CHAPTER II

REVIEW OF LITERATURE
This review includes the scientific information available to date on the flavour compounds (flavours and off-flavours) of milk and milk products with special emphasis in regard to the carbonylic flavour compounds. However, certain other flavour compounds, viz., fatty acids, lactones, alcohols, phenols, esters etc. are also included. The information has been given product-wise namely, milk, cultured dairy products, dried and concentrated milks, cheese, milkfat and related products (butter, butter oil and ghee). This is followed by a section on the 'role of lipids in flavours'. Finally, there is a section dealing with some general aspects of flavour research.

1 MILK

Characteristic flavour - Fresh milk has a typical faint flavour. Forss (1969b), in a recent review, reported low molecular weight compounds such as acetone, acetaldehyde, methyl ketones, 5-lactones, traces of C_4 to C_{10} fatty acids and dimethylsulphide to be involved in the milk flavour. Jenness and Patton (1959), Harper and Huber (1956) and Wong and Patton (1962) also reported the contribution of low molecular weight volatile compounds including C_3 to C_7 methyl ketones, formaldehyde and acetaldehyde in the flavour of milk. Jenness and Patton (1959) suggested that butyric acid and other low
molecular weight acids might also contribute to flavour of milk. Parks et al. (1963) and Wishner and Keeney (1963) identified C₉ to C₁₆ saturated aldehydes in fresh milk. Palo and Ilkova (1971) demonstrated the presence of acetaldehyde, acetone and diacetyl, in addition to compounds such as methanol, ethanol, isopropyl alcohol, n-propyl alcohol, isobutyl alcohol etc. in milk.

Methyl sulphide has also been shown to impart a flavour highly characteristic of fresh milk (Patton et al. 1958). The flavour threshold value of this compound is approximately 13 parts per billion in distilled water.

**Oxidized flavour** - Oxidized flavour is one of the most important flavour defects of milk and it is believed to be due to carbonyl compounds. According to Jenness and Patton (1959), carbonyl compounds involved in the oxidized flavour of milk are formed from unsaturated fatty acids contained in the phospholipids and glycerides. Terms such as cardboard, metallic, oily, and tallowy have also been used to describe various shades of this off-flavour.

Studies of Parks et al. (1963) on the carbonyl compounds in the butter oil, prepared from spontaneously oxidized whole milk, showed the tentative presence of C₅ to C₁₆ saturated aldehydes, C₉ to C₃₁ alk-2-enals and C₈ to C₁₂ alka-2,4-dienals. They suggested that deca-2,4-dienal was the major contributor to the off-flavour of this product, whereas saturated aldehydes were of little
significance in this regard. These carbonylic compounds, according to them, arise by a mechanism typical of classic lipid oxidation.

Forss et al. (1965 a) identified the flavour compounds from oxidised skim milk as acetone, acetaldehyde, hexanal, crotonaldehyde, 2-pentenal through 2-undecenal. They also obtained presumptive evidence for the presence of several 2, 4-dienals of short to medium chain lengths among the various 2-alkenals, 2-octenal and 2-nonenal were considered to be the principal flavour compounds. Forss et al. (1965 b) subsequently identified C₆ to C₆ alka-2,4-dienals in the distillates from skim milk with oxidised flavour, the most abundant 2-enals and 2,4-dienals being 2-octenal, 2-nonenal, 2,4-heptadienal and 2,4-nonadienal. These compounds, which imparted oxidised (cardboard) flavour to skim milk, originated from the oxidation of the more highly unsaturated fatty acids in milk lipids.

Sunlight flavour - Two distinct flavours have been reported to occur in whole milk exposed to sunlight, an oxidised flavour and an activated flavour (Singleton et al., 1963; Parks, 1967). Terms such as burnt and cabbagey have also been used to describe activated flavour. Wishner and Keeney (1961 and 1963) showed that the whole milk exposed to sunlight contained C₃, C₄ and C₆ to C₈ methyl ketones, C₁, C₂ and C₅ to C₁₂ alkanals, and C₄ to C₁₁ alk-2-enals among the oxidised flavour components. The activated flavour was attributed to methional (3-mercaptomethyl propionaldehyde) formed by the reaction of methionine with riboflavin (Patton, 1954; Jenness
Wishner and Keeney (1963), however, could not confirm the presence of methional in the sunlight exposed milk. Ballance (1961) reported that methional was further degraded to methyl mercaptan, dimethylsulphide and acrolein during the Strecker degradation of methionine. On the basis of the loss of various amino-acids on exposure of milk to sunlight, Moor and Hendricks (1966) suggested that methionine, histidine, tyrosine, tryptophan, cystine, lysine and possibly threonine were also important in contributing to the activated flavour of milk.

**Heat induced and irradiation induced flavours** - The flavour of milk is affected by heat treatments such as pasteurization, forewarming, preheating, superheating, heat sterilization etc. Parks (1967) suggested that the flavour of pasteurized milk was as much due to the loss of volatile compounds as to the formation of new flavour constituents at the pasteurizing temperature.

Jenness and Patton (1959) reported that momentary heating of milk at about 74°C produced a distinct cooked flavour due to volatile sulphides, hydrogen sulphide in particular, arising from the sulphydryl group activated by heat denaturation of β-lactoglobulin and to a lesser extent the proteins of the fat globule membrane. Blankengel and Humbert (1963) also referred to the role of β-lactoglobulin in the development of
cooked flavour.

On prolonged heat treatment at 74°C or high heat treatments, cooked flavour gave way to caramelized flavour, the degree of caramelized flavour being correlated positively with the magnitude of browning and associated changes (Jenness and Patton, 1959). They further reported that compounds associated with browning of milk were largely lactose fragments including furfuroil, furfural, hydroxymethyl-furfural, maltol, acetol, methylglyoxal, C₁ to C₄ acids, acetaldehyde, lactic and pyruvic acids, Ammonia, hydrogen sulphide and carbon dioxide also appeared to contribute to caramelised flavour of milk.

Cobb (1964) reported the presence of acetone, pentanone-2, heptanone-2, acetaldehyde, maltol, furfuryl alcohol, 6-decalactone, 8-dodecalactone, vanillin and a homologous series of alkanolic acids containing an even number of carbon atoms (C₄ to C₁₈) in heated milk. They further reported that maltol, vanillin and 8-decalactone were generally associated with caramel-flavour of heated milks.

Scanlan et al. (1968) showed increased concentrations of methyl ketones, lactones, diacetyl, acetaldehyde, benzaldehyde and dimethyl sulphide in the ultra high temperature processed milk. Kirk et al. (1968) found acetone, butanone, ethanol, propanal, furfural, ethanol and butanol in fresh fluid sterile milk. The stored fluid sterile milk also contained butanal, hexanal, heptanal, 2-pentanone and 2-heptanone.
Day and Papionnou (1963) reported the formation of n-alkanals (C₄, C₇, C₈, C₁₀, C₁₂, C₁₅ and C₁₈), iso-alkanals (C₁ to C₅ and C₉) and methyl ketones (C₃, C₄, C₇, C₉, C₁₁ and C₁₃) in milk irradiated with γ-rays under reduced pressure. It was suggested that long-chain aldehydes and methyl ketones were produced via hydrolytic cleavage rather than oxidative mechanisms.

In skim milk, the effect of γ-irradiation (Day *et al.*, 1957a) was fragmentation and aggregation of proteins, activation of sulphydryl groups, browning and odour formation. This effect induced a mild malty and caramel like off-flavour, which were somewhat like sunlight flavour in milk. They postulated methyl mercaptan, methylsulphide, and methyldisulphide to be involved in the off-flavour. Day *et al.* (1957b) concluded that methylsulphide and acetaldehyde were of immense significance in the off-flavour of irradiated skim milk.

**Absorbed flavours** - Terms such as "feed," "mead," "cowy," "barny" and "unclean" have been used for off-flavours that are absorbed into the milk through the cow (Jenness and Patton, 1959) and such absorbed flavours have been reviewed by Strobel *et al.* (1953) and Parks (1967). Honkanen *et al.* (1964) observed that aliphatic alcohols with an odd number of carbon atoms were transferred to milk from the rumen to a greater degree than others. Of the ethyl esters administered, only those with even number of carbon atoms in the fatty acids
entered milk while the methyl esters were not transferred in detectable amounts. Only traces of aldehydes administered, with the exception of 2-methylpropanal, were transferred.

"Cowy" or "barny" flavour, according to Jenness and Patton (1959), is generally prevalent in raw milk during the winter months. Tainted stable air, abnormal silage fermentations and ketosis were three possible sources for this off-flavour. Josephson and Keeney (1947) indicated a direct relation between the concentration of acetone in milk and the incidence of "cowy" flavour. They showed that addition of acetone to milk imparted "cow" flavour; the threshold level of acetone for this off-flavour being approximately 25 ppm. Concentrations up to 100 ppm imparted "cowy" flavours of different degrees.

