out the remnants of solids-not-fat. Ghee prepared by this so-called 'decantation' process had been observed by Bangappa et al. (1946) to have higher induction period than the one prepared by the direct heating method. Bahadur et al. (1950) used the induced-stratification of buttermilk in ghee making. In this stratification method, butter was heated to a temperature below boiling (80°C) and kept at that temperature for sometime to form three distinct layers. The bottom layer of buttermilk was then tapped out. When the bottom layer of liquid fat was heated along with the scum on the top at temperature of 120°C, ghee of acceptable quality could be obtained. The period of holding for desi-butter had been found to be 30 minutes and creamery-butter 15 minutes. These authors also reported that the product obtained by pre-stratification method contained less acid and more vitamin A.

In general, this pre-stratification technique has the following advantages over the direct heating method:
a) economy in fuel consumption to an extent of 60% compared to direct clarification, b) production of ghee with lesser acidity and longer shelf-life and c) greater control over
the clarification process and elimination of chances for over-exposure of normally high acid butter to high temperatures, resulting in reduced stability towards oxidative deterioration.

v) Comparison of different methods

Kothavalla and Cox (1927) studied the comparative advantage of ghee making by desi and creamery-butter methods and concluded that the latter method gave 18% out-turn in ghee over the desi method, and was more economical as it helped in an additional income from the out-turn of the separated milk. Dave (1935) compared the two methods of ghee making from cream and creamery-butter. He observed that the cream ghee method was more economical than the creamery-butter method, although the economy in fuel requirement for the former was more than for the latter, and the loss of fat in ghee making was greater in the former than in the latter. The physical quality of the product as evident from aroma and granular texture had been recorded to be superior in the butter ghee than in cream ghee. Doctor et al. (1940) found that desi ghee was better in flavour, colour and texture than cream-ghee. The method of preparation had no appreciable effect on the physico-chemical constants of ghee. Patel et al. (1949) studied the comparative yield and market quality of ghee prepared from cow and buffalo milks by indigenous (desi), creamery-butter and direct-cream methods. Their results showed that the net out-turn of ghee
prepared from cow milk by desi, creamery-butter and direct-cream methods were 82, 90 and 89% respectively. The corresponding values from buffalo milk were 87, 92 and 86% respectively. The data suggested that, in the production of ghee from cow milk, both creamery-butter and direct-cream methods were capable of giving higher recovery than desi method. The failure in direct-cream method with buffalo milk was observed to be due to the excessive loss of fat in ghee residue. Rangappa and Banerjee (1950b), from a comparison of different methods of ghee-making, found that cream acidified with citric acid yielded the maximum quantity of an acceptable quality product. The yield from cow milk was 92.0%, whereas from buffalo milk it was 92.5%.

2. Keeping quality of ghee

One of the serious problems encountered during the storage of ghee is the development of rancidity. There are two major categories of rancidity, i) hydrolytic and ii) oxidative.

i) Hydrolytic rancidity in ghee

In hydrolytic rancidity, fatty acids are hydrolysed from triglycerides, leaving behind either di- or monoglycerides or glycerol, depending upon whether one, two or three fatty acid radicals have been removed from each molecule. The hydrolysis is caused by the action of lipase on fatty acid esters. Moisture is also a contributing factor. Cream
and butter act as excellent media for the lipase and moisture action. Therefore, improper handling can bring about this type of hydrolysis and liberation of free fatty acids in ghee.

Rangappa and Banerjee (1946a) observed that lipolysis of butter was selective towards lower fatty acids and oleic acid. Paul et al. (1947) stored cow butter for one month to develop an acidity of about 13-19% (free fatty acids) and then converted to ghee. The fatty acids separated from the high acid ghee were subjected to ester-fractionation and observed that lower saturated esters were same as that of normal ghee, higher saturated esters were lower and unsaturated esters (chiefly oleic) were higher than that of normal ghee.

