CHAPTER II

REVIEW OF LITERATURE
A large number of investigations has been carried out to study the effect of ingestion of fats and fatty feeds on the quantitative secretion of milk and butterfat. Increase in the yield of milkfat has been reported when the diet of cows was supplemented with fats or oils poor in polyunsaturated fatty acid, e.g. tallow, butter palm oil or coconut oil (197, 96, 5, 236, 37). Increased yields of milkfat have also been observed when the diet of the cow was supplemented for short periods with vegetable oils rich in polyunsaturated fatty acids (219, 4, 96, 68). Neither, however did partial isocaloric exchange of fat and starch in the concentrate fraction achieve any obvious success even when the fat content of the concentrate mixture was increased from 4.2 percent to 7.5 percent (38, 292, 193, 194, 195, 33, 186, 183, 184, 74, 75). Only milk yield increased a little and with it total fat production. In other cases, however, neither percentage of fat content nor milk yield was affected significantly (254, 152, 205, 130, 185). Even if the fat content of the ration was increased up to 10 percent by the addition of vegetable or animal fats, percentage of milkfat content increased slightly (75, 86, 113, 136, 286, 67, 180, 235). In other instances certain fat supplements increased fat content, for
example, unbalanced feeding of soybeans. The addition of more than 5 kg. soybeans enabled Byers et al. (40) and Bainter et al. (12) to raise the milkfat content considerably. In several experiments different dietary fats exhibited a positive effect temporarily, but this disappeared as the rationing period progressed (238, 270, 41, 100, 196).

Many investigators have observed a decrease in the yield of milkfat when vegetable oils rich in polyunsaturated fatty acids were incorporated in the diet of cows over long periods (96, 5). Maynard and Mc Cay (192) extracted the dietary fat from the lucerne hay, beet and concentrates and replaced it with starch as a result the milk yield dropped with no suitable reduction in the fat content. The decrease in the yield of milkfat was also observed when highly unsaturated oils of marine origin, especially cod liver oil, were incorporated in the diet of cows (51, 239, 96, 269).

The method of incorporating the fats or oils in the diet may determine the effect on milk production. For example, Williams et al. (301) and Williams (304) observed that more milkfat was produced by cows given whole oil seeds than cows given a mixture of extracted seeds and that had been obtained from the seeds. Moore et al. (215) demonstrated that the yield of milkfat was reduced when cod liver oil was given once each day but
was unaltered when the same total amount of code liver oil was given in the form of several smaller doses during the day. Locali et al (183) found that the output of milkfat depended on the levels of fat, starch and roughage in the diet. The addition of fat to the diet resulted in the greatest increase in the yield of milkfat when the diet had either a high roughage or low starch content. These findings were not confirmed by Brown et al (37). However, interpretation of the results of experiments of Brown et al (37) is complicated by the fact that cows on the low roughage treatment received almost 3 times as much supplementary fat in the diet as did the animals on the high roughage treatment.

The feeding of high fat residues from industrial processing of oilseeds produces variable results. In experiments undertaken by Barberlaik and Jucker (17) the milk fat content showed no change although groundnut extract meal containing only 1.1 percent fat. When residues from cottonseed (310, 96, 5, 204, 68, 213, 37) were fed, results were variable. This may be attributable to the varying amounts and composition both of the fat and basic ration fed.

Doctor et al (79) did not find any variation in the fat content of milk when any of the cakes like, groundnut, sesame, linseed, cottonseed, coconut were fed in addition to wheat bran, ragi straw, Rhodes and guinea grass, Patel and Ray (227) studied the
effect of equivalent replacement of dairy mixture by cottonseed on the secretion of milkfat by buffaloes. The results showed a rise in the fat content for the first three weeks after which it gradually declined until the end of 6-7 weeks and thereafter a rapid drop occurred. The results suggested that whenever milk is to be utilized for preparing ghee, cottonseed feeding is advantageous only on temporary basis and secondly green fodder supplement was necessary.

Steele and Moore (277) observed that the addition of cottonseed oil to high roughage diet increased the yield of milkfat in the beginning but decreased the at the end. While the inclusion of tallow in the high roughage diet resulted in an increased yield throughout the period. Gaba (93) as a consequence of feeding cottonseed oil as a supplement to Sahiwal cows did not find any change in milk yield and fat percentage of milk. Angelo (8) reported a reduction in the milk yield and lactation period for Mar- parkar cows and Murrah buffaloes when cottonseed was fed to these animals. Wadsworth (298) studied the effect of periodic feeding of linseed oil and observed a decline in lactation.