Methyisulphide with a flavour threshold slightly above 12 ppb in distilled water has also been reported (Jenness and Patton, 1959) to impart "cowy" flavour to milk. Dunham et al. (1968) also reported that dimethylsulphide appeared to be derived from fodders and methionine was the precursor of dimethylsulphide in cow's milk. Keenen and Lindsay (1968) showed that the dimethylsulphide content of milk was increased by heating, indicating the presence of a heat-labile dimethyl sulphide precursor. This was probably an S-methylmethionine sulphonium salt.

Rancid-flavours - According to Jenness and Patton (1959), free fatty acids formed by lipolysis of milk glycerides, were
responsible for the rancid flavour in milk. Kolar and Mickle (1963) reported a significant correlation of the free fatty acids, especially short-chain ones, with the rancid flavours in milk. They isolated C_1 to C_4 fatty acids in the ether extract of rancid milk. Jensen (1964) has supported the view that rancidity in milk was due to accumulation of free fatty acids, especially free butyric and other short chain fatty acids, released from milk triglyceride by lipases. Hunter et al. (1968) also attributed spontaneous rancidity in milk to the presence of excess free fatty acids resulting from lipase action.

2. DRY AND CONCENTRATED MILKS

Parks (1967) and Forss (1969b) have reviewed the work on the flavours of dry and concentrated milks. Methyl ketones (C_3 to C_7, C_9, C_11, C_13 and C_15) and alkanals (C_1 to C_3, C_5 to C_10, C_12 and C_14) have been identified in fresh as well as stored dry whole milk, instant nonfat dried milk and commercial and aged concentrated milks (Wong et al., 1958; Parks et al., 1959; Dutra et al., 1959; Bassette and Keeney, 1960; Parks and Patton, 1961; Ganguly, 1962; and Muck et al., 1963). The higher concentrations of methyl ketones and alkanals during storage of these products contributed to stale or oxidised flavour (Parks, 1967; and Parks and Patton, 1961).

Bassette and Keeney (1960) believed that the presence of trace quantities of heptanal, octanal, decanal, dodecanal and tetradecanal were largely responsible for the cereal type
flavour which sometimes developed in non-fat dried milks, and the majority of these aldehydes resulted from the autoxidation of lipids in these products. Nawar et al (1963) also showed that stale flavour in dried whole milk was associated with the fat phase and was due to carbonyls, including unsaturated dicarbonyl or hydroxy carbonyl compound.

\[ \delta \text{-Lactones (C}_{10}, \text{C}_{12} \text{ and C}_{14} \text{) and C}_{12} \gamma \text{-lactone have been identified in concentrated milks (Arnold et al, 1966; and Muck et al, 1963). Presence of } \delta \text{-decalactone in dry whole milk and evaporated milk has been shown by Keeney and Patton (1956), and the "coconut-like" off-flavour of these products was attributed to this lactone.}\]

Muck et al (1963) reported the presence of C\text{6} to C\text{16} even chain fatty acids, formed possibly by hydrolysis, in the stored evaporated milk.

Parks and Patton (1961) and Bassette and Keeney (1960) reported the presence of furfural, hydroxy methylfurfural, diacetyl, maltol, methylpropanal and 3-methylbutanal in dry whole milk, nonfat dry milk and evaporated milk, possibly as a result of direct or indirect heat treatment given during the preparation of these products. The presence of benzaldehyde has also been reported by Arnold (1966) and Parks and Patton (1961).
Parks et al. (1964b) identified 0-aminoacetophenone as the flavour compound in stale dry skim milk. The flavour threshold value of this component was about 0.0004 ppm and the highly stale flavoured dry whole milk contained this compound 8-10 times this value. Arnold et al. (1966) also established, the presence of 0-aminoacetophenone in stale flavoured sterilized concentrated milk. In addition, they reported nonanone-2, tridecanone-2, acetophenone, naphthalene, and benzothiazole in the above product.

3. CULTURED DAIRY PRODUCTS

The subject of flavours of cultured dairy products like cultured buttermilk, dahi, yoghurt, butter cultures, cultured cream butter and creamed cottage cheese has been reviewed by Lindsay (1967). Free fatty acids and methyl ketones at optimum concentrations were believed to add to the flavour of cultured dairy products, especially those containing significant quantities of milkfat (Lindsay, 1967). Earlier, Lindsay et al. (1965b) had reported larger quantities of 2-pentanone and 2-heptanone in butter cultures prepared from heated whole milk than in uncultured heated milk. Day et al. (1964) reported dimethyl sulphide in butter cultures, cultured cream butter and sweet cream butter also. According to them, dimethyl sulphide, smoothed out the harsh flavours of diacetyl and acids in culture flavours.
According to Jenness and Patton (1959), the flavours of cultured dairy products were due to certain carbonyl compounds and volatile acids, which were the by-products of lactic acid fermentation. These included diacetyl, acetoin, acetone, acetaldehyde, butyric, propionic, acetic and formic acids. Diacetyl, formed possibly from pyruvic acid, was by far the most typical representative compound of such flavours.

Several recent investigations (Chou, 1964; Winter et al., 1963 and Lindsay et al., 1965b) have demonstrated the presence of aldehydes, methyl ketones, alcohols, ethyl esters of aliphatic acids and lactones in cultured dairy products.

Day et al. (1962) isolated the flavour volatiles from the ripened cream butters and selected starter cultures by distillation at low temperature under reduced pressure. They were tentatively identified as ethanal, propanal, butanal, pentanal, acetone, ethanol, butanol, ethyl acetate, methyl sulphide and acetic acid. In addition, 3-methylbutanal, ethylformate and ethyl butyrate were detected in butter volatiles and 2-methylbutanal in culture volatiles.

Day et al. (1963 and 1964) established the presence of methyl sulphide also in butter cultures, cultured cream butter and high quality sweet-cream butter. They further observed that proper concentration of methyl sulphide constituted the fresh flavour of the butter and smoothed out the flavour of diacetyl and acids. Concentrations in excess imparted a feed flavour to butter.
Chou and Harper (1963) reported the presence of acetaldehyde, propanal, butanal, acetone, butanone-2, ethyl acetate, acetoine, diacetyl, butanol, valeric acid and dimethylsulphide as the volatile flavour compounds in cultured butter milks. Chou (1964) also showed the presence of butyraldehyde, acetic acid, n- and iso-valeraldehyde in cultured butter milk.

Lindsay (1966) proposed that the flavour of cultured butter was due to the combination of compounds contributing to the flavour of non cultured butter and the compounds produced by starter bacteria used to prepare the cultured butter. Lindsay (1967) reported that from 1.0 to 2.5 ppm of diacetyl was the desirable concentration range for diacetyl in both butter culture and cultured cream butter. Lindsay et al (1965a) also established the diacetyl-acetaldehyde ratio (about 4:1) in full flavoured butter cultures.

Lindsay et al (1967) prepared synthetic butter culture concentrates for butter, cottage cheese, sour cream and butter milk by including diacetyl, acetaldehyde, dimethylsulphide, acetic acid and lactic acid in the formulations. They reported that the product flavoured with the synthetic culture flavours was similar to that produced by natural aroma butter cultures.

Corner et al (1971) established the presence of acetaldehyde, acetone, methyl ethyl ketone and ethanol in the GLC analysis of cultured milks of cows, goats and
Acetaldehyde has been considered to be a flavour compound of importance in yoghurt in particular and fermented milks in general (Jenness and Patton, 1959).

El-Hagarawy et al. (1971) reported that increase in the temperatures of ripening of cream was accompanied by an increase in the free fatty acids in butterfat, peroxide value of butter, diacetyl and acetylmethylcarbinol contents of butter.

4. CHEESE

Day (1967) reviewed the flavours of cheeses and reported that complex mixtures of compounds obtained by the degradation of fat, proteins and carbohydrates in their manufacturing and ripening processes gave rise to the varietal cheese flavours. Carbonyls, lactones, esters, alcohols, fatty acids, nitrogenous compounds such as ammonia, amines and amino acids, sulphur compounds such as hydrogen sulphide, methional, methylmercaptan and dimethylsulphide, etc. were involved in the flavours of cheese. The presence of milkfat appeared to be essential for the development of characteristic cheese flavours. The fat not only acts as a solvent for many of the flavour components, but it also serves as a precursor for flavour compounds such as lactones, methylketones, esters, alcohols and fatty acids.

The carbonylic flavour compounds in cheese have been studied extensively (Day, 1965). Methylketones
(C₃ to C₁₁ and C₁₃) have been identified in Cheddar (green as well as ripened) cheese (Day and Keeney, 1958; Day et al., 1960; Lawrence, 1963 and 1967; El-Din and Mattick, 1963; Matsuoka and Trugo, 1963; Bills et al., 1966; Morris et al., 1966). Increase in the concentrations of pentanone-2, heptanone-2 and nonanone-2 has been reported during ripening of Cheddar cheese (Matsuoka and Trugo, 1963).