Dharmani and Lohara (1947) studied the preservative effect of common salt. For that, ghee with varying amounts of moisture and butter-milk (0.5 to 1.5%) was kept in 120 g tins and earthen pots of the same capacity. Salt was used at the rate of 2% of the weight of ghee. The development of rancidity of ghee was judged by the characteristic aroma, acrid taste and increase in free fatty acids. They observed that ghee containing 0.5 to 1.5% of moisture kept well in air tight tins for 15 months, with or without 2% salt. Samples stored in earthen pots became rancid after 5 months, but those stored with salt developed less acidity. Rangappa and Banerjee (1948) found that desi-butter stored in butter-milk and changed daily developed less acidity than samples stored dry. The keeping quality was further improved by storage in
0.8% lactic acid. The quality of ghee was higher from butter stored in lactic acid solution. Paul and Anantakrishnan (1949a) observed that ghee containing 4 to 7% residual moisture rose in acidity from 0.2 to 1.1 - 1.5% (as oleic acid) during six months' storage in glass bottles. The same lot of ghee when clarified to 0.2 to 0.3% moisture level had only half this acidity on storage. The same authors in a later study (Paul and Anantakrishnan, 1949b) observed that butter and ghee prepared from raw milk were of high acidity when compared to those prepared from boiled milk samples.

In a detailed study on the rancidity of butterfat, Mukherjee (1950a,b) observed that, in the absence of microorganisms (under sterile conditions), hydrolytic rancidity with the liberation of free fatty acids was only in the presence of moisture. Increasing the degree of relative humidity at which the fat was stored increased the degree of hydrolytic rancidity. Sterilized butterfat stored for 90 days at 25 and 100% humidity caused the acid value to rise to 1.03 and 2.25 from an initial negligible level.

The activity of micro-organisms is an important factor in butter rancidity. Many bacteria and moulds have lipolytic activity. In ghee also, despite the low moisture content, several micro-organisms are reported to flourish. Bhat and Sethna (1950) examined fifty samples of commercial ghee for their moisture and microbial contents, and the presence of 0.6% or more of moisture was pointed out as one of the causes
in the spoilage of ghee. They also studied the types of micro-organisms and their population and suggested that they are the most potent factors involved in the production of acid. Mukherjee (1951 and 1952) studied in detail the degradation of fats by micro-organisms. The author isolated three Aspergilli (\textit{A. niger}, \textit{A. fumigatus} and \textit{A. flavaryzen}) and one Penicillium (\textit{P. glaucum}) moulds from ghee and used them in his trials. The author observed that even at low level of moisture content (0.05%), these organisms secreted lipases which hydrolysed the triglycerides to free fatty acids and glycerol. The other enzymes secreted by the moulds rapidly oxidised the individual components. Lalitha and Dastur (1953) observed that butterfat prepared at 40°, 65° and 115°C kept quite well, provided care was taken to remove moisture and other impurities properly. Amount of free fatty acids was little higher in 40°C samples at the end of storage period than in 115°C samples. There was no difference in the rate of development of acidity in ghee made by either desi or creamery-butter methods. Small amounts of free fatty acids in ghee (about 1.5% as oleic acid) did not affect its keeping quality. Ghee with an initial acidity up to 2.5% (as oleic acid) developed further acidity only slowly. In high acid ghee samples, there was a tendency for the rapid development of acidity.

ii) Oxidative rancidity

The major type of rancidity which affects ghee is that caused by the action of oxygen. During the storage
of ghee there is a period of time during which relatively little change appears to occur, followed by an increased absorption of oxygen. The period preceding rapid oxidation is called 'induction period'. The reaction which follows seems to be self-catalysed and hence is often referred to as autoxidation. The oxidation of ghee results in loss of nutritional value and also develops strong disagreeable odour, which leads ultimately to great economic loss to dairy industry. Therefore, the mechanism and control of oxidative rancidity are of considerable importance to ghee industry. Here no attempt has been made to review the mechanism of such changes because the subject has been reviewed by many workers. However, an effort is made to give the summary of the important findings in relation to these degradative changes.

a) **Mechanism of autoxidation:** The mechanism of autoxidation of fats and oils has been extensively reviewed (Swern *et al.*, 1948; Powers, 1949; Bolland, 1949, 1950; Skellon and Gordon, 1951; Sims, 1951; Skellon, 1953; Morris, 1954; Holman, 1954; Swern and Coleman, 1955; Riemenschneider, 1955; Badings, 1960; Swern, 1961; Frankel, 1962; Lundberg, 1961-62; Schultz *et al.*, 1962; Skellon and Wharry, 1963; Uri, 1967; Emanuel and Lyaskovskaya, 1967; Artman, 1969).