Story et al (285) observed a significant reduction in the yield of milkfat when coconut oil was fed to cows. Pan et al (222) fed formaldehyde treated casein plus fever oil to lactating cows and observed an increase of 15% fat in milk.
2. EFFECT OF FEEDING DIFFERENT CONCENTRATED AND RAW SEED OILS ON PHYSICO-CHEMICAL PROPERTIES OF MILK

The common belief is that the fat content of milk is an inherited quality of the animal. Variation in fat content of milk from individual milch animal or herds in different seasons and maintained on different feeds, has attracted the attention of many investigators both in India and abroad.

(a) Reischert Meisel and Polesnake value:-

(1) BUTTERFAT:- Eckles and Palmer (84) found that feeding cottonseed whole meal to cows decreased the R.M. value while Geisler (98) on the other hand observed a high R.M. and low Polesnake value when he also fed cottonseed meal. Sutton et al (289) while feeding one pound of corn oil daily observed that the R.M. value came down to half. Williams et al (301) have reported minimum R.M. and Polesnake value when soyabean oil was fed. Hill and Palmer (123) noticed no appreciable effect on the R.M. value of butterfat due to feeding of varying amounts of corn oil, coconut seed oil and coconut oil except linseed oil. Verma et al (275) observed that hydrogenated groundnut oil had a comparatively mere effect on the lowering of R.M. value of Sindhi cows and Murrah buffaloes than that observed by feeding hydrogenated coconut oil.

Cranfield (63) observed a slight rise in R.M. value and a significant rise in Polesnake value of
butterfat whereas Anantakrishanan et al (7) observed a decrease in Peterske value when cottonseed oil was made part of the diet of cows. Doctor et al (79) reported that the composition of milkfat of animals fed oil cakes tended to become similar to that of oil. Groundnut, linseed, sesame and cottonseed cakes had no effect on R.M. value and Peterske value as a result of feeding red palm oil.

Dutta et al (83), Anantakrishanan et al (7) and Maghal et al (208) reported a fall in R.M. and Peterske value as a result of feeding sesame oil. Anantakrishanan (6) observed a general fall in R.M. and Peterske value of butterfat when he fed cottonseed oil, sesame oil, groundnut oil and coconut oil to Sindhi cows and Murrah buffaloes. Steele and Moore (277) while studying the effects of isocaloric replacement of part of dairy concentrate mixture with cottonseed oil observed a fall in R.M. value and Peterske value of butterfat.

(ii) Ghee:- Patel and Ray (227) observed that the R.M. and Peterske value of ghee was lowered significantly when buffaloes were fed with cottonseed in combination with green or dry fodder. A comparative study of the quality of ghee from cow and buffalo under identical dietary conditions, including heavy feeding of cottonseed was carried out by Patel et al (235) and they observed that there was a decrease in R.M. and
the effect was more pronounced in buffalo ghee than cow ghee. Kehar et al. (166) studied the R.M. and Pelenske value of ghee prepared from milk collected from 12 farms in different part of India, from 8 breeds of cows and 1 breed of buffalo during different seasons. The average values for buffaloes and cow's ghee were R.M. 29.44 and 24.29, Pelenske value 1.78 and 1.77 respectively. Gaba and Jain (94) as a consequence of feeding cottonseed oil as supplement to Sahiwal cows observed a significant decrease in R.M. and Pelenske value. Angulo (8) reported a fall in R.M. and Pelenske value of ghee when he fed cottonseed to Tharparkar cows and Murrah buffaloes. Singhal et al. (274) collected ghee samples from different regions of the Country and observed lower R.M. and Pelenske values in areas where cottonseed is fed to Cattle.

(b) Iodine values—

(1) Butterfat:—Holland and Buckley (124, 125) observed alterations in the unsaturation of cow milk-fat when cows were fed the basal ration supplemented by 3/4th pound of coconut, groundnut or soyabean oil. Coconut oil caused a slight diminution while other oils showed a marked decrease in the I.V. of butterfat. Eckles and Palmer (84) and Geisler (98) found that feeding cottonseed whole meal to cows increased the Iodine value of butterfat. Sutton et al. (289),
feeding one pound corn oil daily observed that the
I.V. of butterfat increased by 30 percent. Williams
et al (301) have reported changes in the chemical
composition leading to maximum I.V. when soyabean oil
was fed. Brown et al (36) on feeding one pound of
either expeller, crude or refined soyabean oil observed
an increase in the I.V. Hill and Palmer (423) reported
a drop in I.V. as a result of feeding barley, bran and
hay. Dasgupta (66) found no influence on the iodine
value of butterfat when there was a replacement of
green grass by paddy straw.

Moghul et al (208) fed cottonseed oil, sesame oil
and groundnut oil to Sindhi cows and Murrah buffaloes,
the rate being 1.5 pounds, per head per day for 15 days;
The I.V. for cows increased to 37.5, 44.8 and 39.0 units
at the end of the feeding period and the corresponding
values for the buffaloes being 32.7, 37.5 and 40.5
units respectively.