Methyl ketones, especially heptanone-2 and nonanone-2, have been considered to be the key flavour components of Blue cheese (Bakalor, 1962). Day (1967) described the presence of C₃ to C₁₁, C₁₃ and C₁₅ methyl ketones in the Blue cheese. Schwartz and Parks (1963 a) and Schwartz et al. (1963 b) determined the level of odd numbered methyl ketones from C₃ to C₁₅ in the Blue cheese and Roquefort cheese respectively. 2-Heptanone was the major component in these cheese samples, except one which contained more of 2-nonanone. Similar results were also obtained by Anderson and Day (1966) on Blue and Roquefort cheeses.

Lawrence (1963), however, has questioned the presence of methyl ketones in Cheddar cheese in any significant concentrations. He reported that these methyl ketones were essentially artefacts and resulted from degradation of 3-hydroxyesters, the normal constituents of milkfat. Lawrence (1967) showed that C₆, C₈ and C₁₀
Methyl ketones were present in small quantities in the cheese. It was observed further that C₅ to C₁₃ methyl ketones in Cheddar cheese resulted from the hydrolysis of esterified C₅ to C₁₄ \( \beta \)-keto acids and \( \beta \)-oxidation of the corresponding fatty acids. Butanone-2 has been found in relatively large concentration in Cheddar cheese (Day et al., 1960; Scarpellino and Kosikowski, 1962; Day and Libbey, 1964). According to Scarpellino and Kosikowski (1982), this methyl ketone resulted from reduction of acetoin to 2,3-butylene glycol which was in turn dehydrated to the ketone.

Alkanals from C₁ to C₁₀ have been reported in Cheddar cheese (Day et al., 1960; Badr El-Din and Mattick, 1963). Badr El-Din and Mattick (1963) also reported the presence of alk-2-enals (C₄, C₁₁ and C₁₆) in green and ripened Cheddar cheese. 3-Methylbutanal and 3-methylthiopropanal have been shown to be present in cheese (Day et al., 1960).

Ethanol, 1- and 2-propanol, 1-butanol, 2-methyl-1-propanol, 3-methyl-1-butanol, ethyl and propyl acetate and ethyl butyrate have been reported in Cheddar cheese by Bills et al. (1966) and Morris et al. (1966).

Morris et al. (1968) and Fors and Patton (1968) reported the presence of alkanolic acids (C₂ to C₅) in cheese. Oren and Tuckey (1969) also brought out the importance of fatty acids in the cheese flavours and
flavour reported that typical Cheddar cheese did not develop when the degree of fat hydrolysis was low.

Forss and Patton (1966) and Kristoffersen (1967) reported the significance of sulphur compounds in cheese flavours. They reported the presence of hydrogen sulphide, methyl mercaptan, dimethylsulphide, methional and 3-mercaptopyrrolidone acid in the Cheddar cheese. Badings (1967) reported that "Catty" off-flavours of certain cheeses was due to the presence of 2-mercapto-2-methylpentan-4-one.

The "phenolic" defect in Gouda cheese has been attributed by Badings et al (1968) to the presence of p-cresol.

Egrow and Abdullins (1968) reported the presence of formaldehyde, acetaldehyde, propanal, butanone-2, pentanone-2 and heptanone-2 in different cheeses. Doležalek and Hoza (1971) confirmed the presence of formaldehyde, acetaldehyde, acetone, 2-butanone, 2-pentanone, 2-heptanone, 2-nonanone and 2-undecanone after 14 days of ripening of cheese but 2-undecanone was not found after 30 or 47 days.

Liebich et al (1971) showed the presence of more than 150 flavour components in cheese, 120 of which included ketones, aldehydes, alcohols, acids, esters, lactones, terpenes, alkanes, alkenes and alkyl benzenes.

Palo and Toth (1971) reported that the characteristic
aroma of cheese was due to the carbonyl compounds contained in the fat fraction. Golovnya and Uralets (1971) identified alkanals (C₂ to C₁₁), 2-enals (C₂, C₄, C₅, C₈ to C₁₀), alka-2,4-dienal (C₆) and also methyl ketones (C₄ to C₁₀) in 'Rossiiskii' cheese.

Recently, Chasov (1972) has shown the presence of formaldehyde, diacetyl, acetaldehyde, glycolaldehyde, acetyl methyl carbinol, acetone, propanal, heptanal, and hexanal in small quantities in cheese. He attributed the typical flavours of 'pikant' cheese to the presence of diacetyl and other carbonyl compounds resulting from fermentation of citric acid and its salts by Streptococcus diacetilactis in the starter. Day et al. (1960) had also reported the presence of 3-hydroxy-2-butanone (acetyl methyl carbinol) and 2,3-butanedione (diacetyl).

5. MILK FAT AND RELATED PRODUCTS

Carbonyl compounds have been reported to be largely responsible for the flavours, including off-flavours, of milkfat and related products such as butter, butter oil and ghee etc., although non-carbonylic flavour compounds have also been reported.

Babel (1946) stated that butter flavours depended on the type of cream from which it was made. According to him diacetyl, acetyl methyl carbinol and volatile acids such as acetic, propionic and butyric acids, in low
concentration, gave a pleasing flavour and aroma to butter. At about the same time Jacobs (1946a, b) suggested that diacetyl, acetyl propionyl, dipropionyl and several other dicarbonyls contributed to the flavour of butter. Further he observed that acetyl methyl carbinol (acetoin) was the most significant flavour contributor to butter. Of the fatty acids, butyric, caproic, caprylic and capric acids occurred in free form in small quantities and these also contributed considerably to the flavour of butter.

Andersen (1950) was of the view that diacetyl and acetyl methyl carbinol were inadequate to give true butter aroma. Diacetyl supplemented with low molecular weight fatty acids, esters, lactones, produced true butter flavour. The importance of lactones as flavouring materials grew and Jacobs (1956) suggested the use of \( \gamma \)-undecalactone in cream.

Taylor (1962) has observed that significant flavour compounds in butter serum were diacetyl, lactic acid and its salts, the lower fatty acids (mainly acetic acid) and their salts. In the fatty part of butter, aliphatic \( \delta \)-lactones, which formed a homologous series containing an even number of carbon atoms (say \( C_6, C_8, C_{10}, C_{12}, C_{14} \) and \( C_{16} \)) have been identified. A lactone containing an odd number of carbon atoms, say \( C_{11} \) has also been identified. According to him lactones in butter fat were probably formed from \( \delta \)-keto acids. One unsaturated \( \delta \)-lactone,
and an unsaturated \( \gamma \)-lactone have been identified. Methyl ketones, aldehydes, indole, skatole and dimethyl sulphide were some of the other compounds which contributed to flavour of butter. Monerieff (1965) reported that butter flavour depended on both watery part and the butterfat. Wong and Patton (1962) also established the presence of formaldehyde, acetaldehyde, acetone, butanone-2 and hexanone-2 in the steam distillate of cream. Bolding and Taylor (1962), on complete removal of the volatile carbonyls by high-vacuum degassing and subsequent steam distillation of the butterfat, detected the presence of a homologous series of methyl ketones with odd number of carbon atoms from \( C_7 \) to \( C_{15} \) in the distillate. They found that these carbonyl compounds were present in ppm in butterfat. In addition, they detected the presence of minor quantities of normal aliphatic \( \gamma \)-lactones in the butterfat concentrates. Lawrence and Hawke (1963) confirmed the findings of Bolding and Taylor (1962) that methyl ketones were formed during steam distillation of milkfat. Winter et al (1963) identified twelve carbonylic compounds in the steam distillate of fresh butter and these included formaldehyde, acetaldehyde, iso-butyraldehyde, iso-valeraldehyde, hexanal, nonanal, 2-nonanone, acetone, 2-heptanone, phenylacetalddehyde, diacetyl and acetoin.
Jurriens and Oele (1985) reported the presence of γ- and δ-lactones from C\textsubscript{10} to C\textsubscript{18} in butter. A series of n- (C\textsubscript{3} to C\textsubscript{8}) and iso- (C\textsubscript{4}, C\textsubscript{5}, C\textsubscript{9}) acids, and carbonyl compounds such as diacetyl, propionaldehyde, n- and iso-valeraldehyde, n-hexanal, 2-pentanone, 2-hexanone and 2-octanone were isolated by Kawanish and Saito (1965a) from the flavour concentrate of fresh sweet cream butter. Kawanish and Saito (1965b) identified formic, acetic, propionic, n- and isobutyric, isovaleric, caproic and caprylic acids, diacetyl, propionaldehyde, hexanal, octanal, 2-hexanone and 2-heptanone, together with several unidentified components from the flavour concentrate prepared from ripened butter. Lardelli et al (1966) isolated from butterfat a compound with a strong odour reminiscent of that of celery and assigned it the structure 2,3-dimethyl-2,4-nonadien-4-oxide (bovolide), the enol lactone of 2,3-dimethyl-4-oxo-nonenoic acid. Butters of wide origin, including ghee, were found to contain bovolide at a concentration of about 0.2-0.5 mg per kg butter. Zijden et al (1966) reported the presence of three unsaturated lactones of 5-hydroxy-9-tetradecenoic, 5-hydroxy-9-dodecenoic, and 4-hydroxy-8-dodecenoic acids in butter. In a study on the trace components of butterfat, Boldingh et al (1967) reported the presence in butter of saturated δ-lactones with C\textsubscript{8}, C\textsubscript{9}, C\textsubscript{10}, C\textsubscript{11}, C\textsubscript{12}, C\textsubscript{14} and C\textsubscript{18} chain lengths. They further observed that C\textsubscript{8}, C\textsubscript{10} and C\textsubscript{12} δ-lactones appeared to be the principal contributors to
butter flavours. Kinsella et al (1967b) tentatively identified the δ-lactones of 4-hydroxyoctanoic and 4-hydroxynonanoic acids in butterfat using gas-liquid chromatography. These lactones occurred in quantities of 0.25-0.5 ppm and approximately 0.2 ppm respectively in the butterfat samples investigated. A new lactone compound (C₇H₁₂O₂) moving between the δ-C₆ and δ-C₇ lactones on the gas chromatogram has been reported in milkfat by Honkanen et al (1960).