According to the present concept of autoxidation mechanism, which is due to the work of Farmer *et al.* (1942), when fat is exposed to air there is first an induction period
during which antioxidants present in the fat are consumed and free radicals are formed. The source of the original free radicals is not known, but it seems that initiation of oxidation can be greatly accelerated by copper, iron, manganese and other transitional metals. It is generally presumed that these metals will undergo redox reactions involving one electron valence changes and generate odd-electron species such as hydroxyl radicals. Light and other radiations can also initiate autoxidation. Howsoever formed, these radicals eventually reach such a high concentration that induction period ends and is followed by a period of rapid oxygen absorption. It is believed that the free radicals are the perpetuators of the chain mechanism. The steps involved in the oxidation of mono-unsaturated fatty acids are shown schematically below:

\[
\begin{align*}
-\text{CH} = \text{CH} - \text{CH}_2^{-} & \quad \text{(Unsaturated fatty acid)} \\
& \downarrow \text{H} \quad \text{(Abstraction of hydrogen)} \\
-\text{CH} = \text{CH} - \text{CH} = \text{CH}^{-} & \quad \text{(Free radical)} \\
& \downarrow \text{CH} = \text{CH} = \text{CH}^{-} \quad \text{(Peroxide, Free radical)} \\
-\text{CH} = \text{CH} - \text{CH}^{-} & \quad \text{(Hydroperoxide)}
\end{align*}
\]
In these fatty acids, there are usually two \( \alpha \)-methylene groups which are points of attack in free radical chain reaction. The intermediately formed \( \alpha \)-methylene radicals are stabilized by resonance. Each of the structures contributing to the resonance hybrids gives rise to the formation of a hydroperoxide.

In this mechanism, a hydrogen atom escapes from the \( \alpha \)-methylene carbon atom, leaving an unstable free radical. Oxygen readily adds to the position left by the hydrogen atom, producing an unstable peroxide free radical. The peroxide free radical captures a hydrogen atom from another fatty acid chain producing a hydroperoxide which is fairly stable at low temperatures. Studies of the hydroperoxides produced by the oxidation of methyl oleate have shown four isomeric hydroperoxides, with \(-\text{OOH}\) group in positions 8, 9, 10 and 11.

Oxidation of polyunsaturated fatty acids is more complicated. Among the polyunsaturated fatty acids, those containing conjugated double bonds are generally more resistant to oxidation than the non-conjugated ones. This is probably due to the close proximity of the double bonds which give added stability by enabling electrons to dissipate excess energy through what is called resonance. In the monoethenoic and non-conjugated polyethenoic esters the movement of the electrons is considered to be blocked by the \(-\text{CH}_2\) groups, and hence an electron which has excess energy may expend it by
breaking away from the remainder of the molecule. When it does so, it takes a proton with it, which is equivalent to the removal of hydrogen atom, leaving a free radical.

Oxidation of linoleate is a simple example for non-conjugated polyethenoic esters. It follows the same steps that have been described above, except that the hydrogen which escapes is believed to come from the methylene group separating the two double bonds. The removal of labile hydrogen from \( \cdot \) -methylene group produces a resonance hybrid of three valence bond structure of the following given below:

\[
\begin{align*}
\text{CH}_3(\text{CH}_2)_4 & \quad \text{CH} = \text{CH} - \text{CH} = \text{CH} - \text{CH}_2 - \text{CH} = \text{CH} - \text{CH} = \text{CH} - \text{CH}_2 \cdot \text{COOR} \\
\text{Resonance} & \quad \text{Hybrid} \\
\text{Free} & \quad \text{Radicals}
\end{align*}
\]

Studies on the hydroperoxides have shown that major portion of the isolated peroxides are conjugated hydroperoxides. As the oxidation proceeds, these conjugated hydroperoxides undergo further oxidation and form diperoxides by 1, 4 addition.

Similarly, a mechanism can also be formulated for the autoxidation of linolenate ester which is similar to that for...
linoleate. Linolenate contains two pentadiene systems and they form four isomeric peroxides in which 9 and 16 isomerates predominate than 12 and 13 isomers, since the latter are more easily decomposed. Further hydrogen abstraction at the active methylene side would lead to di-hydroperoxides.