Anantakrishnan et al (7) observed an increase in
I.V. when cottonseed oil was made part of the diet of
cows. Hansen and Steensberg (110) feeding linseed oil-
cake observed an increase in I.V. of the milkfat.
Doctor et al (79) reported that groundnut, linseed,
sesame and cottonseed oil cake, feeding increased the I.V.
of butterfat.
Hilditch and Jaspersen (122) studied the effect of supplementation of basal ration with 8 ounces of groundnut oil, hydrogenated groundnut oil (I.V.45) and hydrogenated groundnut oil (I.V.17) daily. Groundnut oil and hydrogenated groundnut oil (I.V.45) led to an increase in the amount of oleic acid and a slight diminution of the butyric and caproic acids. Hydrogenated groundnut oil (I.V.45) increased the stearic acid content also. All these changes occurred in the case of hydrogenated groundnut oil (I.V.17) as well but to a less extent.

Dasgupta (66) observed a decrease in I.V. of milk-fat as a result of feeding red palm oil. Brown et al (36) reported that in about three weeks of experiment crude and refined soyabean meal increased I.V. by over 5.0 units, hydrogenated soyabean oil caused an increase by nearly 5-6 units.

Butta et al (83), Anantakrishnan et al (7) and Meghul et al (208) have reported a rise in I.V. as a result of feeding sesame oil. Anantakrishnan (6) observed an increase in the I.V. of butterfat when he fed cottonseed oil, sesame oil, groundnut oil and coconut oil to Sindhi cows and Murrah buffaloes, Steele and Moore (277) while studying the effects of isocaloric replacement of part of dairy concentrate mixture with
cottonseed oil observed a rise in I.V., butterfat. Wadsworth (298) studied the effect of periodic feeding of linseed oil on the iodine value of milk-fat.

(ii) Influence of cottonseed and lucerne on the I.V. of ghee. Their results indicated an increase in I.V., the units being 36.5 and 40.0 at the end of 50 percent and 100 percent replacement respectively. Kehar et al (166) studied the Iodine value of ghee prepared from milk collected from 12 farms in different parts of India, from 8 breeds of cows and 1 breed of buffalo during different seasons. The average Iodine values for buffaloes' and Cows' ghee were 30.2 and 34.4 respectively. Gaba and Jain (9%) as a consequence of feeding of cottonseed oil as supplement to Sahival cows observed a significant increase in I.V. of ghee. Angale (8) reported an increase in I.V. of ghee when he fed Tharparkar cows and Murrah buffaloes with cottonseeds. Singhal et al (27%) observed higher iodine values in the ghee samples which were obtained from areas where cottonseed is grown.

(c) Germicidal value:
A comparative study of the quality of ghee made from cow and buffalo under identical dietary conditions, including heavy feeding of cottonseed was carried out
by Patel, et al (225). They observed significantly lower S.V. in cow ghee as compared to buffalo ghee. Kohar et al (166) studied the saponification value of ghee prepared from milk collected from 12 farms in different parts of India, from 8 breeds of cows and 1 breed of buffalo during different seasons. The average saponification values for buffaloes' and cows' ghee were 225.7 and 223.6 respectively. Gaba and Jain (94) as a consequence of feeding cottonseed oil as supplement to Sahiwal cows did not find a significant effect on saponification value.

(d) B.R. reading/Diffractive Index-

(i) Butterfat:- Dasgupta (66) observed a fall in R.I. of milkfat as a result of feeding red palm oil. Dutta et al (83), Anantakrishnan et al (7) and Meghdul et al (208) have reported a slight rise in R.I. of the butterfat as a result of feeding sesame oil. Anantakrishnan (6) recorded a rise in B.R. reading of butterfat when he fed cottonseed oil, sesame oil, groundnut oil to Sindhi cows and Murrah buffaloes.

(ii) Ghee:- Patel and Ray (227) observed that the B.R. reading of ghee was raised significantly when buffaloes were fed with cattansaa4 ail as supplement to Behind cavs an4 torts* af tonffale 4urlnf 4iffarent seasons. lha average saponification values far buffaloes* set caws' fhaa wars 225*7 at* 223** respectively. Gaba an4 Jain as a consequence af fleeting cattansaa4 ail as supplae ta Behind cavs 414 nat fin* a significant affect an sapanlflcatlan rains.

(1) Skti*- Fatal an* Ray (227) observe* that the B.R. mating af gtoea was raise* significantly Stoss touff elaas vara fa* vltli eat tan see 4s in eesblsa-
cottonseed, the B.R. reading increased both in cow and buffalo ghee and the effect was more pronounced in buffalo ghee than cow ghee. Angulo (8) reported an increase in B.R. reading of ghee when he fed cottonseed to Tharparkar cows and Murrah buffaloes. Singhal et al (274) observed a higher B.R. reading in the ghee samples collected from the areas when cottonseed is fed to animals.