Khatri et al (1967) identified alkanals, alkanals, short-chain fatty acid and lactones in the vacuum distillate of milkfat. Abousteit (1967) isolated volatile flavour substances from butter by molecular distillation, fractionated them by TLC and partition chromatography and obtained 11 fractions including pentanone-2, heptanone-2 and nonanone-2. Forss (1969b) suggested that the main flavour components of butter were C₂, C₄, C₆, C₈, C₁₀- alkanolic acid, C₈, C₁₀, C₁₃ δ-lactones, dimethyl sulphide, various phenolic and heterocyclic compounds. Siek and Lindsay (1970) analysed the neutral volatile fraction of fresh sweet-cream butter by capillary gas liquid chromatography and found odd number methyl ketones, several alkanals, δ-lactones, γ-lactones and diacetyl in addition to several other compounds. Steinsholt et al (1971) determined the mean content of acetaldehyde, acetone, ethanol, aceticin and diacetyl by GLC in fresh butter and these were 0.35, 0.82, 1.72, 71.46
and 1.79 mg/kg respectively. Urbach et al. (1972) determined the flavour of \( C_6 \), \( C_8 \), \( C_{10} \), \( C_{12} \), \( C_{14} \) \( \delta \)-lactones, \( C_{12} \ \gamma \)-lactone, \( C_2 \), \( C_4 \), \( C_6 \), \( C_8 \), \( C_{10} \), \( C_{12} \), \( C_{14} \) n-alkanonic acids, phenol, \( m \)- and \( p \)-cresols, \( o \)-methoxyphenol, indole and skatole in synthetic butter prepared from 84% steam-deodorized butteroil and 16% water. They reported that none of the above compounds constituted basic butter flavour on its own, but \( C_8 \), \( C_{10} \) \( \delta \)-lactones, \( C_{10} \) acids, phenol, \( p \)-cresol, indole and skatole contributed to the butter flavour.

Parks et al. (1961) studied the bound aldehydes in butter oil. They tentatively identified \( C_9 \) - \( C_{18} \) straight-chain and branched-chain aldehydes with the exception of \( C_{12} \). These formed the major portion of the bound carbonyls of butter oil. Forss et al. (1966) identified \( \gamma \)-dodecalactone in butter oil and also obtained evidence for the presence of \( \gamma \)-\( C_9 \) to \( C_{11} \) and \( C_{13} \) to \( C_{16} \) lactones and \( \delta \)-\( C_{13} \) and \( \delta \)-\( C_{15} \) lactones.

Forss et al. (1967a) identified the flavour volatiles obtained from butter oil by high vacuum degassing as \( C_3 \), \( C_5 \), \( C_7 \), \( C_9 \) -alkan-2-ones, the \( C_2 \), \( C_4 \), \( C_6 \), \( C_8 \), \( C_{10} \), \( C_{12} \) n-alkanoic acids, \( C_6 \), \( C_8 \), \( C_{10} \) \( \delta \)-lactones (or their hydroxy acids), dimethyl sulphone, butanone, undecan-2-one, 5-dodecalactone, diacetyl, \( n \)-monanoic acid, 9-decenoic acid, \( n \)-methyl\( n \)-decanoate. In addition, they reported the presence of toluene, \( o \)-methoxyphenol, cresol, phenol, benzaldehyde, \( n \)-methylbenzoate.
and benzothiazole. No compound was isolated in an amount greater than flavour threshold. The contents of C<sub>10</sub> to C<sub>14</sub> aliphatic δ-lactones in butter oil, obtained from mixed herd milk, by Dimick and Harner (1968), averaged 98.0 ppm (range 58.6 - 139.0) and 67.2 ppm (range 47.7 - 100.2) on barn feed and pasture feed respectively. Schwartz and Virtanen (1968) detected in butteroil, 37 carbonyl compounds (as 2,4-dinitrophenylhydrazones) and these included eight methyl ketones (C<sub>3</sub> to C<sub>7</sub>, C<sub>9</sub>, C<sub>11</sub> and C<sub>13</sub>), eleven saturated aldehydes (C<sub>1</sub> to C<sub>4</sub> and C<sub>6</sub> to C<sub>12</sub>), ten alk-2-enals (C<sub>3</sub> to C<sub>12</sub>) and an unidentified class containing eight members. Siek and Lindsay (1968) detected 120 volatile compounds in the distillate of butter and identified C<sub>2</sub> to C<sub>6</sub> alkanals, and C<sub>3</sub> to C<sub>9</sub> alkan-2-ones among them.

There is a big change in the flavour of butter after it has been heated to 70°C. Patton and Keeney (1957) postulated that the δ-hydroxy acids present in butterfat were converted, on heating, into δ-lactones, and the marked change in flavour was possibly due to this conversion. This was further confirmed by Boldingh and Taylor (1963). An increase in the lactone content was observed when fat was heated for sometime at 140°C. A compound known as δ-caprolactone was isolated from heated milkfat by Parliment et al. (1965). Subsequently, Parliment (1966) confirmed the presence of even carbon C<sub>6</sub> to C<sub>16</sub> n-alkanoic acids and even carbon C<sub>8</sub> to C<sub>14</sub> δ-lactones. Forss (1968) also concluded that heating of butter
or butteroil increased the amount of lactone two to four fold and the characteristic flavour of these products was partly due to lactones. Nawar et al (1960) isolated volatiles from heated milkfat and showed that there were no qualitative differences in the composition of volatiles up to the temperature of 180°C. By heating the milkfat for 2 hours up to 130 - 185°C, they reported the formation of up to 19 components, eight of which were carbonylic in nature. Formation of acetone, pentanone-2, heptanone-2, nonanone-2 and undecanone-2 was observed in the butterfat heated in the absence of oxygen or moisture (Nawar et al, 1962). In addition, they could detect the presence of small quantities of butanone-2, hexanone-2, octanone-2 and C₆, C₇, C₈, C₉-alkanals. Abousteit (1967), using TLC and partition chromatography, reported the separation of volatile flavour substances from heated butter into 9 fractions containing among others odd numbered methyl ketones C₃ to C₁₅. Duin (1965) observed that no methyl ketones were formed in butterfat heated for 2 hours at 50°C, but after heating for the same period at 70°C, measurable quantities of methyl ketones were formed. Heating at 100°C caused a 6 - 7 fold increase and at 125°C there was a further 5-fold increase in the methyl ketone level. Only a slight further increase in methyl ketone formation was observed at 150°C. Similar results were obtained in sweet
and ripened cream butter. Schwartz et al. (1965) estimated methyl ketones on celite column from dry butterfat heated for various lengths of time at 50 to 115°C. They observed that methyl ketones production was relatively rapid in the vicinity of 100°C. Earlier, Bhalerao et al. (1959) had also reported an increase in the carbonyl numbers during heating (thermal oxidation) of butterfat.