The autocatalytic radical chain reaction involves three important steps namely, free radical initiation, chain propagation and chain termination. This mechanism may be represented by the over-simplified steps where RH is the symbol for an unsaturated fatty acid molecule, H being a hydrogen atom of the methylene group adjacent to the double bond (\(\alpha\)-methylene group). The \(\alpha\)-methylene group is the preferential point of attack in the free radical chain reaction.

\[
\begin{align*}
\text{RH} + O_2 & \rightarrow \cdot R + \cdot OOH \quad \text{Free radical initiation} \\
\cdot R + O_2 & \rightarrow ROO \quad \} \\
ROO + RH & \rightarrow ROOH + R \quad \} \text{Chain propagation} \\
\cdot R + \cdot R & \rightarrow RR \quad \} \\
\cdot R + ROO & \rightarrow ROOR \quad \} \text{Chain termination} \\
\cdot R + \cdot OOH & \rightarrow ROOH \quad \}
\end{align*}
\]

The hydroperoxides formed in the autoxidation of unsaturated fatty acids are usually not stable and will decompose to give rise to many breakdown products which include a large number of saturated and unsaturated carbonyls.
Badings (1960) described the major pathways of hydroperoxide dismutation which is schematically shown below:

\[ \text{ROOH} + \text{-CH} = \text{CH} \rightarrow \text{-CH} = \text{CH} + \text{ROH} \]

\[ \text{ROO} + \text{-CH} = \text{CH} \rightarrow \text{-CH} = \text{CH} + \text{RO} \]

In addition to the above breakdown products, Parmer et al. (1942) have shown the formation of epoxides as a result of reaction of hydroperoxide or its radical with a double bond.

The various acids formed may not be as a result of direct hydroperoxide decomposition, they apparently come from further oxidation of alcohols and carbonyl compounds.

Polymers are also produced during autoxidation of fats. The mechanism of formation of polymers involves the formation
of conjugated hydroperoxides of polyunsaturated fatty acids during autoxidation of fats. These conjugated hydroperoxides produce free radicals which in turn react to form polymers. The monomer units of these are linked through carbon to carbon bonds. The formation of dimers and trimers in the autoxidation of ethyl linoleate is schematically shown below:
b) **Factors affecting oxidation**: In addition to the degree of unsaturation, increasing temperature accelerates not only the chain propagation reaction but the peroxide decomposition also, with or without help of other catalytic factors like light and metals. The accelerating effect of light is dependent on wave length. The effect of the visible light appears to be primarily one accelerating the decomposition of hydroperoxides. The effect of ultra-violet light is more pronounced. High energy radiations, such as $\beta$- and $\gamma$-rays exert pronounced accelerated effects, not only because they split hydroperoxides but they also generate free radicals from unoxidised substrates. Various mechanisms have been proposed to the effect of trace metals, and more than one mechanism may be operative (Uri, 1961). The effects of trace metals, as catalysts of autoxidation, are reviewed by Ingold (1962). A study of the catalytic effect of metals showed that lead, copper, cobalt, iron, tin, nickel and other transitional elements considerably accelerated the fat oxidation. The sequences of the catalytic activities of some of the metals established by various workers were as follows:

- $\text{Cu} > \text{Fe} > \text{Ni} > \text{Sn}$  \hspace{1cm} (Morris *et al.*, 1950)
- $\text{Cu} > \text{Li} > \text{Fe} > \text{Co}$  \hspace{1cm} (Stull *et al.*, 1951)
- $\text{Pb} > \text{Cu} > \text{Brass} > \text{Sn} > \text{Zn} > \text{Fe} > \text{Al} > \text{Stainless steel} > \text{Ag}$  \hspace{1cm} (Huṣaini and Saletore, 1953).

In addition to the above factors, presence and absence of antioxidants will also affect the oxidation of fats.
c) **Mechanism of action of antioxidants**: Antioxidants are substances which, when present in small quantities (naturally or by addition) in an oxidisable substance, are able to prevent or delay the rate of oxidation of that substance.

All antioxidants are structurally similar in that they contain unsaturated benzene rings plus either hydroxy or amino groups. Numerous proposals have been made on the mechanism of antioxidant action and the subject have been amply reviewed (Uri, 1961; Stuckey, 1962a; Emanuel and Lyaskovskaya, 1967).

When an antioxidant is added to an oxidising hydrocarbon they interfere either with initiation step or the early stages of propagation steps in the hydroperoxide formation. Therefore, an antioxidant reacts with either the original free radical or with one formed in the early stages to give an intermediate which is not capable of continuing the chain reaction. It reacts with $\cdot \text{RO}_2$ and $\cdot R$ radicals which propagate oxidation chain.

