CHAPTER II

REVIEW OF LITERATURE

Trace Components in Milk Fat and Other Dairy Products
REVIEW OF LITERATURE

Although milk lipids contain mainly triglycerides there are a number of other components present in trace quantities which play an important role in the quality of milk and other dairy products. Hence, a study of the qualitative and quantitative nature of these trace components has formed the subject of intensive research work. The trace components of importance, encountered in milk and milk products include carbonyl compounds, alcohols and glyceryl ethers. These trace components are usually natural constituents of milk or milk lipids, but some of them are also produced during processing and storage of milk and milk products. The study of these trace components involves their isolation from milk and milk products, followed by their separation and identification. Investigation in the field of these trace components of milk and dairy products is now made possible due to the availability of advanced and at the same time easier techniques of chromatography and various spectral methods. The importance of the trace components in the flavour and off-flavour of milk and milk products has received considerable attention. Studies involving the biological origin of the significance of the trace components in milk and milk products is also of considerable interest.

A review of literature on the research connected with these trace components given below indicates that so far the research has been done mostly on cow milk and its products and there are only a few references to the work done on buffalo milk and its products.
A. CARBONYL COMPOUNDS:

Among the various families of flavour compounds of dairy products, extensive studies have been made so far, in respect of carbonyl compounds. They are considered as natural constituents of milk fat (Wong et al 1953). These compounds occur in flavour isolates from most dairy products and depending upon their nature and quantity and the particular dairy product involved, assume different characteristic flavour. The same carbonyl compounds while producing pleasantness and palatability in a particular product may have deleterious effect upon a different milk product. For example, the same methyl ketones which give good flavour in blue cheese, give an unpleasant flavour in butter when present in high concentration. Besides, an increase in the concentration of the same carbonyl compounds in fresh milk, produces a change from pleasantness to an off-flavour.

c). Techniques in Isolation and Estimation of carbonyl compounds:

1) Concentration of the volatiles:

In earlier studies, since the carbonyls were isolated from the distillates, various methods have been used to concentrate the volatiles in the distillates. In experiments with aqueous solution, to concentrate the volatiles, steam distillation at reduced pressure and temperature at or below 40°C was adopted inorder to avoid heat induced changes (Patton and Tharp 1959). In the case of fatty products quantitative recoveries of volatile carbonyl compounds (carbon number C3 - C14) have been obtained for analysis by gas-liquid chromatography by short path vacuum distillation and condensation with liquid
Gas-liquid chromatography is considered to be the best method for the analysis of volatiles. On account of extreme volatility of the carbonyl compounds, their separation at low temperature is possible through gas-liquid chromatography. The high resolving capacity of the capillary columns in gas-liquid chromatography is well illustrated by the separation of about 130 neutral compounds in the volatile fraction of cheddar cheese (Day and Libbey 1964) and the separation of aldehydes and methyl ketones (Nasar and Fagerson 1962). The gas-liquid chromatographic pattern of the total neutral volatiles from biological materials is often very complex and the identification of components by retention time alone is not reliable.

However, there has been much progress in combining gas-liquid chromatography with rapid scan mass-spectrometry which can provide a better means for identifying carbonyl compounds in flavour isolates (Day and Libbey 1964; Day and Anderson 1965).

The property of the carbonyl compounds to form stable derivatives with 2,4-dinitrophenyl hydrazine (2,4-DNPH) which are characterized by their melting points and absorption maxima, is quite useful in the isolation, identification and estimation of these compounds in dairy products. If the carbonyls isolated are present in an aqueous solution they could be converted to their 2,4-DNP hydrazones by adding an equal volume of aqueous 5N HCl saturated with 2,4-DNPH.
Though this reaction is not entirely quantitative, as pointed out by Cheronis and Levy (1957), it is often the only way the carbonyls can be estimated in aqueous solution. The estimation and identification of the carbonyl compounds from neutral volatiles can be made after their conversion into 2,4-DNP hydrozones by paper and thin-layer chromatographic procedures followed by colorimetry in conjunction with gas-liquid chromatography. Evaluation of the odour and flavour properties of parent compounds has been made possible to some extent by the development of procedures for regeneration of the carbonyl compounds from their 2,4-DNP hydrazones (Keeny 1957; Bagsette and Day 1960)

iii) Liquid-liquid column and paper chromatography:
Some excellent chromatographic procedures have been recently described for the preparation and separation of 2,4-DNP hydrazones of carbonyl compounds occurring in dairy and other food products. As these compounds occur at very low levels, it is necessary to ensure the elimination of the carbonyl compounds in solvents used for their extraction and chromatographic analysis. Schwartz and Parks (1961) described a procedure using celite column impregnated with 2,4-DNPH, phosphoric acid and water for the elimination of contaminant carbonyls in solvents.

Lawrence (1963a) observed that methyl ketones are readily produced as artifacts during extraction involving heating of dairy products containing milk fat. Further he found that on steam distillation at atmospheric pressure cream, butter-oil
and cheese fat all gave the same range of methyl ketones with odd number of carbon atoms from C₃ to C₁₅. On the basis of his observation, the data on carbonyl compounds obtained by the use of steam distillation in extraction has to be evaluated. For these reasons the direct method of isolation of carbonyl compounds from milk fat is preferable. Schwartz et al (1963) described a direct method for the isolation and quantitative estimation of carbonyl compounds which occur at very low level in milk fat. In this method, by passing a hexane solution of fat through a celite column impregnated with phosphoric acid, 2,4-DNP and water, the carbonyl compounds in it are quantitatively converted to their 2,4-DNP hydrazones. The monocarbonyl derivatives are further separated from the neutral fat solution by adsorption chromatography on magnesia and aluminium oxide columns. The total carbonyls and monocarbonyls are estimated in the form of their derivatives by colorimetry. The monocarbonyl derivatives are separated into classes on magnesia celite column (Schwartz et al 1963). Any contaminant present in the classes are identified while separating the individual members of the classes by means of partition or reverse phase column chromatography (Monty 1968; Corbin et al 1960; Day et al 1960).

iv) Thin-Layer Chromatography:

There has been considerable progress made in the application of Thin-layer Chromatography (TLC) for the separation of 2,4-DNP hydrazones of carbonyl compounds in dairy products. With limited subfraction, silicagel, aluminium oxide or other adsorbents in general separate carbonyls or their derivatives into chemical classes.
Urbach (1983) using aluminium oxide-G containing AgUO$_3$ (w/w) as adsorbent and diethyl ether - light petroleum as developing solvent mixture separated the 2,4-DNP hydrazones of alkamals, alk-2-enals, alk-2,4-diennals, methyl-vinyl ketone and methyl-ethyl ketone. Thin-layer chromatography on silver nitrate impregnated Silica gel-G plate was used to separate 2,4-DNP hydrazones of monocarboxyls into classes by Badings and Wassink (1963). Schwartz et al (1968) developed a method for the separation of 2,4-DNP hydrazones into four classes. Using air dried TLC plates coated with magnesia : calite (3:7) and developing it in hexane-chloroform (95:25) they found that 2,4-DNP hydrazones of monocarboxyls can be separated into methyl ketones, alkamals, alk-2 enals and alk-2,4-diennals without the overlapping of lower members in another class.