Preparation of ghee involves heat treatment of creamery butter or 'makhan' i.e. desi butter or cream at 100 - 130°C. Clarification temperatures as high as 150°C have been used, though rarely as reported by Rangappa et al. (1946). Davies (1940) suggested that it was possibly the charring of the casein in the clarification process which was responsible for ghee flavour. Ramaswamy and Banerjee (1948) also felt that an unidentified protein had a possible role in the development of ghee flavour. According to them, the flavour in ghee developed possibly through the interaction among the protein (probably casein degradation product), a reducing sugar (probably lactose) and minerals, when butter was heated. Rao (1954) heated butter oil to 120°C with different amounts of pure and crude lactose and casein, both taken singly and in different combinations, and found that when pure lactose and pure casein were used singly and in combination, ghee flavour was not produced, but when crude lactose and crude casein (isolated from milk)
were used singly and in combination, characteristic ghee flavour was produced. The intensity of flavour was maximum with the use of 1.5% of a mixture of both crude casein and crude lactose (in equal proportions) of the clarified butterfat. It was concluded from this that it was neither pure casein nor pure lactose nor the combination of the two that was responsible for the characteristic ghee flavour. One or more of the minor constituents of milk present in the non-fat phase of milk was possibly responsible for the ghee flavour. Recent reports from this laboratory have brought out the possible role of carbonyl compounds in the flavour of ghee. Jain and Bindal (1968) showed that carbonyl compounds contributed significantly to the flavour of ghee, and that the flavour volatiles isolated from ghee consisted of complex mixtures of carbonyls tentatively identified as ethanal, butanal, propanone and butanone-2. Jain and Singhal (1969) further reported the presence of propanone-2, pentanone-2, heptanone-2, octanone-2, or nonanone-2 among the volatile carbonyl compounds isolated from deai ghee. More recently, Jain et al. (1971) detected similar eleven volatile carbonyl compounds in ghee prepared by different methods. Six of these were tentatively identified as propanone, butan-2-one, pantan-2-one, heptan-2-one, octan-2-one and nonan-2-one.
Oxidized and related off-flavours - The most widely encountered off-flavours in milkfat are oxidized and related off-flavours which are produced by oxidative action (autoxidation). Terms such as cardboard, metallic, tallowy, fishy, oily, mushroom, cucumber, painty, etc. have been used to describe the specific oxidized off-flavours.

All the reports in literature on the off-flavours resulting from the autoxidation of milk and milk products agree that the main components of these off-flavours were carbonylic in nature and that most of the oxidative carbonyls belonged to four classes, namely, alkanals, alk-2-enals, alka-2,4-dienals, and alkan-2-ones. Keeney and Dean (1951 a) vacuum distilled the oxidized milkfat, collected the material with characteristic flavour and odour of oxidized fat, and presented evidence that the flavour volatile material (C₉H₁₈O) was a 2-hydroxypropanal. In another report, Keeney and Dean (1951 b) showed that the volatile neutral material from oxidized milkfat consisted of carbonyl compounds of the empirical formulae: C₃H₄O₂, C₇H₁₂O, C₉H₁₈O, C₁₂H₂₀-₂₂O. Theoretical considerations indicated that the first compound was 2-hydroxypropanal. The C₇ and C₉ compounds were unsaturated ketones with the former containing an isolated ethylenic bond, while the latter was an α,β-unsaturated ketone.

Neither of the ketone was methyl ketone. The C₁₂ compound
was an α,β-unsaturated carbonyl compound, but it could not be shown conclusively whether it was an aldehyde or ketone. Tamsma (1959) suggested that carbonyl compounds appeared to be related to oxidized flavour in milkfat.

The volatile fraction concentrate exhibited two characteristic absorption maxima at 215 - 220 nm and at 260 - 265 nm indicating the presence of monoene carbonyl compounds and diene carbonyl compounds.

Day and Lillard (1960) identified C₁ to C₁₀ alkanals, C₅ through C₁₁ alk-2-enals and acetone in the volatile material from oxidized milkfat. In addition, they identified, tentatively, but-2-enal and the odd numbered C₅ to C₁₅ alkan-2-one. Using distillation followed by gas chromatography, the flavours of oxidized butteroil were partially characterized by Še-Negoumy et al (1961). The main contributor to the characteristic off-flavour was in the vicinity of octanal and 2-heptenal. Other carbonyl compounds produced in relatively large amounts made only a limited contribution to the oxidized flavour. Lillard and Day (1961) also reported that carbonylic compounds were most effective in producing the oxidized flavours. Most of the volatile carbonyls in oxidizing milkfat belonged to four classes, viz., alkanals, alk-2-enals, alka-2,4-dienals and alkan-2-ones. Otake (1962) reported that carbonyl compounds were not detected during the induction period of autoxidation of butterfat but increase.
occurred with rise of peroxide value. Of the carbonyls formed in oxidized fat, 63% were saturated. Parks et al. (1963) studied the carbonyl compounds in the butteroil of spontaneously oxidized whole milk and identified them as C₅ to C₁₆ saturated aldehydes, C₆ to C₁₁ alk-2-enals and C₈ to C₁₂ alka-2,4-dienals.

Stark and Forss (1965) identified alkan-1-ols (C₁ to C₈) in the steam distillate of oxidized butter. Stark and Forss (1966) observed in the steam distillate of oxidized butter C₁, C₂, and C₅ to C₈ alkan-1-ols in approximately equimolar quantities and in concentrations of approximately the same order of magnitude as those of C₆ to C₉ alkanalns and greater than those of alk-2-enals. In addition, they reported the presence of C₃ and C₄ alkan-1-ols, though in minor amounts, in oxidized butter. They suggested that these alkanols were formed by oxidation of unsaturated fatty acids, according to the theory of Farmer et al. (1943), from primary alkoxy radical resulting from the decomposition of lipid hydroperoxides. They suggested that the formation of primary terminal hydroperoxides as a result of decomposition of lipid hydroperoxides (secondary) appears to occur at an early stage of oxidation:

\[
\begin{align*}
R-\text{CH}_2-\text{CH}_2-\text{CH}-R' & \quad \xrightarrow{O_2} \quad RCH_2^\cdot + R'\text{CHO} + \text{OH} \\
& \quad \xrightarrow{\text{R=CH}_2-\text{OOH} + \text{R}''} \\
\text{Secondary hydroperoxide} & \quad \text{Primary hydroperoxide}
\end{align*}
\]
These may then form alkan-1-ols and/or alkanals via alkoxy radical (Bell et al, 1951). The following mechanism depicts the formation of hexanal, pentanal, and pentan-1-ol from the 13-hydroperoxides of linoleic ester.

\[
\begin{align*}
\text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CH} = \text{CH} - \text{CH} - \text{CH} &= \text{CH} - \text{R} & \\
\text{O}_2 &\rightarrow \text{R}^\dagger \text{H} & \\
\text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CH} - \text{CH} = \text{CH} - \text{CH} &= \text{CH} - \text{R}^\dagger \text{H} & \\
\text{O} &= \cdot \text{O}^\dagger & \text{H} &= \cdot \text{H} \\
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 &\rightarrow \text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CHO} & \text{Hexanal} & \\
\text{O}_2 &\rightarrow \text{R}^\dagger \text{H} & \\
\text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 &\rightarrow \text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 & \text{Pentanal} \\
\text{O}_2 &\rightarrow \text{R}^\dagger \text{H} & \\
\text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 &\rightarrow \text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{CHO} + \text{R}^\dagger \text{H} & \\
\text{OH} &\rightarrow \cdot \text{OH} & \\
\text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 &\rightarrow \text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 & \text{Pentan-1-ol} \\
\text{OH} &\rightarrow \cdot \text{OH} & \\
\end{align*}
\]

Stark et al. (1967) identified pent-1-en-3-ol and pent-1-en-3-one in approximately equal amounts from buttermilk obtained from oxidized cream. They have also given the scheme for the formation of pent-1-en-3-ol and pent-1-en-3-one.
Forss et al. (1987b) reported the presence of C₁ to C₇ alkanes; C₂, C₄ to C₆, C₈ alk-1-enes; C₇, C₈ alk-1-ynes; C₂ to C₆ n-alkanoic acids; ethanol; C₂ to C₇
alkanals; 3-methylbutanal; 4-methylpentanal; C$_1$ to C$_4$ alkylformates; ethylacetate; butanone-2 etc. In the oxidized butter, Steinsholt et al (1971) observed that contents of acetaldehyde and diacetyl decreased, acetone and ethanol remained nearly constant and acetoin levels increased markedly during storage of butter. Recently, Ahmed et al (1971) isolated and identified carbonyl compounds from the oxidized milkfat. Relative proportions (as percentage of the total monocarbonyls) of saturated aldehydes, methyl ketones, 2-enals and 2,4-dienals were reported to be 55, 25, 12 and 7 respectively in oxidized butter oil.

Specialized oxidized off-flavours

Fishy flavour - Pont et al (1960) reported that during storage fishy flavour was produced in butterfat containing an antioxidant preparation of nordihydroguaiaretic acid (NOGA) and citric acid in propylene glycol. It was observed that the development of fishy flavour was favoured by the addition of citric or lactic acid in solution. Copper was not an essential factor though it intensified the defect when other factors favoured its development. Gas chromatographic analysis of the flavour concentrate (Forss et al, 1960a) showed that the fishy flavour was composed of an oily flavoured fraction containing hexanal, heptanal, hex-2-enal, and
heptan-2-one and a metallic fraction containing a single carbonyl compound present in relatively small amounts. It was considered that these compounds with the exception of heptan-2-one were mainly responsible for the fishy flavour. Other compounds isolated included propanal, pentanal, octanal, nonanal, decanal, acetone, pentan-2-one, nonan-2-one, undecan-2-one, acrylaldehyde, pent-2-enal, hept-2enal, oct-2-enal, non-2-enal, hepta-2,4-dienal, a compound resembling mushroom flavour, and a compound that formed 2,4-dinitrophenylhydrazine of methyl glyoxal. Forss et al (1980b) fractionated flavour concentrate from the fishy butter, obtained from fishy cream, by gas chromatography on silicone oil column into 6 characteristic fractions, which in order of elution were described as painty or drying oil, oily, mushroom, metallic, tallowy and cucumber-like. The flavours of all fractions were predominantly caused by carbonyl compounds and the oily and the metallic fractions were the essential major contributors to the fishy flavour. Pentanal and pent-2-enal occurred in the painty fraction; hexanal, heptanal and hex-2-enal in the oily fraction; hept-2-enal and a compound with mushroom odour in the mushroom fraction; carbonyl compound with metallic odour in the metallic fraction; octanal, nonanal, oct-2-enal and hepta-2,4-dienal in the tallowy fraction; and non-2-enal
in the cucumber-like fraction. Diemair and Sehams (1962) reported observations similar to those of Forss et al. (1960b) on the analysis of fishy flavour.