An antioxidant molecule $Q$ possessing a multiple bond (e.g. quinones) will inhibit oxidation by linking $\cdot R$ or $\cdot \text{RO}_2$ radicals to this multiple bond as follows:

$$\text{RO}_2(\cdot R) + Q \rightarrow \text{RO}_2Q \text{ (or RQ)}$$

It gives $\text{RO}_2Q$ or $\text{RQ}$ with little or no activity.
However, the main group of antioxidant is made up of compounds which do not possess double bond but an active hydrogen atom (e.g. phenols). Their molecule can be represented by $\text{AH}$, where $\text{H}$ is the active hydrogen. The mechanism of action of antioxidant will therefore be:

$$\text{RO}_2 (\text{or } \cdot \text{R}) + \text{AH} \rightarrow \text{RO}_2 \cdot \text{H} + \cdot \text{A} (\text{or } \text{AH} + \cdot \text{A})$$

in which $\cdot \text{A}$ is the inactive radical.

Thus, antioxidant action is due to the donation of hydrogen and substitution of active $\text{RO}_2$ or $\cdot \text{R}$ radical by the inactive $\cdot \text{A}$ radical. The antioxidant radical, unable to continue the chain reaction, will decay and form stable products, mainly by dimerization:

$$\cdot \text{A} + \cdot \text{A} \rightarrow \text{A} - \text{A} \text{ (terminated)}$$

or by reaction with a second chain radical

$$\text{RO}_2 \text{ (or } \cdot \text{R}) + \cdot \text{A} \rightarrow \text{ARO}_2 \text{ (or } \text{AR})$$

Therefore, antioxidant furnishes hydrogen atom to terminate the initiation and propagation mechanism of autoxidation. The action of antioxidants tends to support the Farmer's free radical chain reaction theory. Antioxidants have affinity for free radical and they will more readily give up a hydrogen atom to the free radical than a fatty acid molecule. When the free radical captures the hydrogen atom, its action is terminated and consequently the chain reaction is prevented.
Most of the antioxidants used in the prevention of oxidation are phenolic in nature. The oxidation of phenol (and its participation in the antioxidant process) starts with dissociation of a hydrogen atom and the formation of a phenoxy radical which may be represented in three forms:

The free radical is called semiquinone and has much greater stability than a free radical of the type shown for fatty acids. The reason for this is that the electron is capable of a movement through the ring structure as illustrated by the structures given above for semiquinone. This resonance structure imparts stability to the semiquinones. The phenoxy radicals of many phenols have markedly different life times (stability). The radical formed from \( \alpha \)-tocopherol persists only a few seconds and is then converted to \( \alpha \)-tocopheryl quinone. The radical formed from butylated hydroxy toluene has a slightly longer life while that from butylated hydroxy anisole is fairly stable. This free radical does not possess strong enough attraction for a hydrogen atom to remove it from an unsaturated fatty acid molecule. If it should encounter a free hydrogen atom, however, the original antioxidant would
be restored. They must also undergo other unknown chain reactions which is obvious from the fact that eventually they are used up and fats go rancid.

The degenerating chain branching reaction is retarded not only by chain termination as with phenols, aromatic amines and quinones, but can also be retarded by reducing the rate of free radical formation. The addition of compounds which are capable of reacting with hydroperoxides and thereby preventing free radical formation, must also reduce the rate of oxidation. Some of the organic sulphur compounds have this property. Hydroperoxides react with dialkyl sulphides to form a sulphoxide which reacts further with the hydroperoxides forming a sulphone, the latter form at much lower rate:

$$R_1-S-R_1 + ROOH \rightarrow ROH + R_1 - SO - R_1$$

$$R_1-SO-R_1 + ROOH \rightarrow ROH + R_1 - SO_2 - R_1$$

The antioxidant activity of these sulphur compounds is much less effective than alkyl phenol or amine type antioxidant.