Individual members of homologous series from classes are identified by reverse phase system according to carbon number. Adopting the paper chromatographic method of Lynn et al (1956) in the case of thin-layer chromatography on Kieselgur-G plate Urbach (1963) separated all the members of normal homologous series of C$_1$ - C$_{14}$ alkamals, C$_3$ - C$_{13}$ alk-2-ones, C$_4$ - C$_{11}$ alk-2-enals, C$_5$ - 12, 14, 16, 18 alk-2,4-diennals and C$_6$, 7, 10 alk-3-2-ones 2,4-DNP hydrazones by multiple ascent technique. The individual members of each class of DNP hydrazones were also separated on carbowax-400 impregnated Kieselgur-G plates (Badings and Wassink 1963) and basic zinc carbonate plates (Badings 1964).
v) Spectral Methods:

Infrared absorption spectra have proved of value for the identification of carbonyl groups in unknown mixtures (Dutra et al 1959; Winter et al 1963) and in purified fractions (Wong et al 1953). Concentrations of 2,4-DNP hydrazones in total extracts or in column eluates may be determined by measuring the absorbance in hexane or chloroform solution at 340 µm (Schwartz et al 1963). Each class of monocarbonyl-2,4-DNP hydrazones exhibit major maxima which are useful for identification purposes. Absorption spectra of derivatives in ethanolic-KOH are also useful criteria for establishing their identity. Differences in the fading ratio of the aldehyde and ketone chromophores in ethanolic KOH can be used to distinguish the classes: (Jonnes et al 1966)

b) Carbonyl Compounds in Milk:

1) Fresh milk.

Some low molecular weight carbonyl compounds such as acetone, acetaldehyde present in trace quantities are known to contribute to milk flavour. Forss et al (1955a) isolated acetaldehyde and acetone in the steam distillate of skim milk. The presence of formaldehyde and acetaldehyde was also detected and estimated in raw skim milk by Harper and Huber (1956). They ascertained the quantity of acetaldehyde in normal milk to be 1.87 µg/lit. The presence of acetone bodies were detected in milk from cows of normal health. (Knoedt et al 1942) Bertoni 1965). This contributes to the relatively large amounts of acetone found in the distillate from normal skim milk.
(Morgen et al 1957). Acetone, butanone, acetaldehyde and formaldehyde were also identified in normal milk by Wishner and Keery (1961).

Noble et al (1962) applying low temperature reduced pressure distillation technique showed the presence of formaldehyde, acetaldehyde, acetone, butanone, pentanone and hexanone-2 in milk and cream. From the fact that these are found in milk which has received no heat treatment, they confirmed that these components should be normal constituents of milk and are initiated during the process of formation of milk in the udder. Further they concluded that the spontaneous decarboxylation of \( \beta \)-keto acids which are intermediates in \( \beta \)-oxidation of fatty acids may account for the formation of these ketones. Woods and Aurand (1963) used low temperature high vacuum method, with liquid nitrogen trapping to separate the volatiles from milk. The carbonyls were then precipitated as 2,4-DNP hydrazones. After regenerating the carbonyls and fractionating by gas-liquid chromatography they identified acetone and butanone as normal constituents of normal milk.

ii) Heated milk:

Acetaldehyde and furfural are the principal heat generated carbonyl compounds found in milk. Morgen et al (1957) from their experiments on heated milk came to the conclusion that acetaldehyde should arise through a Strecker degradation of alanine which is presumably one of the predominant free amino acids in milk. Additional alanine which could be converted to acetaldehyde may be obtained during heating of
milk by degradation involving pyruvic acid and such free amino acids as glutamic acid, glycine, serine and aspartic acid.

Carbonyl compounds were also isolated from evaporated milk. Acetone, Pentanone-2 and heptanone-2 were identified as the principal volatile carbonyl compounds in distillates, obtained at low temperature and reduced pressure, from commercial evaporated milk (Wong et al 1958; Parks and Patton 1961; Muck et al 1963). Dutra et al (1959) from their experiments came to the conclusion that a part of the acetaldehyde present in evaporated milk originates from alanine by Strecker degradation. The presence of pentanone-2 and and heptanone-2 was also detected in evaporated milk by them.

iii) Oxidized off-flavoured milk:

The most important flavour defect of milk and its products is commonly called as oxidized flavour (Green-bank 1943) although such terms as cardboard, metallic, oily and tallowy are used to describe the off-flavour. The off-flavour in milk is accelerated by catalytic effect of sunlight and contamination by copper and iron (Greenbank 1940; Forss 1955a, Unnikrishnan et al 1976). The off-flavour development in milk is caused by the formation of aldehydes. Forss et al (1955a; 1955b) isolated acetone, acetaldehyde, crotonaldehyde and C5 - C11 unsaturated aldehydes from the steam-distillate of skim milk possessing oxidized off-flavour. They found that only 2-enals in particular the oct-3-enal and non-3-enal are the principal flavour determining compounds and they impart an oxidized flavour resembling cardboard flavour even when they are present in milk at very low concentration of 1 part in $10^7$ to $10^9$. 
The off-flavour compounds in milk are formed from the oxidation of (di- and) polyunsaturated fatty acids. The cleavage products of low molecular weight produced by the oxidation of these fatty acids are usually 1,5-unsaturated aldehydes (R-CH=CH.CH=CHO) which have been found to be the significant off-flavour compounds. Copper even when present in milk at levels of a fraction of a part per million is a very potent catalyst for the oxidation of these fatty acids. Hence, the role of copper in oxidized flavour has been studied extensively.

Pont (1962) observed from the steam distillate of whole or skim milk that oxidized flavour caused by adding copper to milk was composite in character. He could distinguish two main types of off flavour in copper treated milk: one as an oily metallic flavour caused by the oxidation of milk fat, another as card board which came from the skim milk portion. Bassette (1976b) observed increases in concentrations of n-hexanal, n-pentanal, n-propanal and acetaldehyde in raw, laboratory pasteurised and pasteurised-homogenised milk exposed to 5 ppm added copper. The maximum changes were in the case of copper treated pasteurised milk and least in pasteurised-homogenised milk. Considerable increase in acetaldehyde in copper exposed skim milk indicate that the precursor of this compound is a nonfat component. The production of carbonyls in copper treated milk was found to be inhibited by the addition of ascorbic acid (Bassette 1976b).

Milk exposed to sunlight contained C1-C12 alkanals and C5-C9 alk-2 enals (Wishner and Keery 1961). On exposure to sunlight the quantities of n-hexanal and n-octanal in milk increased but that of formaldehyde decreased. Among the
carbonyls produced by exposing milk to sunlight, alcohols were considered as responsible for off-flavour. Pagetti (1976a) observed maximum increase in acetalddehyde, pentanal and hexanal in skim milk exposed to sunlight for an hour.

Allen and Parks (1959) detected the presence of C\textsubscript{15}, C\textsubscript{13} and C\textsubscript{11} and traces of C\textsubscript{7} methyl ketones in evaporated milk. From their experiment on storage of sterile concentrated milk they came to the conclusion that the development of off-flavour in SCM depends on the total methyl ketone potential of milk fat, composition of methyl ketones in milk, the extent of hydrolysis, and decarboxylation of the \( \beta \)-ketocids governed by the initial heat treatment of the product, storage temperature and storage time. Arnold and Lindsey (1963) determined the concentration of odd numbered \( n \)-methyl ketones (C\textsubscript{3} - C\textsubscript{11}) in stored sterilised concentrated milk. The \( n \)-methyl ketones were found to exceed their average flavour threshold values after 13 weeks of storage at 27°C imparting off-flavour. Among \( n \)-methyl ketones 2-heptanone is considered to be important in off-flavour of sterile concentrated milk.

iv) Cultured milk.