(c) Melting range/opacity:

(i) Butterfat:- Eckles and Palmer (84) and Goisler (98) observed that the melting point of butterfat was raised when cottonseed meal was fed to animals. Kuhlman and Gallup (174) and Bal and Misra (13) found that feeding of cottonseed tended to make the butter hard with a higher melting point. Delby (80) studied the softening point of original fat and its solid and liquid fractions which were 34.1, 38.6 and 24.8 respectively.

(ii) Ghee:- Singhal et al (274) found that ghee samples increased in their opacity only below a temperature of 22°C while animal body fat samples exhibited opacity above 22°C. They also observed that storage of adulterated ghee samples between 15-35°C did not affect the opacity profile.

3. UNSAPONIFIABLE MATTER:

Butta et al (83) observed that the carotene
content of the butterfat was lowered by feeding oils with no effect on vitamin A content. Doctor et al. (79) investigated the effect of feeding groundnut, sesame, linseed, cottonseed and coconut cakes in addition to ragi straw grass and wheat bran. They reported that the carotene and vitamin A contents of the milkfat were slightly lowered in all cases and considerable lowering occurred as a result of coconut cake feeding.

Dharamani and Chopra (71) studied the vitamin A potency of buffaloes' ghee throughout one lactation. The buffaloes were fed definite quantities of fuzzy, delinted American cottonseeds and Indian cottonseeds with green fodder ad-libitum. The carotene content varied from 1.70 to 5.94 μg, while the vitamin A content was 138 to 336 more blue units per 100 ml, of milk. They did not observe any difference in the vitamin A content of the milk produced when the buffaloes were fed with three different types of cottonseeds. The effect of heavy ingestion of carotene on the vitamin A content of the milkfat of Haryana cows was studied by Sarkar and Sen (265) and the average values for carotene and vitamin A content of the butterfat were 5.7 and 8.31 I.U./g., respectively.

The periodic fluctuation in the carotene content of cows and buffaloes milkfat due to change in the intake of green feeds was studied by Bal and Srivastava (124). Carotene content of cow milk fat varied between
2.0 to 5.7 ug/g while that of buffalo 0.2 to 0.3 ug./g. The amount of both carotene and vitamin A was lowered considerably under a low intake of carotene. Ingestion of green grass raised the values immediately. The carotene content of the milkfat was usually more sensitive to the quality of feed and showed greater and more immediate response than vitamin A.

The rate of transmission of carotene, in the butter-fat of Kankrej cows on a progressive feeding of large quantities of green fodder was studied by Patel and Ray (228). The results seem to indicate that (i) in the cottonseed dietary the increase in the carotene content was slow and quantitatively less on a liberal supplementation of green grass and the decrease was faster and quantitatively more when the grass was withdrawn, (ii) when the green fodder was introduced in the ration, the increase in vitamin A value was quick under conditions of feeding of both the test mixtures, but the quantitative increase in a given time was comparatively less under the cotton seed dietary. The rate as well as the extent of decrease was more pronounced when the green fodder was withdrawn from the ration of animals fed on the mixture containing cottonseed.

In a low carotene diet, feeding vitamin A supplement to cows and buffaloes increased the vitamin A
content of milkfat (155), the response was better in cows than in buffaloes. Ramaswamy et al. (247) developed a TLC method for the unsaponifiable matter for the detection of vegetable fats in ghee. Gaba (93) reported no significant change in unsaponifiable fraction and total cholesterol when they fed cottonseed oil to Sahiwal cows as supplement. Angele (8) reported an increase in the unsaponifiable matter content of milk-fat of cows and buffaloes when fed with cottonseed.

Hendrickx and Huyghebaert (120) reported the amount of sterols in substituted fats lower than the quantity found in butter. They also observed that the substituted fats had only bound sterols. In an earlier investigation Van Ginkel and Reese (294) had reported that sterols are present in butter as free sterols and in lower quantities as bound sterols. Huyghebaert and Hendrickx (137) in their first experimental series found in substituted fats several constituents besides cholesterol which were not identified as natural sterols. Later on Ross et al. (257) showed that these substances were not sterols, but belong to a group which was precipitated with digitonin during the determination of the content of sterols and were later designated as unsaponifiables.

Huyghebaert and Hendrickx (138) while examining the total unsaponifiable fraction by thin layer
chromatography noticed a group of substances with substitute fats which was not found in butterfat. They have further suggested that by this method minimum quantities of substituted fats could be detected in butterfat.

Bindal and Jain (25) found that buffalo milk ghee gave higher level of unsaponifiable matter as compared to cow ghee. They also found that the TLC patterns of ghee prepared by desi and direct creamery methods from cow milk were identical.