d) **Synergists and their mechanism of action:** Synergists are substances which have little or no antioxidant activity of their own, but when present with an antioxidant markedly enhance its protective properties. As synergists a number of polyhydroxy or acidic compounds such as ascorbic (Columbic and Mattill, 1941; Calkins and Mattill, 1944; Columbic, 1946;
Clausen et al., 1947; Morris et al., 1950; Privett and Quackenbush, 1954b, Reiff and Free, 1959), citric (Evans, 1935; Dutton et al., 1948; Evans et al., 1954; Privett and Quackenbush, 1954b), tartaric (Roy, 1954), phosphoric and some salts (Olcott and Mattill, 1936; Emerson et al., 1937; Olcott, 1941; Golumbic, 1942a; Bailey and Feuge, 1944; Golumbic, 1946; Calkins, 1947) were reported to enhance the stability of fats. In addition to these acids various amino acids (Chow, 1934; Clausen et al., 1947; Olcott and Kuta, 1959), sulphhydrlys (Schwab et al., 1953), carbohydrates (Dutton et al., 1949) and various other compounds also have synergistic effect when used in edible fats.

The mode of action of synergists is not well understood. Probably more than one function may be involved. Mostly they seem to function as metal deactivators. However, remarkable synergistic effects are often observed in the absence of heavy metals, and hence other explanations are required. The synergists seem to have two important functions in antioxidant formulations.

1. It is believed to increase the effectiveness of the antioxidants by supplying hydrogen atoms to the free radical formed and thereby regenerating the original antioxidant so that it can function again.

2. It sequesters or chelates with the trace metals, which are fat oxidising catalyst by forming complex stable compounds called chelates.
Ascorbic acid has metal deactivating properties (Morris et al., 1950) and also some characteristics of synergist and antioxidant (Columbic and Mattill, 1941; Privett and Quackenbush, 1954b). Citric and phosphoric acids also function as metal deactivators (Dutton et al., 1948; Morris et al., 1950). Some of the proteins, carbohydrates and surfactants can also act as chelating agents if they form a complex with heavy metals.

Most of the acidic compounds like phosphoric (Columbic, 1942b, 1943, 1946; Privett and Quackenbush, 1945b), ascorbic (Columbic and Mattill, 1941; Calkins and Mattill, 1944; Calkins, 1947; Privett and Quackenbush, 1954a, Reiff and Free, 1959) and other acids may also play the part of hydrogen donors and thus cause the reduction of oxidised form of the antioxidant and regenerates it.

Phospholipids both from vegetable and animal sources have been widely studied and used as fat oxidation inhibitors. These are complex mixtures containing lecithin, cephalins, sphingomyelin, cerebrosides, inositol, plasmalogens etc. The inhibitive effect of phospholipids have been studied by many workers (Evans, 1935; Swift et al., 1942; El-Rafey et al., 1944; Richardson et al., 1947; Riemenschneider et al., 1944; Smith et al., 1958; Haab, 1959; Rama Murthy et al., 1968; Pruthi et al., 1970; Hector and Narayanan, 1972; Kuchroo and Narayanan, 1972). The cephalin components of commercial lecithin (phospholipid) appear to be responsible
for most of the synergistic effect or inhibiting effect (Olcott and Mattill, 1936; Smith et al., 1958; Haab, 1959). However, Stuckey (1962b) found that even lecithin fraction had synergistic properties; and Olcott (1962) found that both cephalins and lecithin had synergistic activity. Effectiveness of lecithin (phospholipids) including its similarity to the effect of phosphoric acid has been described by many investigators (Olcott and Mattill, 1936; Olcott and Emerson, 1937; Olcott, 1941; Oliver et al., 1944; Columbic, 1946; Calkins, 1947; Privett and Quackenbush, 1954a).

Tocopherols, which occur naturally in oils and fats have also been shown to possess antioxidant property. The use of tocopherol as synergist has been reported by Mukherjee and Goswamy (1947) and Bector and Narayanan (1972).

e) Oxidative rancidity in ghee: The mechanism of fat oxidation so far presented has found general acceptance and is the result of a variety of experimental observations on olefines and polyolefines, including fats and oils. Here an attempt is made to review the findings on oxidation of ghee.