It will be logical to conclude that the odour and flavour of cultured dairy products are ascribable to certain carbonyl compounds which are the byproducts of the lactic acid fermentation. Principal among these are diacetyl, acetoin, aceton and acetalddehyde. Single strain culture \textit{S. lactic} and \textit{S. diactiactina} produce acetalddehyde, acetoin and butanone in skim milk. (Saknishi Mills and Oyu 1960; Saknishi and
Formaldehyde was detected in butter milk and yogurt to the extent of 0.3 – 3.0 µg/kg. (Möhrer and Denbisky 1970), gas chromatographic data obtained from butter culture distillate showed the presence of pentanone-2 and heptanone-2 in much greater amounts than in heated milk (Lindsay et al 1965). The greater quantities of methyl ketones in butter culture residues may be explained from the fact that the rate of decarboxylation of acetoacetic acid is 50 times greater in acid solution than in alkaline solution (Midmark 1932). The keto-acid decarboxylation reaction is accelerated in acid medium because of a partial carboxyl proton transfer through intramolecular bonding, to the keto-group where it aids the decarboxylation reaction. On this basis Lindsay et al (1965) concluded that because of the acid nature of the medium β-keto acids exist in the anion form in butter culture and hence the methyl-ketone production was enhanced.

c) Carbonyl compounds in milk fat:

Milk lipids play a significant role in the flavour of dairy products. When fat is removed from milk, it will not have that rich pleasing flavour. It appears that most of the components responsible for the flavour of milk are concentrated in the fat phase. To obtain a better understanding of the contribution of milk fat to the flavour of milk Tamsna et al (1969) prepared a series of beverages containing 1 – 3% different fat, with skim milk. They observed that untreated milk fat in whole concentrate and partially deodourised milk fat were the only fats which improves the flavour of skim milk.
1. Monocarbonyls.

The carbonyl compounds normally encountered in milk fat can be broadly classified into monocarbonyls and dicarbonyls. Monocarbonyls which consist of methyl ketones, alkanals, alk-2-enals, and alk-2,4-dienals play a prominent role in the flavour of milk fat. The monocarbonyls associated with dairy products are identified as n-alkanals C₁ - C₁₀, alk-2-enals C₃ - C₁₁, alk-2,4-dienals C₇, C₉, C₁₀ and C₁₁ and n-methyl ketones C₃ - C₁₅ (Winters et al 1963). These compounds are extremely flavourful and are organoleptically detected at very low concentration. Though they are responsible for the good flavour at optimum levels, cause various off-flavours when their respective concentrations attain abnormally high levels (Winsella 1969). The flavour threshold values (FTV) of these compounds were determined by Day et al (1963). The FTV of the oxidative aldehydes decrease with increase in their chain length and unsaturation. They found that when oil is used as the carrier the aldehydes can be tasted at much lower concentration than the concentration required to detect their smell. Seik et al (1969) measured the taste threshold of volatile compounds in butter and deodourised butter oil, and inferred that the synergistic interaction exhibited by the carbonyl compounds are important in butter flavour.

The flavour potency of the methyl ketones were also found to vary with their chain length. Langler and Day (1964) showed that heptanone-2 has the lowest FTV and hence is most important in butter flavour. The concentration of methylketone in milk of 4% fat prepared from heated milk fat was 6.30 ppm whereas
the concentration of the mixture of methylketones to give a
detectable flavour at the average threshold of the mixture was
found to be 1.55 ppm. Thus the methyl-ketone level in heated
milk fat was enough for detectable flavour. Further they con­
firmed that the methyl ketones could play a significant role in
non-oxidative type flavour deterioration of milk fat.

1) Origin of methyl ketones:

The importance of methyl ketones as a constituent of
biological materials has in some aspects undergone considerable
reappraisal in recent years. It was earlier recognised that
methyl ketones of intermediate chain length were metabolic pro­
ducts of certain fungi and are important in flavour of dairy
products. It is now established that methyl ketones may be
derived from naturally occurring constituents of milk fat mainly
the triglyceride bound β-ketoacid and hence are normal consti­
tuents of milk. The presence of bound ketoacids were found in
volatiles of butterfat by Bolding and Taylor (1962). After
removal of the volatile carbonyls by high vacuum degassing and
subsequent steam distillation at 180°C of the fat they identified
a homologous series of methyl ketone with odd number of carbon
atoms from the distillates. This evidence suggested that the
β-ketoacids, a normal intermediate in the biological fatty acid
cycle, were present in bound form in butter. This view was
supported by Lawrence and Hawke (1936). They found that the
methyl ketones formed from the milk fat obtained from milk after
injecting the cow with (Carboxyl C14) acetate showed greatest
activity in C11, C13, C15 and C9 ketones. From this they
concluded that the β-ketoacids arise biosynthetically by
acetate condensation and are apparently unfinished fatty acids which, during synthesis become dissociated from their acyl carrier protein (ACP) prior to completion of the normal biosynthetic steps. The precursors of methyl ketones, even numbered β-keto-alkanoic acid esterified in the glyceride, constitute about 0.041% of the butterfat. The methyl ketones are shown to be generated from β-ketoacid esters by hydrolysis and decarboxylation (Parks et al 1964; Schwartz et al 1965).

11) Nature and quantity of methylketones:

As long back as 1933 Thauffel found that alk-2-ones were generated from fat through the action of heat and water. From the spectral changes after adding 2,4-DNP to the steam distillate of milk fat Patton and Tharp (1959) confirmed the presence of ketogroup in milk fat. They were the first to demonstrate the presence of a homologous series of saturated methylketones with an odd number of carbon atoms (C₃ - C₁₅) in several dairy products, especially those containing heated milk fat. Lawrence (1963) observed that a large amount of distillate should be collected in order to obtain the maximum yield of methyl ketones. It was found necessary to collect 10 l of distillate to ensure maximum yield of methyl ketones from 10 g of butterfat. Mono-carbonyls present in fresh milk fat are composed of 75 to 95% methyl ketones with trace amount of aldehydes (Dimic and Walker 1968). Methyl ketones are the integral flavour components of good quality butter and they impart cooking flavour in food in which butter fat is used as shortening (Winter et al 1963).
iii) Factors influencing methylketone formation:

The quantity of methylketones produced from \( \beta \)-ketoacid esters depends on the extent of water present, temperature and time, duration of heat treatment (Langler and Day 1964; Schwartz et al 1965). Experiments by many workers have shown the significance of water in the formation of methylketones from its precursors in milk fat. Removal of water from milk fat prior to heat treatment inhibits methylketone formation in milk fat. The milk fat dried over calcium hydride even after heating for 24 h at 101°C did not show any methyl ketone formation. On the other hand maximum levels of methylketones were obtained with as little as 0.008% water. (Schwartz et al 1965). According to Langler and Day (1964) the extent of water necessary was found even still lower i.e., 0.0031%, for the complete conversion of \( \beta \)-ketoesters to methyl ketones in milk fat. Vacuum degassing of dry butterfat yielded only traces of methyl ketones in the distillate whereas heating in the presence of water in ampoules for 6 hours at 101°C yielded considerable amount of methyl ketone (Van der Ven et al 1963). Over 95% of the carbonyls formed under these conditions were found to be methyl ketones (Schwartz et al 1965). Van Duin (1965) did not obtain methyl ketones when butter fat was heated for 2 hours at 50°C although heating for 2 hours at 75°C produced measurable quantities. Nearly maximum yield were obtained at 125°C. He found that the recovery of \( C_3 \), \( C_{13} \) and \( C_{15} \) methyl ketones was more difficult as compared to that of \( C_5 \) to \( C_{11} \) methyl ketones.
Schwartz et al (1966b) showed that the formation of methyl ketones in butter fat from its precursors follow the first order reaction kinetics. The rate of reaction being $1.16 \times 10^{-7}$ at 50°C, $1.68 \times 10^{-6}$ at 77°C, $3.39 \times 10^{-5}$ at 100°C and $9.8 \times 10^{-5}$ at 115°C. However, the proportion of various individual methyl ketones produced was same irrespective of the reaction time. For instance, C$_{15}$ and C$_7$ methyl ketones are produced in larger quantity throughout the reaction.

The potential for the formation of methyl ketones in low and high melting fractions of milk fat which have softening points of approximately 25°C and 45°C respectively was determined by Walker (1972). The concentration of methyl ketone precursors in low melting fraction was somewhat higher than that in the anhydrous milk fat. He observed that the high melting fraction contained only 50 - 70% of the methyl ketone potential of low melting fraction.

iv) Variation in monocarboxyls:

It has been observed that the monocarboxyls of milk fat fall within wide variations. These variations are attributed to many factors such as feed, season, stage of lactation etc. Dimic and Walker (1966) found that the monocarboxyls in milk fat vary with season, being higher in winter (barnfed) and lower during the summer months (pasturefed). The variation in methyl ketone is very much related to the variation in shortchain fatty acid composition of the fat as the precursors of methyl ketones namely the 2-keto acids are synthesized during the biosynthesis
of fatty acids from acetate. The monocarboxyls and methyl ketones also were found to increase as the lactation period progressed (Dimic and Walker 1968).

v) Carbonyl compounds in ghee:

Carbonyl compounds were also found to be responsible in ghee flavour. Propanone-2, pentanone-2, haptanone-2 and nonanone-2 and C3 - C12 alkanals were identified in cow and buffalo ghee clarified at 110 - 115°C (Jain and Bindal 1968). Mono-carbonyl content being the same in buffalo and cow ghee, total carbonyls were found to be higher in buffalo than in cow ghee. The average total carbonyl contents were found to be 4.3 μ mole/g and 7.0 μ mole/g fat in cow and buffalo ghee respectively (PL-430 project N.D.R.I. 1971). Malhan et al (1975) observed an increase by about 14% in carbonyl content of ghee prepared from cream which was ripened for 33 h at room temperature. This may explain the rich flavour of desi ghee. They also observed a 20% increase in carbonyl content of ghee by heating it to 130°C.

2. Ketoglycerides:

Analyses of carbonyl compounds led to the isolation of ketoglycerides, the glycerides containing a ketoacid esterfied by glycerol along with two fatty acids. They were found to occur in milk fat in a concentration of 5 to 15 μ moles/g. The keto group was distributed in all positions of the chain but mostly in the 9 and 10 positions. Ketopalmitic and keto-stearic acids were found to be the predominant keto acids in milk fat (Keery et al 1962). Animal fats, vegetable oils and nut oils were also examined and found to contain substantial amounts of keto-glycerides. Ketoglyceride content of buffalo milk fat
is distinctly higher than that of cow milk fat (PL-430 Project N.D.R.I. 1971). The average ketoglyceride content in cow and buffalo milk fat is estimated to be 2.4 μ moles/g and 4.4 μ moles/g fat respectively.

The β-ketoglycerides deserve special mention since they are the source of methyl ketones arising in many dairy products. Schwartz and Virtanen (1967) accurately quantitated β-ketoglyceride in milkfat via methyl ketone formed when milkfat is heated in the presence of water. The β-ketoglycerides occurred in milk fat to the extent of 0.4 to 1.7 μ mole/g. The β-keto acids are hydrolyzed from the glycerides under various heating and storage conditions (Schwartz et al. 1968b) and the free β-ketoacid is then decarboxylated to give methyl ketones.

d) Autoxidation of milk fat:

1) Off-flavour development.

Autoxidative deterioration is one of the main causes of flavour defects in dairy products. The mechanism of autoxidation in milk lipids involves the formation of hydroperoxide on the carbon of the methylene group adjacent to the double bond of unsaturated fatty acids. These hydroperoxides are mostly odourless and tasteless (Menick et al. 1954). The substances which result from simultaneous partial breakdown of peroxides have the potency for the formation of off-flavour. In fats that have suffered flavour deterioration, the components that have received the most attention are, the volatile carbonyls. Some of these especially the unsaturated ones possess high off-flavour
(Patton et al 1959, Sroboda and Lea 1955). Therefore eventhough they are present at only low concentrations in off-flavoured fats, their contribution to the flavour has been recognised. Most of these oxidative carbonyls belong to four classes namely n-alkanals, alk-2-emals, alk_2,4-dienals and alk_2-ones.

Despite the great similarity in qualitative carbonyl content of oxidized dairy products, flavour differences are very noticeable. Attempts, however to correlate the off-flavours with specific compounds or groups of compounds are made difficult for several reasons. These include i) the multiplicity of compounds produced, ii) differences in the threshold values of individual compounds, iii) similarity of flavours imparted by individual aldehydes near their threshold, iv) a possible cumulative effect of mixtures of the same members of a homologous series, and v) difficulties involved in adding pure carbonyl compounds to dairy products inorder to evaluate the flavour characteristics. Day and Lillard (1960) concluded that because of large number of aldehydes identified in oxidized fat, the odour defect can not be attributed to a few specific compounds, rather the presence of complete spectrum of compounds is necessary for the characteristic odour associated with oxidized fat.

Wilkinson (1964) observed that the particular flavour compounds which are formed depend upon the conditions and duration under which the oxidation takes place, the oxygen tension, presence of metals, heat, water and the lipid material which undergo oxidation. He obtained precise information regarding the development of oxidized flavour in dairy products
and emphasised the important role of secondary oxidations in the oxidative deterioration of lipids and the function of water in supplying hydrogen, hydroxyl ions and radicals which control the products generated. He also noticed that low temperature, metal catalytic dehydration quickens the production of unsaturated compounds from which octeneone can be formed by hydration and further oxidation.