Forss (1964), in a review of the work on the identification of compounds responsible for fishy flavours, has stressed upon the remarkable similarity between the volatile compounds isolated from stored fish and from dairy products with fishy flavours.

**Painty flavour** - Forss et al. (1960b) isolated a volatile fraction with painty flavour fraction from fishy-washed cream and showed that pentanal and pent-2-enal accounted for the painty flavour. Forss et al. (1960c) obtained the same range of carbonyl compounds as from fishy flavoured butterfat or washed cream. They consisted of C₅ to C₁₀ alkanals, C₅ to C₁₀ alk-2-enals, hepta-2,4-dienal and a compound with metallic flavour. Heptan-2-one, present only in small amounts in washed cream, was also isolated. Three factors distinguished the painty from the fish oil flavoured butterfat. There was a relative increase in pentanal and C₅ to C₁₀ alk-2-enals. Secondly, the metallic compound was present in the butterfat in amounts too small to have an effect on the flavour. Thirdly, the total weight of carbonyl compounds was about 100 times greater in painty butterfat than in fish-oil flavoured butterfat. Flavour tests revealed that pentanal and
pent-2-enal were important in the painty flavour. A flavour concentrate from the painty butter oil was subjected to gas chromatographic analysis (El-Negoumy et al., 1961) and it was shown that the painty flavour in butter oil seemed to be due to a single component. This component was possibly similar to the painty compound reported by Forss et al. (1960, b, c); and not entirely due to pentanal and pent-2-enal.

**Oily flavour** - Forss et al. (1960, a, b) reported the compounds hexanal, heptanal and hex-2-enal to be responsible for the oily flavour. In addition, heptan-2-one has also been reported in the oily fraction separated from fishy butter (Forss et al. 1960a). El-Negoumy et al. (1961) however, could not confirm these observations.

**Mushroom flavour** - Forss and Stark (1963a) attributed the mushroom flavour in oxidized milkfat and dairy products to the presence of oct-1-en-3-ol (vinyl-n-amyl-carbinol). According to some workers oct-2-en-3-one also had mushroom flavour. However, oct-1-en-3-ol had more characteristic mushroom flavour and actually occurred in certain species of mushrooms. In addition, oct-1-ene, oct-2-ene and octa-1,3-diene also imparted mushroom odours. Earlier, Forss et al. (1960b) had believed this flavour was due to a carbonyl compound and his data on its 2,4-dinitrophenylhydrazone closely
resembled that of oct-1-en-3-one. Forss (1964) stated that the formation of this 2,4-dinitrophenylhydrazone might have arisen from oxidation of alcohol or from its contamination with the ketone.

Metallic flavour — Forss et al (1960 a, b) could separate the volatile fraction with metallic flavour from the concentrate of butter and washed cream using gas chromatography. They observed that the fraction with metallic flavour contained a single carbonyl compound which was subsequently identified as oct-1-en-3-one (n-amyl vinyl ketone) (Stark and Forss, 1962). The compound had a flavour threshold value of one part in $10^9$ butterfat and one part in $10^{10}$ in water. Later, Hammond and Hill (1964) also observed that oct-1-en-3-one accounted for the metallic flavour of autoxidized milkfat. They suggested that this in combination with small amounts of aldehydes imparted oxidized flavour to milk. El-Negoumy et al (1961 and 1962) showed that the vinyl amyl ketone responsible for the oxidized-metallic flavour came from linoleate. Wilkinson and Stark (1967) suggested that oct-1-en-3-one was likely to be derived from linoleic or arachidonic acids or both. Pathways for the production of intermediate giving rise to oct-1-en-3-one were suggested for systems with free and with limited access to oxygen. These mechanisms involved secondary
oxidations of the initial monomeric oxidation products.

With free oxygen access, primary oxidation of carbon atom 13 in linoleate, or 15 in arachidonate, would result in the following structures:

\[
R'-R^*-\text{CH=CH-CH=CH-R}''
\]

where \(R'\) is \(\text{CH}_3-(\text{CH}_2)_4\)

\(R^*\) is either \(-\text{CH(OOH)}\), \(-\text{CH(OH)}\), or \(-\text{C}=\text{O}\)

\(R''\) is the remainder of the ester chain.

Secondary oxidation may occur over the conjugated C-C structure of the primary product, particularly in linoleate which no longer contains activated methylene groups. Attack by a radical at the cis double bond creates a new radical site to which in turn molecular oxygen may add with eventual formation of a hydroperoxy group or polymers:

\[
\begin{align*}
R'-R^*-\text{CH=CH-CH=CH-R}'' &\xrightarrow{\text{OH (or RO)}} R'^*-\text{CH=CH-CH=CH-R}'' \\
R'^*-\text{CH=CH-CH=CH-R}'' \times 2 &\xrightarrow{\text{O}_2} R'^*-\text{CH=CH-CH-CH-R}'' \\
R'^*-\text{CH=CH-CH=CH-R}''+R_* &\xrightarrow{\text{OOH}} R'^*-\text{CH=CH-C} &\xrightarrow{\text{O}_2} R'^*-\text{CH=CH-CH=CH-R}'' \\
R'^*-\text{CH=CH-CH=CH-R}'' &\xrightarrow{\text{OOH}} \text{ (dimeric and polymeric products)} \\
\text{(monomeric product)} &\xrightarrow{\text{OOH}} \text{ where } R^*_R'\end{align*}
\]
Decomposition of the monomeric product with cleavage of the C-C chain at the 10-11 bond in linoleate, or the 12-13 bond in arachidonate, followed by propagating hydrogen exchange reaction with substrate lipid, may then result in the formation of oct-1-en-3-one or oct-1-en-3-ol, the latter compound being responsible for mushroom flavour.

\[
\begin{align*}
R^*-R^+_n-CH=CH+CH-CH-R^+ \quad & \quad \downarrow \quad \text{Cleavage} \\
\quad & \quad \text{OH} \\
R^*-R^+_n-CH=CH + R^+_n-CH-CH=O+OH \quad & \quad \downarrow \quad \text{Cleavage} \\
\quad & \quad \text{OH} \\
R^*-R^+_n-CH=CH_2 + R, \quad & \quad \text{(Oct-1-en-3-one or Oct-1-en-3-ol)}
\end{align*}
\]

The compound \(R^*-R^+_n-CH=CH_2\) is either oct-1-en-3-one or oct-1-en-3-ol depending upon whether the \(R^+_n\) is \(-C=O\) or \(-CH(OH)\). If \(R^+_n\) is \(-CH(OOH)\) it may be dehydrated or reduced to a keto or hydroxy group before or after the chain cleavage shown above.

Another mechanism by which oct-1-en-3-one or oct-1-en-3-ol may be formed under these conditions relates specifically to arachidonic esters provided the \(C_{12}\) mesomeric radical may be formed along with the preferred \(C_{15}\) species. This pathway is thus of special importance in oxidizing membrane lipids.
The mechanism by which oct-1-en-3-one may be formed from hydroxylated keto derivatives of linoleate or arachidonate in aqueous media is shown in the nett reactions:

\[
\begin{align*}
\text{CH}_3-(\text{CH}_2)_4-\text{CH}=&-\text{CH}=-\text{CH}=-\text{CH}=-\text{CH}=-\text{CH}- \\
\text{\downarrow}
\text{\phantom{CH}_3-(\text{CH}_2)_4-}\text{CH}=&-\text{CH}=-\text{CH}=-\text{CH}- \\
\text{\phantom{CH}_3-(\text{CH}_2)_4-}\text{CH}=&-\text{CH}=-\text{CH}- \\
\end{align*}
\]