Naegamwala et al. (1954) studied forced oxidation of ghee at 98°C. They observed that the reaction during the oxidation of ghee with gaseous oxygen was the formation of peroxides. They found that the peroxides had the hydroperoxide form. Small quantities of ring peroxides were also formed at the double bond, but being very unstable they immediately broke
They observed that the presence of free acids favoured the decomposition of hydroperoxides. The hydroperoxides were decomposed to hydroxyl compounds, the active oxygen being utilized to produce epoxides/or aldehydes. Kartha (1957, 1958, 1960) found that a high content of saturated acids in a fat such as in ghee, had little effect on the induction period, which preceded autoxidation. A proportionate decrease in iodine value was noticed with an increase in peroxide value. Then, a long period called 'maximum rate autoxidation period' came, and at this period, the rate of decrease of iodine value became constant, the unsaturation being lost at a constant rate. The peroxide value at this 'maximum rate autoxidation period' was first increased and then decreased. The author postulated two types of peroxides, i.e. hydroperoxides and cyclic peroxides, in the oxidised ghee. Vachha et al. (1957, 1958) studied the autoxidation of cow and buffalo ghee at 98°C. From their studies on quantities of oxygen absorbed and carbon dioxide produced, they observed that absorption of oxygen by both samples followed the typical sigmoid curve characteristic of the autocatalytic reaction. Buffalo ghee absorbed twice as much oxygen as cow ghee during the same interval of time. They observed three distinct stages of autoxidation, i) an initial induction period with an exceedingly slow rate of oxidation, ii) a buffer period during which the rate of oxidation increased linearly with the extent of oxidation and iii) a rapid oxidation period during which the oxidation
proceeded uniformly at a maximum rate. Water and carbon dioxide were formed from the very start of the autoxidation, although during the first few hours there was no absorption of oxygen. Therefore, they concluded that the production of carbon dioxide and water was due to the oxidation by internal oxygen. At any stage of oxidation, in both the samples, the quantities of water produced were nearly 6 to 12 times more than those of carbon dioxide.

Different factors have been found to influence the keeping quality of ghee. The method of preparation of ghee is one of them. Ghee prepared by desi method has been found to have less keeping quality than by creamery-butter or direct-cream methods (Rangappa and Banerjee, 1946a,b; Rangappa et al., 1946; Persai and Barnicoat, 1949; Paul and Anantakrishnan, 1949b; Paul et al., 1949; Patel et al., 1949; Lalitha and Dastur, 1953, 1954).

The initial acidity of ghee is another factor which influences the oxidation of ghee. Mukherjee (1950a) and Lalitha and Dastur (1953) reported that higher initial acidities in ghee showed higher development of peroxides on storage.

Temperature of storage of ghee also affects the oxidative stability of ghee. Govindarajan and Banerjee (1940) observed that ghee samples stored at 75°, 85° and 95°C had
induction periods (the end of induction period was taken to cor-
responding to a fall in pressure of 5 mm of mercury, in-
dicating the absorption of 0.6 ml of oxygen) of 20, 8 and 2 hour respec-
tively. Mukherjee (1950b) studied the peroxide development of ghee samples stored at 37°, 60°, 85°, 100° and 120°C in the absence of light and observed that they had induction periods (induction periods taken as the time required to reach a peroxide value of 2.0 units) of 1080, 5, 3, 1.5 and 0.75 hour, respectively.

Light is also found to affect the development of peroxides in ghee during storage. Lalitha and Dastur (1953) found that ghee exposed to air, but stored in the dark at 30°C developed peroxide value of only one unit in four months, but when stored in the diffused daylight the peroxide value increased to 2 to 3 in one week and 5 in three weeks.

In addition to these factors addition of antioxidants will also influence the keeping quality of ghee.

f) Application of antioxidants in ghee: The studies on the use of gallates (Lea, 1944; Tollenaar, 1949; Lalitha and Dastur, 1953; Sampath and Anantakrishnan, 1957; Ramanujam and Anantakrishnan, 1958; Kuchroo and Narayanan, 1972), butylated hydroxy anisole (BHA) (Sampath and Anantakrishnan, 1957; Ramanujam and Anantakrishnan, 1958; Kuchroo and Narayanan, 1972), butylated hydroxy toluene (BHT) (Kuchroo and Narayanan, 1972, 1973), and gum guaiac (Lea, 1952) for dry milk fat have
shown that the action of these antioxidants for inhibiting the development of peroxides were somewhat similar to those reported in other oils and fats.

In India, according to Prevention of Food Adulteration Rules (PFA rules) 1955 (as amended till February, 1974) antioxidants butylated hydroxy anisole and butylated hydroxy toluene are permitted to be added to ghee either singly or in combination in a concentration not exceeding 0.02%.