Polyunsaturated fatty acids play a predominant role in autooxidative deterioration (Vasistha et al 1963). Their rapid autoxidation increases the oxidation susceptibility of lipid containing product as a whole. Flavour defects which occur in initial stages of autoxidative deterioration are due mainly to volatile carbonyl compounds derived originally from polyene fatty acids. (button et al 1951; Naftan 1961). Flavour defects at a later stage of autoxidative deterioration may be caused by volatile carbonyl compounds originated from the less unsaturated fatty acids and also by those resulting from a further degradation of the compounds formed in the early stages of autoxidation (Smouse and Chang 1967). Milk and butter while containing relatively low quantities of unsaturated fatty acids possess adequate amounts of polyenes for autoxidation and development of rancidity. The oxidation of the unsaturated fatty acids associated with the neutral fat produce more saturated aldehydes whereas that of phospholipids generate unsaturated aldehydes and ketones. The oxidation of oleic, linoleic, linolenic and arachidonic acids which occur in milk fat at 30.0, 2.0, 0.30 and 0.25 percent respectively, do produce a large number of carbonyls in dairy products (Kinsella 1969).
ii) Carboxyl compounds associated with off-flavour:

Each type of off-flavour is attributable to a particular group of carboxyl compounds depending upon their quantity and quality. Boyd et al (1965) came to the conclusion that C7 - C10 alkanals possessed oily tallowy odour; C7 - C11 alk-2-enal gave oxidized painty odour; nut-meg spicy odour was due to C6, C8 alkanals and alk-2-enals. Importance of the proportional occurrence of carboxyl compounds in the oxidized fats was emphasised by Forss et al (1960a; 1960b) in a comparative study made on fishy, tallowy and painty flavours of butter fat. They showed that there was a relative increase in n-heptanal, n-octanal, n-nonanal, heptanone-2, 2-heptenal and 2-nonenal in the tallowy butterfat and a relative increase in the n-pentanal and the C5-C10 alk-2-enals in butter fat with painty flavour. The total weight of the carboxyl compounds was found about ten times greater in the tallowy and 100 times greater in the painty butter fat than in the fishy flavoured butter fat.

All the carboxyls except the alk-2-enones occur during lipid oxidation (Ryöss et al 1964; Cobb and Day 1965). Out of the aldehydes formed during oxidation some are saturated ones, some unsaturated conjugated and others unsaturated nonconjugated. In lipid oxidation owing to the degradation of hydroperoxides, both saturated and unsaturated aldehydes are formed. In majority of cases the n-alkanals occur in maximum concentration with a small amount of alk-2-enals and alk-2,4-dienals. But according to the theory of antioxidation more unsaturated aldehydes should be formed from the fatty acids (Badings 1930). Lillard and Day (1961) observed that unsaturated aldehydes formed are further
oxidized to saturated aldehydes. This explains the large resulting quantity of these compounds in oxidized lipids. Further they noticed that the conversion of saturated aldehydes into carboxylic acids and 2,4-dienals in to malonaldehyde are possible by further oxidation of these compounds.

Ahmed et al (1969) found saturated aldehydes and methyl ketones as predominant classes of monocarbonyls in oxidized buffalo saum. The higher quantities of methyl ketones (40%) in oxidized saum than in that of oxidized butter oil (25%) was found to be due to the effect of heating the butter in the presence of air during the processing of saum. The development of carbonyl compounds during storage of cow and buffalo ghee was studied by Gaba and Jain (1974). A two-fold and three-fold increase in the total carbonyl content of ghee was noticed after 100 and 200 days of storage at 37°C, respectively. They observed that there was a relative decrease in the percentage of methyl ketones and an increase in that of aldehydes especially saturated aldehydes, during storage of ghee. Changes to a greater extent were noticed in buffalo ghee than in cow ghee.

Parks et al (1933) indicated that the mechanism involved in the production of the carbonyl compounds in the butter oil of spontaneously oxidized whole milk was typical of classic lipid autoxidation. In this product they identified tentatively C5 - C13 saturated aldehydes, C5 - C11 alk-3-ones and C8-C12 alk-2,4-dienals. Among these compounds alk-2,4-dienals especially 2,4-decadienal was found to have a significant role in the off-flavour of this product whereas saturated aldehyde did not contribute significantly to the off-flavour.
It was observed that aldehydglycerides, unsaturated aldehydglycerides are formed during autoxidation of fat (Crossley and Thomas 1964; Horikx 1965). These compounds are expected from the break-down of fatty peroxides. If the fatty acid chain breaks towards glycerol group side of the hydroperoxy group, then short chain volatile aldehydes and ketones are formed. If the chain breaks towards methylene group side of the hydroperoxy group, then the same aldehyde and ketone functional groups are formed but remain attached to the glyceride end of the chain.

It is noteworthy that aldehydes and alk-2-ones interact at subthreshold concentrations to give perceptible flavours (Day et al 1983; Lengler and Day 1994). The typical oxidized flavours are due to the combined effect of different carbonyls produced and not due to any single component.

iii) Measurement of oxidation:

Various methods have been devised to measure the extent of autoxidation in lipids and lipid containing food products. The degree of oxidative deterioration is expressed in terms of hydroperoxides per unit weight of fat. The method very commonly used is the one, involving the liberation of iodine from potassium iodide (Lee 1933) wherein the amount of iodine liberated by the hydroperoxides is used as the criterion for the extent of the oxidative reaction. The colorimetric ferric thiocyanate method by Hills and Theil (1946) involves the conversion of ferrous ion to ferric state in the presence of ammonium thiocyanate by the hydroperoxides present to yield
ferric thiocynate. The degree of lipid deterioration in dairy products is also measured by thiobarbutrylic acid test (Dunkley and Jonning 1951). This test is more sensitive to oxidative changes (Patton et al 1961).

Any method based on direct or indirect determination of hydroperoxides does not consider previous dismutations of primary reaction products and are therefore not necessarily truly indicative of the extent of oxidative reaction. It is to be noted that organoleptic detection of off-flavours in dairy products usually occurs at peroxide values approaching 1.0 (Hills and Thiel 1946; Smith et al 1953).

Although the above methods are commonly used, gas chromatography is of great promise as an analytical aid in measuring the extent of oxidation, though method is expensive. Using temperature-programmed column Apics et al (1960) first correlated differences in the various oxidised flavours of butter. Such methods to determine the extent of oxidation in different varieties of dairy products were later developed by Patton (1961) Scarpellino and Kosikowski (1961).

iv) Dissolved oxygen:

Dissolved oxygen in milk fat react with unsaturated fatty acids producing peroxides which in turn breakdown to give undesirable carbonyl compounds. McDowell (1963) devised a chemical method to estimate dissolved oxygen in milk fat. In this method the oxygen in milk fat was allowed to oxidise ferrous-manganous ions. The extent of oxidation of ferrous-
manganese ions was determined iodometrically. He estimated about 3.0 ml of dissolved oxygen per g milk fat. Buffalo milk fat was found to absorb more oxygen than cow milk fat (Vachha et al 1957).

e) Cheddar cheese:

Cheddar cheese is the most popular of all cheeses. Changes which occur in milk and subsequently in cheese during manufacturing process have intrigued dairy scientists for many years. The flavour of cheddar cheese is due to the presence of a large number of organic compounds in it. Among these, carbonyl compounds and fatty acids are considered to be the most important.

i) Carbonyl compounds in cheddar cheese:

Among the carbonyl compounds the C5 - C13 methyl ketones which are extremely flavourful are considered to contribute to the flavour of cheddar cheese (Day et al 1960; Walker 1961). Kristoffersen and Gould (1959) examined commercial cheddar cheese samples for carbonyl compounds and concluded that the numbers and concentrations of these compounds varied between different cheese samples. They could not correlate flavour to the different quantities of carbonyl compounds present in the cheese. The importance of methyl ketones in blue cheese flavour was first noticed by Patton (1959). Harvey and Walker (1960) Scarpellino and Kosikowski (1963) identified acetaldehyde, acetone, butanone-2 and pentanone-2 in one day old cheese.
Development of carbonyl compounds in cheddar cheese was studied by Harvey and Walker (1960). They observed the formation of odd number methyl ketones progressively during cheese ripening. By distillation under reduced pressure at 45°C, heptanone-2 after 2 - 4 weeks, nonanone-2 after 20 - 24 weeks, and undecanone-2 after 36 weeks were found during ripening of cheddar cheese. They concluded that the flavour of mature cheese was apparent after 6 - 12 weeks and afterwards it became more pronounced as the concentration of pentanone-2, heptanone-2 and nonanone-2 increased.