Under the pathway requiring limited access to oxygen, the secondary oxidation may take place without involving the addition of molecular oxygen:

\[
\begin{align*}
\text{R}^*\text{R}^*\text{C}=&\text{CH}=-\text{CH}=-\text{CH}=-\text{CH}=-\text{CH}=-\text{CH}- \\
\text{OR} \\
\text{OH} \\
\text{OH}
\end{align*}
\]

where R may also be H and R*, R" and R** are as previously.
Forss et al. (1960b) isolated a tallowy fraction from fishy washed cream and attributed this flavour to octanal, nonanal, oct-2-enal and hepta-2,4-dienal. Forss et al. (1960c) isolated and identified the carbonyls from butterfat with tallowy flavour. These compounds consisted of C$_5$ to C$_{10}$ alkanals, C$_5$ to C$_{10}$ alk-2-enals, hepta-2,4-dienal, and an unidentified compound with a metallic flavour. Three factors distinguished the tallowy from fish-oil flavoured butterfat. There was a relative increase in heptanal, octanal, nonanal, heptan-2-one, hept-2-enal and non-2-enal in the tallowy butterfat. Secondly, the 'metallic' compound was present in too small amounts to have an effect on the flavour. Thirdly, the total weight of the
volatile carbonyl compounds was 10 times greater in the tallowy butterfat than in the fish oil flavoured butterfat. Flavour tests confirmed the hypothesis advanced previously (Forss et al., 1960b) that octanal and nonanal provided elements in the tallowy flavour. This was shown most effectively by observing the change in flavour on removing these compounds from the flavour extract. El-Negoumy et al. (1982) obtained flavour concentrate by high vacuum distillation of the fat from autoxidized butter and fractionated the concentrate on butanediol succinate. They reported that tallowy flavour of the concentrate could be reproduced by recombining three of the 12 gas-chromatograph fractions. The first of these components migrated close to oct-2-enal, the second components migrated close to undecanal and the third component was 2,4-decadienal. Badings (1970) has reported that tallowy flavour in butter may be caused by 2-trans-noneral and 3,5-octadien-2-one.

Cucumber-like flavour - Forss et al. (1960b) separated a volatile fraction, having cucumber-like flavour, from flavour concentrate of washed cream. This fraction was shown to consist of non-2-enal (which is also found in cucumbers; Forss et al., 1962). Another compound, nona-trans-2, cis-6-dienal, responsible for the cucumber-like flavour, has also been isolated in small amounts from...
oxidized washed cream (Forss and Stark, 1963 b).

**Grassy flavour** - El-Negoumy et al. (1962)
fractionated the concentrate of tallowy flavour obtained from autoxidized milkfat by gas chromatography. It was observed that the fraction containing undecanal gave milk a grassy flavour. They further showed that the grassy flavour originated from linolenate. The distillate from methyl linolenate was fractionated by them into various fractions and they stated that the grassy fraction appeared to contain oct-2-enal and hepta-2,4-dienal. However, they observed that neither of these aldehydes gave a grassy flavour to milk and suggested that the compound identified as oct-2-enal by paper-chromatography was some other compound.

Hammond and Hill (1964) found trans-cis-2,6-nonadienal to be responsible for the grassy flavour observed in autoxidized milk fat. The following mechanism for the production of 2,6-nonadienal from linolenate has been proposed:

\[
\begin{align*}
\text{H} & \quad \text{H} & \quad \text{H} & \quad \text{H} \\
\text{CH-C-C=CH-CH} & \quad \text{C=CH-CH} & \quad \text{C=CH} & \quad \text{CH} & \quad \text{CH} \\
\text{O} & \quad \text{H} & \quad \text{H} \\
\text{O} \\
\text{H} & \quad \text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} & \quad \text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} & \quad \text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} \\
\end{align*}
\]
El-Negoumy et al. (1962) observed that the distillate from autoxidized methyl linolenate had strong beany flavour like reverted soybean or linseed oil. They fractionated the volatiles from autoxidized methyl linolenate into 15 fractions by gas-chromatography and showed that beany flavour was reproduced by combining the first four fractions, which tasted like the C_2 to C_5 saturated aldehydes, and the grassy component.

**Bacon-like flavour** - El-Negoumy et al. (1962) reported the presence of bacon-like flavour fraction in the flavour concentrate of autoxidized butter. They detected that this fraction contained C_5-aldehyde.

**Creamy flavour** - Bemmann and Koster (1964) isolated and purified a compound responsible for the creamy flavour of autoxidized butter and identified it as 4-cis-heptenal. This was estimated as 2pp 10^9 for autoxidized butter and 1.5pp 10^9 for fresh butter. Badings (1965 and 1970) also established the presence of 4-cis-heptenal in the butter with cold storage defect. Addition of 1 part of 4-cis-heptenal to 10 parts of degassed butter gave a creamy flavour but at 10 times this concentration a flavour reminiscent of the cold storage defect was produced. Jong (1969) also found by means of chromatography, ultraviolet and infra-red spectral measurements that the creamy flavour of autoxidized
butterfat was due to an aldehyde, 4-cis-heptenal, occurring in amounts of 0.002 ppm.

**Non-oxidative off-flavours**

**Rancid flavour (hydrolytic rancidity)** - Rancidity problem in milk and milk products has been reviewed by Herrington (1954) and Parks (1967). It has been reported that lower saturated fatty acids (such as butyric, caproic and caprylic), produced by lipolysis of glycerides, are responsible for the rancid flavour in milk and milk products. Of the several lower fatty acids the most characteristic rancid flavour is imparted by butyric acid (Jenness and Patton, 1959). The essence of the reaction causing rancidity may be represented as follows:

\[
\begin{align*}
\text{CH}_2\overline{O}\overline{C}R' & \quad \xrightarrow{\text{Hydrolysis}} \quad \text{CH}_2\overline{O}\overline{C}R'' \\
\mid & \\
\text{CH}O\overline{C}R'' & \quad \xrightarrow{\text{Hydrolysis}} \quad \text{CH}_2\overline{O}\overline{C}R'' + \text{HO-C-CH}_2\overline{C}R''
\end{align*}
\]

The lipases hydrolyze glycerides, either partially or completely, with the result that free fatty acids are liberated. It is evident from the above reaction that the quantitative conversion would involve the formation of three moles of fatty acid and one mole of glycerol from one mole of triglycerides; hydrolysis only to the diglyceride and one mole of fatty (butyric) acid is shown.
Khan et al. (1966) studied the hydrolysis of milkfat by partially purified microbial lipases and showed that fatty acids released by the microbial lipases contained traces of butyric, 42-65% oleic, 13-17% stearic and 2.8% other unsaturated acids compared with 1.49% butyric, 25% stearic, 27.3% oleic resulting from lipolysis of milkfat by milk lipases. Bills et al. (1969) reported from flavour panel studies that C_4 to C_12 acids were responsible for the rancid off-flavours of whole milk. In butter the additive flavour interaction of certain acids has been demonstrated.

Coconut-like off-flavours - Keeney et al. (1954) indicated that lactones, principally δ-decalactone, were responsible for coconut-like off-flavours which developed during storage of certain dairy products including milkfat. They stated that C_{10} to C_{12} and C_{14} monounsaturated fatty acids were the logical source of lactones and lactone formation appeared to be best explained by shifting of double bonds in acids followed by ring closure. The mechanism of this reaction appears to be non-oxidative. Vacuum packing did not hinder the development of coconut-like off-flavour. Hydrogenation of milkfat or low temperature storage of products prevented
the defect, whereas high temperature processing and storage at elevated temperature aggravated it. Keeney and Patton (1958a) isolated and identified the coconut-like off-flavour compound, which developed in butter oil during storage or heating, as $\alpha$-decalactone (the lactone of 5-hydroxy decanoic acid). They used infra-red spectroscopy and paper-chromatography in establishing the identity of off-flavour compound with $\alpha$-decalactone. Keeney and Patton (1956b) obtained coconut-like flavour extracts from dried cream and other milk products. They established the presence of $\alpha$-decalactone in these flavour extracts by paper chromatography, and attributed the characteristic coconut-like flavour defect of these products to this lactone. Mattick et al. (1959) presented evidence, experimental and theoretical, which tends to indicate that the precursor of the coconut-like flavour compound, $\alpha$-decalactone, in fat rich dairy products was 5-hydroxy-decanoic acid. This compound was postulated to exist in native butterfat as a simple ester. $\alpha$-Decalactone was derived by hydrolysis which liberated 5-hydroxydecanoic acid. They suggested following equations representing possible reactions whereby $\alpha$-decalactone would be formed in products containing butterfat.
Reaction A

\[ \text{CH}_3-(\text{CH}_2)_4-\text{CH}-(\text{CH}_2)_3-\text{C}=-\text{O} \rightarrow \text{CH}_3-(\text{CH}_2)_4-\text{CH}-(\text{CH}_2)_3-\text{C}-\text{OH} + \text{H}_2\text{O} \]

Reaction B

\[ \text{CH}_3-(\text{CH}_2)_4-\text{CH}-(\text{CH}_2)_3-\text{C}=-\text{OH} \rightarrow \text{CH}_3-(\text{CH}_2)_4-\text{CH}-(\text{CH}_2)_3-\text{C} \]

Reaction A and B show lactone precursors in which either carboxyl or hydroxyl group of 5-hydroxydecanoic acid is esterified. A third type of precursor in which both the groups are bound is yet another example. However, they stated that none of the precursors was clearly precluded by the data. The presence of \( \gamma \)-dodecalactone in the steam distillate of fresh milkfat has also been shown by Tharp and Patton (1960). They indicated that this lactone occurred in the distillate at a lower level than did \( \delta \)-dodecalactone.