Francesco (92) found that the unsaponifiable matter of butter fat contained in variable proportions a polyene fraction absent from that of the synthetic fats.

4. ULTRAVIOLET SPECTRUM:

Morris et al (211) and Lembke et al (181A) used ultraviolet spectrophotometry for the detection of foreign fats in milkfat. Rege, Ma et al (250) examined the U.V. spectra at 220-220 nm of butter, margarine and mixtures of butter and margarine samples and found a peak with a maximum at about 232 nm in the case of genuine butter samples. They developed a constant which could help the detection of margarine in butterfat.

Clemenar et al (59) studied the U.V. spectra of the milkfat obtained from the milk of cows, ewes and goats and found significant difference between the studies.
Basile and Taralle (20) studied the U.V. absorption maxima of cow and buffalo milkfat and established that the degree of buffaloes's milkfat in cow's milk could be found by using the above technique.

Bay (260A) studied the U.V. and visible absorption spectra of diethyl ether extracts of cow and buffalo milk and developed a procedure for detecting the adulteration of one with the other.

5. PHOSPHOLIPIDS:

The phospholipid content in milk products has been studied by various workers. Cusick (65) 43.3-72.3 mg., Rewald (253) 95 mg., Mehr and Nuss (209) 140-160 mg., Helm et al (126) 224 mg., Baliga and Basu (14) 73-217 mg., Mc Dowell (199) 133 mg., Deutsch et al (70) 200 mg. and Ramamurthy and Naryanan (246) 206 mg. of phospholipid per 100 g of butter obtained from cow milk. Not much information is available on the phospholipid content of buffalo milk products. Baliga and Basu (15) found 122-234 mg% of phospholipids in cream obtained from buffalo milk. Ramamurthy and Naryanan (246) found 200 and 232 mg% of phospholipids in cream and butter obtained from buffalo milk. The phospholipid content of milk of different species has also been reported by various workers.
El Rafey (85) found that inspite of buffaloe's milk having more fat, its phospholipid content was lower than that of cow's milk, the respective values being 0.021 and 0.032 g/100 g.

Rawat (246) has reported the average values for the phospholipid contents of buffaloe's, cow's, goat's and ewe's milk to be 39.9, 30.15, 43, 85 and 43.04 mg/100 ml respectively. Ramkrity and Daryan (246) have also reported 39.2 and 38.7 mg. phospholipids/100 g of milk of cow and buffaloe respectively.

6. CHROMATOGRAPHY

(a) Paper chromatography of fats and unsaponifiable matter

Adulterant margarine in butterfat was estimated from the butyric acid content. The mixed fatty acids as ammonium soaps were developed on Whatman No.1 paper by the ascending technique using butanol saturated with 2N ammonia for 16 hours (251). Quantitation was achieved by comparison of the area of the spot with that of a standard. Though the sensitivity of the method has not been determined, claims have been made for better detection of adulteration by this means than by determination of Reichert Neissel and Pelenske values. Similar systems using either fatty acids (88, 42) or potassium soaps (10) for spotting were employed to detect as low as 1.5% of hydrogenated
dolphin oil in butter, based on isovaleric acid content. Sulzer and Hegel (287) and Peereboom and Reese (234) used paper chromatographic methods for separation of cholesterol and phytosterol.

Ramchandra and Desur (244, 245) used radial paper chromatography for differentiation of ghee from other fats based on differences in the unsaponifiable matter. The authors claim that 10% of Vanaspati (hydrogenated fat), 5-20% of tallow, peanut, sesame and coconut oils can be detected.

Janicek et al (151) suggested a method for the detection of substitutes in cocoa butter based on detection of C12 and C14 acids present in the adulterant.

For identification of animal and plant oils, a paper chromatographic system has been described by Jaky (150) which is based on the basis of fatty acid separation. For quantitation, the developed paper was soaked in a 1% solution of silver nitrate. A Geiger-Müller counter was used to count the radioactivity of material eluted from each spot.

The difference in the composition of triglycerides was made use of to differentiate natural oils from rearranged oils (156, 46, 48). The purified triglycerides were chromatographed on paraffin-impregnated paper using acetone-acetonitrile (3:1)
for 5 hours. The fatty acid composition of the fraction obtained was also determined by paper chromatography (156).

Cholesterol and phytosterol acetates were separated in 20-24 hours on paper impregnated with butanolic-ethanol-paraffin (10:88:2) using methanol with or without water or ethanol as developer (116) whereby animal or vegetable oil adulterants (20 parts) could be detected. A similar separation of the sterols was achieved on paraffin-impregnated paper using 8% acetic acid, permitting the detection of 5% animal fat (287, 288, 61 and 62). The development took longer (24 hours) when 95% ethanol was used (252). Infrared-impregnated paper and an acetic acid-water-ethyl acetate (6:2:2) system also was found to be equally sensitive (112). Rf values of sterols were found unsatisfactory for the detection of animal fat as an adulterant in olive oil (296).