Earlier data on carbonyl compounds reported by Harvey and Walker (1960) and other workers were obtained by use of steam distillation methods but the results of studies by Lawrence (1933) indicate that methyl-ketones are readily produced as artifacts during extraction procedures which involve heating of dairy products containing milkfat. He observed that on steam distillation at atmospheric pressure cheddar cheese one day to 13 months old, gave the same range of methyl ketones with odd numbers of carbon atoms from C3 - C15. Using the method for quantitative isolation of carbonyl compounds from fats and oils (Schöflitz et al 1933), Boothen et al (1973) observed an increase in non-carbonyl content as the age of cheese increased. However, ketoester content remained the same during cheese ripening. A slow formation of odd numbered methyl ketones (C3-C15) was also observed in cheese fat during ripening for an year by Walker and Keen (1974).
ii) Mechanism of methyl ketone formation during cheese ripening:

It is well established that milk fat plays an important role in the development of flavour in cheese and serves as an essential substrate for production of flavour compounds. The desired flavour and aroma in the finished product is understood to develop from the break-down product of fat. Mabbitt and Ziellinska (1965) did not observe the development of the typical flavour in cheddar cheese made from skim milk, which suggests that milk fat contains the precursor of characteristic cheddar flavour. Similar observations have been made by a number of investigators in this field (McKean 1963; Patton 1963; Ohren and Tuckey 1965) and are supported indirectly by the enhancement of cheddar flavour by the addition of lipolytic organisms to the milk prior to cheese making (Roberston and Perry 1961).

On the basis of the occurrence of a series of odd number methyl ketones, nonane-2, heptanone-2 pentanone-2 and acetone found in cheese volatiles even in the absence of mold growth, Day et al (1960) inferred that these compounds are normal products of the curing phenomenon. Compounds such as acetone might result from an ubiquitous fermentation process or might simply be incorporated into the cheese from the milk at the time of manufacture (Bills et al 1966).

It was once thought that the microflora of cheddar cheese might be implicated in the formation of methyl ketones from fatty acids via β-oxidation and decarboxylation during ripening
(Walker and Harvey 1969; Gihrig and Right 1963). Later the presence of precursors of methyl ketones, \( \beta \)-ketoacids were identified in milk fat (Van der Ven et al. 1963; Parks et al. 1964). Hydrolysis of these esters and subsequent decarboxylation to methyl ketones might be expected readily since they break down even spontaneously at low temperature used for ripening the cheese (Lawrence 1963; Day and Libbey 1964). Scarpellino and Kosikowski (1962) observed methyl ketones with odd carbon number during ripening of cheddar cheese and postulated that the fatty acids are converted to \( \beta \)-ketoacids during ripening, which on decarboxylation give rise to methyl ketones; since the fatty acids in normal triglycerides are almost even chain acids they expected only odd numbered methyl ketones.

Synthetic triglycerides containing fatty acids which either do not occur at all in milk fat or present only in trace amounts were incorporated into cheddar cheese in order to investigate the mechanism operating during ripening of cheddar cheese. These synthetic triglycerides were assumed to be as susceptible to hydrolysis as the natural triglycerides in cheese fat (Lawrence 1964; Bills and Day 1964). Lawrence (1964) incorporated synthetic triglycerides containing heptanoic, nonanoic and undecanoic acids at the time of manufacture of cheddar cheese. He observed that the methyl ketones with even number carbon atom were absent in steam distillate of the above cheddar cheese, indicating that methyl ketones are not derived from \( \beta \)-oxidation of fatty acids. By determining the decrease in methyl ketone potential in 'cheese fat' and comparing it with the increase in
methyl ketone concentration during ripening of cheddar cheese, Lawrence (1967) concluded that the methyl ketones in cheddar cheese are formed only by degradation of \( \beta \)-keto acids of milk fat in cheese. Walker and Keen (1974) also noticed a slow increase of C\(_3\) - C\(_{15}\) odd number methyl ketones by break-down of esterified \( \beta \)-ketoacids even at relatively low temperature of cheese ripening.

The methyl ketones have been shown to be produced from fatty acids by P. roqueforti mold spores. Each ketone produced does not depend directly on the amount of available fatty acid precursor. Compared to the quantity of butyric acid, the precursor of acetone, the formation of acetone was low whereas the concentration of heptanone-2 was high as compared to the quantity of its precursor octanoic acid (Anderson and Day 1963). During curing of cheese the mold spores were found to convert the 8:0 acid to C7 methyl ketone most readily while 4:0, 6:0, 10:0 and 12:0 acids are converted to the C\(_3\), C\(_5\), C\(_6\) and C\(_{11}\) methyl ketones respectively to a smaller extent.

Scarpillino (1961) observed the production of butanone during the ripening of cheddar cheese and found to contribute to the flavour of the cheddar cheese. Scarpillino and Kosikowski (1962) postulated that acetoin present in cheese was the precursor of this compound. They believed that acetyl methyl carbinol present in cheese is converted to 2,3 butanediol, which on subsequent dehydration and reduction yield butanone. Walker and Keen (1974) explained the formation of butanone as a result of metabolism by nonstarter micro organisms.
iii) Fatty acids in cheddar cheese flavour:

Even though methyl ketones are important in cheddar cheese flavour, it was noticed by Patton (1963) that the removal of volatile carbonyl compounds from steam distillates of cheddar cheese did not lower the cheddar aroma. However, removal of the volatile (C₂ - C₃) fatty acids resulted in lowering the cheddar aroma. From this he inferred that these fatty acids are the main contributors to the basic cheddar flavour as compared to the carbonyl compounds.

Several studies have been carried out in the production of free fatty acids during ripening of cheddar cheese (Peterson and Johnson, 1949; Bills and Day 1964; Garg and Verma 1966; Garay and Walker 1972; Koen and Walker 1974) in order to understand their role in the flavour of cheese. Considerable increase in acetic acid (Bills and Day 1964; Gray and Walker 1972) was found during ripening of cheddar cheese as a result of fermentation of lactose and protein (Harper and Kristoffersen 1968).

There have been conflicting opinions regarding the role of fatty acids in the flavour of cheddar cheese. Bills and Day (1964) found similar levels of free fatty acids in several normal cheeses in spite of their differences in flavour, age and manufacturing conditions. Gray and Walker (1972) considered that C₄ to C₁₂ fatty acids do not play an important role in the typical flavour of the mature cheese because C₄ - C₁₂ acids are found at the same concentration in both cheese having good flavour and those lacking in the typical flavour. Mannigs and Robinson (1973) analysed head space vapours over the cheese
distillate and concluded that among the volatile fatty acids only 4:0 acids may have a role in the flavour of cheese.