Because of its characteristic fruity flavour, \( \gamma \)-dodecalactone has been considered to be of possible significance in flavour changes of processed and stored dairy products.

Role of lipids in the flavour of dairy products - Lipids are not only a major source of natural flavour compounds in dairy products but they also act as a solvent for many of the flavour compounds derived from changes in the other
constituents of dairy products. The literature on the origin of flavour compounds from lipids has been reviewed by Kinsella et al. (1967) and Fors and Kinsella (1969). The bulk of the flavour compounds occurring in dairy products are generated from the lipids by non-oxidative and oxidative mechanisms (Kinsella et al., 1967a and Kinsella, 1969).

Nonoxidative flavour compounds are generated from triglyceride fraction of milk lipids. Hydrolysis of glyceride ester bond liberates fatty acids, some of which are highly flavourful. The liberation of short chain, volatile fatty acids, C_4 to C_{12} (Jensen, 1964) generally causes hydrolytic rancidity in dairy products. However, in certain dairy products, e.g., fluid milk, fresh cream butter, cheddar cheese, the presence of limited amounts of volatile low molecular weight acids is important for optimum flavour development. These, at low levels, impart characteristic flavour to milk and cream.

Methyl ketones and lactones play a very prominent part in the characteristic flavour of certain dairy products e.g., heated milkfat, certain varieties of cheese and stale flavour of whole dry whole milk and stored butters.

The precursors of methyl ketones have been reported to be the corresponding even numbered $\beta$-ketoalkanoic acids.
esterified in the glycerides (Vander Ven et al., 1963; and Parks et al., 1964a). The methyl ketones are generated from β-keto acid esters by the hydrolysis and decarboxylation (Day, 1966). Schwartz et al. (1966) reported that the reaction was catalysed by heat and occurred spontaneously in stored dairy products. Langrner and Day (1964) also demonstrated that heating of milkfat for 3 hours at 140°C in the presence of trace amounts of water generated maximum quantities of methyl ketones. The mechanism of formation of methyl ketones has been shown (Kinsella et al., 1967a) as follows:

$$
\text{CH}_2-O-C-R \\
| \\
\text{CH} \quad O-C-R_1 \\
| \\
\text{CH}_2-O-C-C-(CH_2)_n-Me
$$

$$
\text{DG} \quad \text{H}_2\text{O} \\
140^\circ C
$$

$$
\text{HO} \quad O-C-C-(CH_2)_n-Me \\
\text{CO}_2 \\
\text{Me}-(CH_2)_n-Me
$$

Free lactones are present in almost negligible quantities in the freshly secreted milkfat. Heating or acidification increases the lactones potential of milkfat.
suggesting that precursors of lactones are present in bound form. Lactones precursors were suggested to be corresponding ω- and ϵ-hydroxy alkanolic acids esterified in glyceride molecule (Patton, 1958). This was further confirmed by infrared spectrometry, column-, thin-layer- and gas liquid chromatography (Jurriens and Ole, 1965; Kinsella et al., 1967 b; and Parliment et al., 1966). When the glycerides containing these hydroxy acid esters are heated, the hydroxy acids are liberated and spontaneously lactonize with the elimination of a molecule of water (Kisella et al., 1967 a). The presence of traces of water has been reported to be essential in the formation of lactones (Whitt et al., 1966). Maximum yield of lactones has been reported by heating butterfat at 190°C for five hours under the conditions of vacuum-steam-distillation (Dimick and Walker, 1967).

Oxidative flavour compounds are mainly carbonyls and they belong to the four classes, viz., alkanals, alk-2-enals, alka-2,4-dienals and alkan-2-ones. They are the products of the autoxidation of unsaturated fatty acids, mainly oleic, linoleic and linolenic acids associated with phospholipids and glycerides. Day (1966) reported that oxidation of unsaturated fatty acids associated with the neutral fat produced more saturated
aldehydes whereas oxidation of polar lipids generated unsaturated aldehydes and ketones.

The specificity of the perceived oxidative off-flavours depends, as already seen, upon the differences in the quality and quantity of carbonyls formed. Some of the descriptive off-flavours are due to the action of many compounds or groups of compounds, and a few are attributed to one single compound. C_7 to C_10 alkanals possess oily tallowy odours; C_7 to C_11 alk-2-enals exhibit oxidized painty odours; alkanals C_8, C_9, C_10 and alk-2-enals possess fruity, waxy aromas; 2,4-dienals exhibit nutmeg spicy odours; nona-2,6-dienal gives cucumber-like flavour and oct-1-en-3-one imparts metallic flavours to oxidized milk fat (Kinsella et al. 1967 a).

The mechanism (Farmer et al. 1943) of autoxidation in milk lipids involves the formation of hydroperoxides on carbon of the methylene group adjacent to the double bond of unsaturated fatty acids. These hydroperoxides, by dismutation and scission, via alkoxy radicals, give rise to various secondary products, viz. aldehydes, ketones and alcohols etc. The main products of hydroperoxide decomposition, however, are saturated and unsaturated aldehydes. The various mechanisms of the formation of the secondary products have been reviewed by Badings (1960), Keeney (1962) and Emanuel and Lyaskovskaya (1967).
The hydrocarbons may be derived from oxidised unsaturated acids (Forss, 1969a).

In addition to generating wide varieties of flavouring compounds, lipids also act as solvent by directly absorbing some of the flavour compounds derived from changes in the other constituents of dairy products or transferred from feeds and weeds. Honkanen et al. (1964) studied the transfer of several compounds from the rumen of cows into their milk. n-Alkanols with odd numbered carbons, lower alkan-2-ones and esters of even numbered fatty acid entered the milkfat in appreciable quantities. Alkanals and some alkan-2-ones and alk-1-en-3-ols were transferred in only trace amounts. They also suggested that oct-1-en-3-ol from clover might enter in the milk and be oxidised to oct-1-en-3-one causing a metallic flavour. 'Bovolide', an unsaturated 4-lactone with an odour like celery isolated from milkfat was presumed to come from the fodder (Boldingh and Taylor, 1962). Wong and Patton (1962) identified chloroform, acetonitrile and ethylene chloride from milk and cream and pointed out that these might have passed via solvent extracted oil seeds used in stock feeds. Dimethyl sulphide identified in butter (Day et al., 1964) has also been reported to be derived from fodders (Dinham et al., 1968).

Amines, especially the longer chain ones which
are soluble in lipid phase, are mostly derived from proteins or aminoacids. Trimethylamine in milk probably is liberated from lecithin by the action of bacteria. Other flavour compounds in dairy products, eg. dicarbonyls, sulphur compounds, furfural and hydroxymethyl furfural and phenols etc. are derived from non-lipid material, such as lactose, proteins or from the reaction of lactose with amine, aminoacids and proteins etc. (browning reaction), occur predominantly in lipid phase (Forss, 1969a).

It is evident from the foregoing survey that the flavour substances, mainly odorous, can be of many chemical types, important being aldehydes, ketones, esters, acids, lactones, alcohols, hydrocarbons, amines and sulphur compounds. These compounds are organoleptically detectable at the level of parts per million or parts per billion and may arise from enzymatic reactions of one kind or other (as in fresh, or uncooked natural foods) or from non-enzymatic reactions (as in cooked, or heat processed or stored foods).

The flavours of dairy products, like other food stuff, cannot generally be attributed to one single compound, but they are due to complex mixtures of several compounds. These compounds show different
flavour characteristics at various concentrations and in different combinations. The compounds responsible for desirable flavour may cause off-flavours in the same dairy product at different concentrations. Furthermore, the flavour thresholds of these compounds are affected by various parameters such as dispersing medium, the molecular weight, polarity, degree of unsaturation, geometric isomerism, solubility, etc. For example, substances of low polarity (say carbonyls or long hydrocarbons or higher chain fatty acids) have a much lower flavours threshold (stronger flavour potential) in an aqueous (lipophobic) than in an oily (lipophilic) medium, whereas more polar substances (say, lower chain fatty acids) have lower flavour threshold in an oily than an aqueous medium. Thus, a blend of normal flavour components of one milk product may cause off-flavours in other milk products.

The lipid fraction plays an important role in imparting typical flavours to dairy products. The bulk of the compounds contributing to the flavour of dairy products emanate from the lipids by the mechanisms involving autoxidation, hydrolysis, decarboxylation and dehydration which produce aldehydes, fatty acids, ketones and lactones, respectively. Alcohol and hydrocarbons may also originate from oxidised
unsaturated acids. Further, it acts as a solvent for many of the flavour compounds derived from the changes in other constituents of dairy products.

It is apparent from the review that carbonyls are rather significant contributors to the flavours of milk and milk products, and that indigenous dairy products like ghee have been little investigated for their flavours including off-flavours.