(b) Thin-layer Chromatography of whole fats and unsaponifiable matter:

In recent years, considerable research work has been carried out using TLC as a technique for the detection of adulteration in ghee. Further, since a considerable reduction of time is desirable for analysis, application of thin layer chromatography has been found to be very useful. Mc Ogan (200) has
described a method for detecting foreign fats in butter-fat by chromatography of the unsaponifiable matter extracted from butteroil using chromeplate technique. He could detect adulterants in butter at 10% level except for samples which contained 20% coconut oil. Peereboom (231, 232) and Peereboom and Boekes (233) used Kieselguhr G plates for separating sterol acetates. Harke and Vogel (112) used paper chromatography in detecting the presence of animal fats in vegetable oils, however the steroid fraction was isolated from the vegetable oil or from the unsaponifiable fractions by means of preventive thin layer chromatography. Kaufmann et al (160, 161) have differentiated between cocoa butter and cocoa butter substitutes, and Corbulis and Zittle (43) have detected milkfat in other fats. Barrett et al (19) used TLC on silica gel impregnated with silver nitrate for the separation of synthetic and natural glyceride mixtures. Chakarbarty et al (46, 47) applied thin layer chromatography for the detection of hydrogenated groundnut oil, tallow and mohua oil in ghee. Ramanurthy et al (247) used calcium carbonate/starch plate impregnated with liquid paraffin for the detection of adulteration of ghee with vegetable fats. Hendrickx and Huyghenbaert (118) on examining the total fat by TLC observed a remarkable difference between butterfat
and substitute fats. Fairly large quantities of mono-glycerides were demonstrated in the substitute fats but they were not found in butterfat of normal acidity. Later Hendrickx and Huyghebaert (119) observed that the qualitative composition of fatty acids of mono-glycerides was identical to the composition of substitute fats and there was a quantitative difference also. Hendrickx and Huyghebaert (120) developed a method in which the presence of mono-glycerides is determined by thin layer chromatography permitting the detection of 2.5 percent of added adulterant.

Van Ginkel and Rees (294) observed that sterols are present in butter as free sterols and in lower quantities as bound sterols. Huyghebaert and Hendrickx (137) found in the substitute fats several constituents besides cholesterol and several of these substances were not identified as natural sterols. Rees et al. (257) showed that these substances were not sterols but belong to a group which is precipitated with digitonin during the determination of content of sterols. Hendrickx and Huyghebaert (120) using TLC observed that the amount of sterols in substituted fats was lower than the quantity found in butter.
Boes (259) while discussing the classical and modern methods for detection of foreign fats in milkfat concluded that TLC was a very useful technique.

Sandra (264) used reversed phase TLC for the detection of vegetable fats added to butter.

Glass et al. (101) using TLC observed that milk-fat of 15 species of ruminants exhibited the distinct triglyceride spots while those of 40 species of non-ruminants only a single spot.

Bindal and Jain (25) used Pet.ether. 60-80, pet. ether 40-60, ether and acetic acid (50:25:15:1) for the separation of ghee into 7 components on silica gel 'G' plates. Bindal (261) observed that when the unsaponifiable matter of ghee obtained from milk of different breeds of cows and buffaloes was subjected to TLC, there was a general similarity of pattern in all the cases.

Joshi et al. (154) studied the carbamylic flavour pattern of ghee samples using TLC technique.

(c) Gas liquid chromatography of whale fats:

Heavy feeding of cottonseed caused an increase in palmitic, stearic and oleic acids and marked reduction in the percentage of C4-C14 acids (203).

The combined use of GLC, dilatometric curves and the Römer index was found by Jasini et al. (148)
necessary for the detection of 10% tallow in lard. The C14 and C16 branched chain acids in tallow (103) and myristoleic and C15 acids present in tallow and hersefat (82, 141) were used as an indication of adulterants up to 10-20% in lard. The analysis of the acids which come between C14 and C16 by means of a hydrogen flame ionization detector is more sensitive than the Böser index in detecting tallow (9, 241).

Determination of isovaleric acid provided Morgantini (217) a basis for the detection of dolphin oil in butter. The pattern of the chromatogram obtained for the methyl esters on polyester columns at 205° or 220-240 reveals the presence of lard (216) or even 1.5% of palmoil, 2% coconut oil, and 4% of margarine (307) in butterfat. A comparison of a chromatogram obtained under almost similar conditions with that of short chain esters on celite 22 impregnated with 23.7% silicone oil containing 1.23% stearic acid at 125°, using helium as carrier gas, indicated the presence of some adulterants in butterfat (29). Haenni and Ritter (107) separated the short chain acids on a column containing bis (2-ethylhexyl) sebacate plus 10% sebacic acid as stationary phase. Patel and Ray (229) found that when cottonseed or cottonseed cake with green fodder were fed to animals the amount of lower molecular weight fatty acids had increased and palmitic and stearic acids were increased
to an extent of 25 and 50% respectively while oleic and linoleic acids were decreased. They also observed that when the animals were kept on dry fodder instead of green they produced butterfat having lower amount of short chain fatty acids and among the long chain fatty acids stearic acid was higher to the extent of about 50% while 14:1 was significantly lower.