Buffalo milk shows considerable compositional differences when compared with that of cow milk (Dastur 1966). Therefore it is reasonable to expect differences in the flavour of cheese made from buffalo milk compared with that from cow milk. With regard to studies on physical constants of fat similar observations were made in buffalo milk cheese as in the case of cow milk cheese (Garg and Verma 1966) except that the changes were slightly slower. The development of desired flavour is very much slow in cheddar cheeses made from buffalo milk than in that made from cow milk (El-Sokkary et al 1962, Burde 1963; Godberon 1964).

B. ALCOHOLS:

Alcohols, though present in trace quantities, have significant role in the flavour of dairy products especially in the fermented ones. n-alkanols are volatile and they accompany flavour volatiles; usually they are identified by gas liquid chromatography of the volatiles obtained from low temperature and reduced pressure distillation of dairy products.

a) Milk:

Aliphatic alcohols were identified in raw and heated milk by many investigators (Loney et al 1963; Scarpellino and Kosikowski 1961; McCungahand Howsann 1962). Ethanol, methanol and n-propanol were tentatively identified in milk by their retention time on gas-liquid chromatography obtained from
total neutral volatiles. Alcohols were found to be produced during heating of milk. Oct-1-en-3ol, heptanol, maltol and 2-butoxy ethanol which were absent in raw milk were found in heated milk (Sealan et al 1963). Kirk et al (1963) identified ethanol and methanol in sterilized milk stored for 3 months. Vacuum steam distillation of stale sterilized concentrated milk (SCM) was shown to contain 2-furfural which increased rapidly during storage at 27°C. This suggested the contribution of 2-furfural in off-flavour deterioration of SCM (Arnold and Lindsay 1962).

b) Cultured dairy products:

Alcohols are important constituents of some cultured milks. In fermentation the acetaldehyde produced from the intermediate pyruvic acid is reduced to ethyl alcohol (Koshland et al 1960; Green et al 1941; Racker 1950). Nabanji et al (1974) showed that Streptococcus lactis has higher capacity than Streptococcus lactis to reduce acetaldehyde to ethanol. Ethanol and 2-butanone were tentatively identified by gas-liquid chromatography of the volatile fractions of selected starter culture and ripened cream butters made with starter culture (Day et al 1962).

c) Oxidized milk fat:

Alkanols may be formed as the decomposition of secondary hydroperoxide during autoxidation of lipids (Bell et al 1951). Evans (1961) obtained gas chromatographic and mass spectral evidence for the presence of ethanol as a result of decomposition of 13-hydroperoxy linolenate. In an investigation of oct-1-en-3ol as a compound responsible for a mushroom flavour
in oxidized dairy products, Stark and Forss (1964) found n-heptan-1-ol always associated with the gas chromatographic fraction containing oct-1-en-3-ol. Stark and Forss (1966) have identified a number of n-alkanols in oxidized butterfat in small quantities. They observed an increase in C\textsubscript{1}, C\textsubscript{2}, C\textsubscript{5} and C\textsubscript{8} n-alkanols during storage of butter. About 1 µ mole/kg increase in alkanol content was measured in oxidized butter after a 4-week storage, although they increased only slowly in amount thereafter, since these compounds have relatively high flavour threshold, they are of only secondary consideration in oxidized flavour. The formation of these alkanols is explained by the theory of Farmer et al (1943), according to which alcohols are formed by the oxidation of unsaturated fatty acids in which primary alkoxy radicals resulting from the decomposition of lipid hydroperoxides split up to give alkanols. C\textsubscript{1} - C\textsubscript{5} n-alkan-1-ols and propan-2-ols were found responsible for off-flavour in irradiated stored butter oil (Merrit 1963).

d) Cheddar cheese:
Alkanols were also identified in the flavour volatiles from blue cheese samples. Heptan-1-ol, nonan-1-ol and pentan-1-ol were identified in much lower concentration (Coffman et al 1960; Anderson and Day 1963). These alcohols were found to be present in approximately same ratio as in the case of methyl ketones. Increase in ethanol, butanol and n-propanol were noticed during ripening of cheddar cheese (Scarpellino 1961; Bills et al 1966). The presence of high quantities of 2-butanol (Keen and Walker 1974) in any type cheddar cheese suggested that this compound
might have arisen from fermentation. The possible mechanism for the formation of butanol in cheddar cheese was explained by:

(i) an initial formation of 2,3-butylene glycol from acetyl methyl carbinol, (ii) conversion of 2,3 butylene glycol to 2-butanone and (iii) finally the reduction of 2-butanone to 2-butanol (Keen et al 1974).

e) Isolation from milk fat:

Van Duin (1954) prepared pyruvyl chloride 2,4-dinitro phenyl hydrazone which forms esters with alcoholic compounds. But these esters always exist in two isometric form. Schwartz and Bromingim (1965) found that the pyruvyl chloride 2,6 dinitro phenyl hydrazone react instantaneously and quantitatively with a large variety of alcohols, in presence of a catalyst, triethylene diamine, forming esters without isomeric forms. This method of isolating alcohols revealed the presence of a large number of complex alcohols, that are normal constituents of milk fat (Schwartz 1970). Trimmen et al (1970) modified the above method for the quantitative estimation of hydroxy compounds in bovine milk lipids. They quantiated about 1.2 μ moles/g aliphatic alcohols which contained mainly methanol and ethanol in milk lipids.

C. GLYCERYL ETHERS:

It can be conjectured that the naturally occurring glyceryl ethers are triglycerides containing two esterified fatty acids.

\[
\begin{array}{c}
\text{CH}_3 \quad \text{CH} \quad \text{CH}_2 \\
\text{OCH}_2\text{CH}_2\text{R} \quad \text{OCOR} \quad \text{OCOR}
\end{array}
\]
They are generally named after the monohydric alcoholic group forming the ether linkage e.g., Chymyl alcohol-(16:0), Bathyl alcohol-(18:0) Selachyl alcohol-(18:1) etc.

However, investigations on this class of compounds have been made on material isolated from the unsaponifiable fraction of milk fat; hence any fatty acids originally present would have been removed by the preliminary saponification. The presence of the ether linkage on the α-carbon of glycerol has been demonstrated by Davies et al (1933) and Karnovsky and Brumm (1955).

a) Importance of glyceryl ethers:

Glyceryl ethers are of pharmacological and therapeutic importance in animals and humans. Evidence for showing stimulation of growth by glyceryl ethers have been established. Brohult (1952) observed that the alkyl-glyceryl ethers do promote the growth rate of rats. Intraperitoneal injections of O-alkyl glyceryl ethers caused rats to gain 1.3 g per day whereas controls gained 1.1 g per day. On the basis of his experiments Brohult (1950) evaluated the effect of selachyl alcohols on growth of Lactobacillus lactis; concentration of 2 to 20 μg/ml were found to increase the number of bacterial cells as determined by turbidity measurements.