In addition to the chemical antioxidants, many other natural substances have also been reported to possess antioxidant properties. The residue obtained during preparation of ghee is also reported to have antioxygenic properties.

3. Role of ghee-residue on the preservation of ghee

The oxidative stability of ghee is also affected by the degree of heat treatment given and the method of clarification applied. Ritter and Nussbaumer (1939) found that butter-serum had a protective influence on fat, but when it was removed by filtration the fat became unstable. They obtained better keeping quality of butterfat when filtration was done at 100°C than at 45°C, possibly due to the effect on the phosphatides which was regulated by the water content of the ghee-residue. They observed that a substance other than phosphatides played a more important role in the keeping quality of fat. El-Rafey et al. (1944) reported that the butter oil
made by the process in which butter was heated to 110°C to drive-off moisture, was found to be more resistant to oxidation than when low temperatures of preparation were used. The improved quality was shown to be due to the transfer of greater amounts of phospholipids from non-oil phase of butter to the oil phase by the boiling-off process. Similarly, Rama Murthy et al. (1968) found that ghee samples prepared by heating butter to 120°C contained a lower level of phospholipids than those prepared by heating to 120°C for 10 and 20 minutes. The ghee samples containing low phospholipid contents had poorer keeping quality than those containing higher phospholipid contents.

During the clarification of butter or cream the nonfatty solids of these products settle down in the form of a heat denatured brown-sediment. This brown-sediment (ghee-residue) is also known as chhas or matha in India, mourta in Egypt and lurs in Germany. Earlier reports of Imperial Dairy Research Institute (Annual Report, 1942) showed that ghee samples in which ghee-residue was retained had comparatively longer keeping quality than the ones from which it was completely removed. Similar observations have also been made by El-Sokkary and Ghoneim (1951) and El-Sokkary and Zaki (1953). Rama Murthy et al. (1969) found that when ghee-residue was added at different levels (1 to 5%) to ghee and kept at 37°C for 5 months, the peroxide development in ghee
was at a lower rate than the control samples. Ghee samples with 5% residue showed better oxidative stability than those with 1-2% ghee-residue. They presumed that the antioxidant property of ghee-residue might be due to its high phospholipid content.

Apart from mentioning the antioxygenic components in ghee-residue, none of the above authors have carried out any detailed study on the nature of components present in ghee-residue and their influence on the preservation of ghee. However, few informations are available on the general composition of ghee-residue and the factors influencing them.

Rewald (1939) reported that a certain portion of the phosphatides in the butter residue existed in association with the proteins, and it was extracted only in presence of alcohol. This was also found to reduce on heating of butter. Ramaswamy and Banerjee (1948) analysed the scum which was obtained when butter was left in an oven at 100°C for one hour and reported protein 52.5%, lactose 24.7% and ash 7.9%.

Few reports are also available on the effect of different treatments of cream on the yield and composition of ghee-residue. Mani (1952) studied the effect of different treatments, like washing and ripening of cream, on the composition of ghee-residue. He observed that washing of the cream increased the moisture and protein contents but reduced the lactose content of ghee-residue. He also studied the effect of ripening of
cream on the composition of ghee-residue and observed that ripening of cream reduced the fat content and also the lactose content of ghee-residue. Ash content of the residue was almost the same in samples obtained from both ripened and unripened cream. Prahlad (1954) studied the effect of method of preparation on the quantity and quality of ghee-residue. He observed that the yield of ghee-residue was the maximum in the direct-cream method. Among the different types of creams used for clarification, the maximum yield was with sweet and unwashed cream. He also studied the effect of method of preparation on the composition of ghee-residue. The ghee-residue from the unwashed cream had maximum, and that from desi-butter had minimum fat contents. Protein was maximum in unwashed-sour-cream ghee-residue and minimum in sweet-cream and creamery-butter ghee-residues. Moisture, lactose and ash contents were maximum in desi-butter ghee-residue and were minimum in washed-sweet-cream ghee-residue.

Rama Murthy et al. (1969) found that ghee-residue is rich in phospholipids.

Apart from these, no other reports are available on the detailed composition of ghee-residue. It is also not known which are the constituents responsible for its antioxidant property. A systematic study was, therefore, carried out to identify the constituents of ghee-residue and also to find out their role on preservation of ghee.