Boes (259) discussed classical and modern methods for the detection of foreign fats in milkfat and concluded that detection of animal body fat in milkfat is very difficult unless several methods are employed, including the whole fatty acid pattern. Singhal et al (274) studied the fatty acid composition of different layers of ghee and observed that the liquid fraction was richer in short chain acids (C4 to C12) and unsaturated fatty acids against solid fractions rich in long chain fatty acids. Cask et al (60) fed formaldehyde treated and untreated casein-safflower oil to lactating cows and observed increased proportions of 18:12 (treated supplement) and 18:1 (in untreated supplement) with a decreased proportions of palmitic (16:0) and myristic (14:0) acids in the milkfat.

(4) Fatty acid ratio of whole fat:

A second method depends on finding out the ratio between certain acids, characteristics of some fats. The increase observed in the C14/C16 ratio from 5-6
to 8–10 on addition of 10–20% tallow and the linoleate/oleate ratio from 0.5–1.5 to 3, on the addition of 10–20% horsefat were suggested as a means of detecting tallow and horsefat in lard (141, 9, 217). Any increase over 5.5 in the ratio of (C14 + C16 + C18)/C18' was caused by the addition of tallow (23%).

The variation observed in the saturated to unsaturated ratio on the addition of olive oil to lard provided a basis for the detection of 10% of the former (220). A 20% polyethylene glycol succinate column at 210°C and helium were employed.

To detect adulteration of butter with 1.5% of coconut oil or palm kernel oil (307), 5% of margarine (104) and 20% of intersterified fat (177), the C10/C12 ratio was found to be suitable. Analysis of a large number of samples of butter and artificial butter revealed the usefulness of the C4/(C6 + C8), C12/C10 and C18 unsaturated/C18 ratios in detecting the adulterant (217, 292, 91). The ratio C10/C12 to C12/C8 also was suggested for the control of the quality of butter by Dore and Gabucci (81). The C12/C10 ratio was shown to vary depending on the source of fat (176). The unreliability of this ratio was further proved by the fact that out of 16 mixtures of margarine and butterfat, the adulterant was only detected in seven (81). Eukis and Mc Carthy (175) employing a column
of 2.5% SE-30 on chromosorb W at 200-325°C, nitrogen as carrier gas and a flame ionization detector could detect 5-10% adulteration of butterfat, based on the distribution patterns of C24-C54 triglycerides. Adulterant coconut or palm kernal fats when interesterified with other fats, were detected qualitatively from the additional peaks observed for C40-C48 triglycerides (181).

As little as 1% margarine in butterfat was detected by GLC of the sterol concentrate obtained by florisil column chromatography of the unsaponifiable matter. GLC of a sterol concentrate obtained by TLC of unsaponifiables, using a silicon oil column at 235°C and an argon ionization detector could detect 5% of animal fat in vegetable oil (58, 249). In the case of palm oil, which contains a sterol with the same retention time as cholesterol, the animal fat adulterant was detected by comparing the peak of cholesterol with those of other sterols, Cannon (41) employed GLC of sterol acetates to detect 2-3% of margarine in butter. The method failed to detect beef tallow in palm-kernal or coconut fats.

7. LIPOLYSIS OF FAT:

Comparative investigations of the rates of hydrolysis of various natural triglycerides by
Pancreatic lipase have been carried out by several workers (115, 201, 1, 272). As a general rule, it is found that vegetable fats such as coconut oil, palm oil, peanut oil and rice bran oil, are hydrolyzed more rapidly than animal fats, such as beef fat or whale oil. These studies do not shed much light on the mode of action of lipase, but tend to support the often quoted view that unsaturated fatty acids are split off more readily than saturated acids, they are useful when the nutritional values of natural fats are under consideration.

The discovery of the specificity of pancreatic lipase for hydrolysis of 1 and 3 positions of triglycerides has led to the use of the enzyme in studying the constitution of some natural fats.

Several studies have been made of lipase stability to elevated temperatures. Pancreatic lipase loses 36% of its activity after 10 minutes at 50°C, and 43% after 14 hours at 35°C (305).