Brohult and Holmberg (1954) stated the beneficial effect of butyl alcohol given to cancer patients treated with radiation. A brief tabulation (Brohult 1933) of survival data accumulated from irradiated patients with uterine-cervical cancer indicated that those treated with glyceryl ether diesters survived for
longer period than irradiated patients not receiving them. Rusarov et al (1962) felt that butyl alcohol helped to prevent some of the changes in the blood elements that characteristically accompany radiation damage. Alkyl glycercyl ethers are also shown to be capable of promoting wound healing in rats and humans (Bodman and Maisin 1962). Butyl alcohol was found useful as a therapeutic agent in bracken poisoning of cattle (Evans et al 1959). They found that injection of butyl alcohol and antibiotics cured 23 out of 31 cattle affected with this disease.

b) Biosynthesis and Bio cleavage of glycercyl ethers:

A considerable number of metabolic studies have provided information about the bio cleavage-interconversion of alkyl and alk-1-encyl ethers. Studies connected with incorporation of labeled precursors are helpful in understanding the mechanism of formation of glycercyl ethers. Data from in vitro experiments with bovine red marrow and glucose - C\textsuperscript{14} suggested the possibility of formation of glycercyl moiety of alkyl glycercyl ethers from glucose. Thompson and Hanahan 1953 proposed that the glycerol of the glycercyl ether might be derived from \(\alpha\)-1-glycerophosphate in a manner analogous to that by which the glycercyl moiety of phosphotidyl chol ine phosphotidyl ethanolamine and triglyceride is derived from the same precursor. Earlier work on incorporating \(1\)-C\textsuperscript{14} labeled glycerol into the alkyl glycercyl ethers of starfish diverticula (Karnovsky and Brum 1955) supports this involvement of \(\alpha\)-1-glycerophosphate in the biosynthesis of alkyl glycercyl ether.
Ellingboe and Karnovsky (1957) showed that the fatty alcohol was a better precursor for the biosynthesis of alkyl glycerol ether, and fatty aldehyde served as a better precursor for alk-1-etyl ether. The enzymatic reduction of alk-1-etyl ether to corresponding alkyl ether was shown by Thompson and Hanahan (1963). Further from the results of a series of experiments, Thompson (1966) suggested that the alkyl ethers were the immediate precursor of alk-1-etyl ethers. Malins (1966) has also found that alkylglyceryl ether can be enzymatically dehydrogenated in dogfish liver to form corresponding alk-1-etyl ethers.

Blomstrand (1953) showed that glyceryl ether can be adsorbed and metabolized in mammalian system. He found that acyl groupings of the glyceryl ether diester fed to animals were hydrolysed in the same way as those of triglycerides during lipolysis. Later Snyder and Pfleger (1966) confirmed that fatty alcohol and fatty acids formed were metabolised to triglyceride and phospholipids.

c) Glyceryl ethers in lipids other than milk:

The existence of glyceryl alkyl ethers in biological materials are known for over 45 years. Recent studies almost exclusively done by gas-liquid chromatography have indicated that glyceryl ethers are found widely distributed in the tissues of fish and animals.

Investigations have disclosed that the alkyl chain in ether linkage with glycerol can be saturated or unsaturated.
Baty! alcohol was isolated by Holmes et al. (1941) from yellow bone marrow of cattle. The nonsaponifiable fraction isolated from different fish oils were analysed for alkyl glyceryl ethers by Karnovsky and Rapson (1946). They measured the formaldehyde produced by per-iodic acid oxidation and found that all the oils contained alkyl glyceryl ethers. Bodman and Maisin (1953) reported that the 0-alkyl glycerol present in peripher- al fat from neonatal calves decreased rapidly after birth. According to Karnovsky and Rapson (1946a) tundra oil contained less than 0.1 percent ethers but the other vegetable oils examined, peanut, cottonseed, sesame seed and castor seed oil contained none. Schwartz (1970) also did not obtain any glyceryl ether in vegetable oils.

The first successful resolution of 10:0-1, 12:0-1 and 13:1-1 alkyl glyceryl ethers by gas-liquid chromatography was reported by Blomstrand (1959). Hallgren and Larson (1962) determined the nature of the 0-alkyl moieties in the glyceryl ethers from the nonsaponifiable fraction of fish and mamalian lipids. They quantitatively determined the concentration of total alkyl glyceryl ethers in human red bone marrow (0.25), spleen (0.05%) and red blood cell (0.01%).

The early investigation in the field of alkyl glyceryl ethers was done generally by saponifying the lipid extracts and working on the nonsaponifiable lipid fraction obtained. Later it was realised that this procedure did not entirely saponify phosphate esters.
The use of acetone-MgCl₂ precipitation of phospholipids and of silicic acid adsorption chromatography on column and thin-layer have been responsible for quantitation of the alkyl glyceryl ethers in specific class and fraction of lipids. The occurrence of alkyl and alk-1-etyl ethers of glycerol in the neutral lipids of various species were studied in detail by Sichberg et al. (1961) and by Gilbertson and Karnovsky (1963). These authors firmly established that alkyl and alk-1-etyl glycerol ethers exist in nature as diesters in large number of tissues. The amount of glyceryl ethers in the neutral lipid fraction of tumors is significantly higher than the corresponding value for healthy tissues (Wood and Snyder 1963).

The presence of alkyl-glyceryl ethers were also demonstrated in the phospholipid fraction of total lipid (Carter et al. 1963; Tanhan and Watts 1961). Thompson and Tanhan (1963) found that alkyl-ether phospholipids in bovine red marrow accounted for over 10 percent of the total phospholipids which in turn consists ethanalamine and choline phosphatides. The alkyl chain of the ethers in the ethanalamine were 16:0 (33%), 12:0 (29%) and 18:1 (37%); in the choline fractions they were 16:0 (40%), 13:0 (15%) and 18:1 (43%).

d) Glyceryl ethers in milk:

Work on glyceryl ethers in milk lipid is very limited. Brohult (1966) has shown that selacyl alcohol has a growth stimulating effect on Lactobacillus lactis. This indicates that the glyceryl ethers in milk might stimulate the growth of
new born individual. Schogt et al (1960) showed experimental evidence for the formation of \(\alpha\)-glycerol ether when lipid material not containing phosphorous was saponified. They detected saturated \(\alpha\)-glycerol ethers of butyl alcohol type in cream and estimated 45 mg per kg of glycerol ether in a sample of milk fat. The glyceryl ether content of human milk was found to be 10 times higher than that of cow milk. Hallgren and Larson (1932) estimated 0.1 and 0.01% glyceryl ether content in human and cow milk respectively. GLC analysis of glyceryl ether showed more quantity of unsaturated glyceryl ethers in human milk than in cow milk. They identified butyl, chmyl and sebacoyl alcohols in cow and human milk. The ratio between the unsaturated (sebacoyl) and saturated (butyl and chmyl) was found to be 0.72 for human milk and only 0.34 for cow milk. Schwartz and Welbrauch (1970) developed a procedure for quantitative isolation of glyceryl ether from milk fat and they isolated about 70 glyceryl ethers in the unsaponifiable matter of milk fat. Milk fat was found to contain approximately 0.27 mg/g of glyceryl ether, of which 70 percent were saturated.

From the above review it would be evident that the trace components namely carboxyl compounds, alcohols and glyceryl ethers have been studied extensively in cow milk and its dairy products. Information similar to that gained in the case of cow milk would be of considerable importance in unravelling the role of these components in buffalo milk and its dairy products, which would be of importance from both the compositional and technological aspects.