The optimum pH of milk lipase lies between pH 8.0 and 9.0 (237, 99, 102). Milk lipase can be activated by cooling, warming and then cooling again (173, 291, 172). Chaudhuri and Shahani (52, 53, 54, 55) isolated milk lipase in a pure form but could get only limited information concerning the characteristics of the enzyme molecule.
Lipases have been used to study the triglycerides structure of milk fat by various workers. Mattson and Lutter (191) studied the specific distribution of fatty acids in the glycerides of animal and vegetable fats by means of pancreatic lipase. Patton et al. (230) studied the action of pancreatic lipase on milkfat to study its glyceride structure. Jensen and Gander (153) studied the fatty acid composition of the monoglycerides from lipolyzed milk fat.

Harwalker and Cobert (108) used milk lipase to study the fatty acid composition of milk fat. Clement et al. (57) used pancreatic lipase to study the hydrolysis of triglycerides of butter. Blank and Privett (27), Dimick et al. (77) and Sampugna et al. (262) used pancreatic lipase to study the triglycerides structure of milk fat.

Beuadrearn and Duman (32) studied the composition of milk fat diglycerides and partial glycerides obtained by the pancreatic lipase hydrolysis. Sampugna and and Jensen (26) studied the effect of hexane and dimethylformamide on pancreatic lipolysis of milk fat. Recently Stewart and Otterby (273) studied the lipolysis of milk fat by pregastric esterases in vivo.

8. DETECTION OF ADULTERATION IN MILKFAT AND CHEESE

Krienke (171) used the method of fractionation by selective solidification as an aid in detecting
butterfat adulteration. Harper and Armstrong (114) developed a chromatographic technique for the estimation of butyric acid to detect substitute fats in dairy products. Malerao and Kummerov (22) developed a refractive index method using alcohol soluble and insoluble portions for detecting coconut oil in butterfat. Later on they (23) modified the method for better efficiency. Prakash et al (240) used critical temperature of dissolution for the detection of adulteration of animal fat in ghee. Malerao and Kummerov (24) reviewed methods for the detection of foreign fats in dairy products and concluded that one cannot rely on the determinations based on either the mixed fatty acid composition or unsaponifiable fraction as a method suitable for detecting all conceivable mixture of substitute fats. Gupta et al (106) found that the presence of 10-15% of tallow in ghee could be detected by estimating the critical temperature of dissolution. Hoos (258) described detection of adulteration in milkfat as "a difficult problem, almost comparable with the detection of pacific water in a sample of Atlantic water."

Sander and Bird (263) used molecular distillation as a tool for the detection of milkfat adulteration.

Mc Gugan (200) observed that the unsaponifiable matter of butterfat containing 10% vegetable oils
yielded more spots than genuine butterfat with a silica gel 5% ethyl acetate in hexane system, but even 20% coconut oil could not be detected.

Bege and Olmedo (254) estimated adulterant margarine in butterfat from butyric acid content. The mixed fatty acids as ammonium soaps were developed on Whatman No.1 paper by the ascending technique using butanol saturated with 2M ammonia for 16 hours.

Cerbulis and Zittle (43) reported that the presence of milkfat upto 1% in other fats could be detected using silica gel 'G' plates. Guillamin (104) has reviewed the different methods of detecting adulterant margarine in butter, including that based on sterol acetates.

Lakshminaryanan and Kaimal (179) detected vegetable oil adulterants (5-20%) in butterfat using argentation thin layer chromatography.

Claveren (56) estimated milkfat and animal fat in cocoa butter using cholesterol content as a criteria.

Bees (259) discussed classical and modern methods for the detection of foreign fats in milkfat and concluded that detection of animal depot fats in milkfat is very difficult unless several methods are employed, including the whole fatty acid pattern.
Iwaida et al. (145) using GLC, suggested the presence of compersterol and β-sitosterol to indicate blending of vegetable oils in milkfat. Ramanurthy et al. (247) developed a TLC method for the detection of adulteration of ghee with vegetable fats.

Rees et al. (257) used sterol analysis as a tool for the detection of foreign fat in milk fat while Puruthi et al. (242) have discussed various methods for the detection of animal body fat in ghee. TLC of saturated triglycerides after random rearrangement enabled Chakarbarty et al. (50) to ascertain the effects of various ghee adulterants. The interesterified fat portion of foreign fat was detected by TLC of a monoglyceride band by Hendrickx and Huyghensbaert (118). These authors (1190 further analysed the monoglycerides by GLC to characterize them.

Paredi et al. (223) detected tallow in butterfat by tri saturated triglycerides. Infrared spectral characterization of Dutch butterfat provided a reference for adulteration analysis for De Ruig (69). Rees et al. (257) found DTA solidification useful for the same purpose. Singhal et al. (274) found that adulteration of ghee with sheep, goat or buffalo body fat upto 5 percent could be detected on the basis of opacity of samples provided the ghee was not from cotton tract areas.