SECTION - VIII

SUMMARY
Isolation and characterisation of unsaponifiable matter constituents. The unsaponifiable matter of buffalo ghee, on chromatography, showed the presence of cholesterol, lanosterol, squalene, glyceryl ether, ubiquinone, and Vitamin A.

TLC pattern of unsaponifiable matter. There was general similarity in TLC patterns of the unsaponifiable matter isolated from ghee prepared by different methods ('desi'/ 'DC' ghee) or at different clarification temperatures (110°C/145°C). TLC patterns of unsaponifiable matter of 'desi' ghee prepared from milk of different breeds of cows and that of Murrah buffaloes were also similar.

Unsaponifiable matter content of cow and buffalo ghee.

i) The level of unsaponifiable matter in cow ghee (449mg%) was significantly higher than that in buffalo ghee (398mg%).

ii) The variations in the level of unsaponifiable matter due to season were significant (P≤ 0.05) both in cow and buffalo ghee. Comparatively higher level was
noticed during spring and winter (465, 462mg% for cow ghee and 410, 398mg% for buffalo ghee respectively). Lower levels (442, 428mg% for cow ghee and 391, 392mg% for buffalo ghee) were observed respectively during autumn and summer.

iii) There were highly significant variations (P< 0.01) in level of unsaponifiable matter of three types of milk fats viz. 'desi' ghee, 'DC' ghee and butter fat of both the species. In case of cow, the increasing order of unsaponifiable matter content of three fats was 'desi' ghee (403mg%), 'DC' ghee (419mg%) and butter fat (435mg%). In case of buffalo, the trend was similar viz. 'desi' ghee (373mg%), 'DC' ghee (394mg%) and butter fat (410mg%).

8.4 Total cholesterol content of cow and buffalo ghee. A direct colorimetric method, which not only obviated the need for prior saponification of milk fat but also eliminated the use of degitonin, had been developed for the estimation of 'total' cholesterol in milk fat (ghee). This method was much more rapid than the conventional methods and yet gave comparable results. The veracity of the method was further established through recovery experiments.
8.4.1 Cholesterol level (330mg%) in cow ghee was significantly higher than that in buffalo ghee (275mg%).

8.5 Factors affecting 'total' cholesterol content of ghee.

8.5.1 Variations in the cholesterol content of ghee due to season in both the species of cow and buffalo were highly significant (P< 0.01). In case of cow ghee, the cholesterol level was highest in spring (342mg%) and declined to minimum during summer (315mg%). In case of buffalo ghee, peak cholesterol values were noticed in spring (285mg%) and then fell to minimum (269mg%) during autumn.

8.5.2 In case of cows and buffaloes, the colostral fat contained exceptionally higher level of 'total' cholesterol (554mg% for cows and 516mg% for buffaloes) as compared to normal milk fat (336mg% for cows and 265mg% for buffaloes).

8.5.3 'Total' cholesterol content of ghee showed two peak values, one at the mid lactation stage and the other towards the end of lactation. The increase towards the end of lactation was very sudden. The trend in both species was almost identical.

8.5.4 Cholesterol content of milk fat prepared from mastitic milk was of the order of 729mg% for cows and 625mg% for buffaloes. These values were
exceptionally high when compared with those of normal milk fat (318mg% for cow butter fat and 294mg% for buffalo butter fat).

8.5.5 'desi' ghee prepared from foremilk (367mg% for cows and 297mg% for buffaloes) contained significantly higher level of cholesterol (P≤ 0.01) than that from strippings (334mg% for cows and 262mg% for buffaloes).

8.5.6 The clarification temperature of butter during the preparation of 'desi' ghee did not appear to have any effect on the cholesterol content. 'Desi' ghee prepared at 110° and 145° from cow milk contained cholesterol to the level of 338mg% and 336mg%. The corresponding values for buffalo ghee were 276 and 270mg%. These variations were not significant statistically.

8.5.7 There were highly significant variations (P≤ 0.01) in the cholesterol content of three types of fats, viz. 'desi' ghee, 'DC' ghee and butter fat. In case of cow milk, the increasing order of cholesterol content of three fats was 'desi' ghee (322mg%), 'DC' ghee (306mg%), butter fat (318mg%) and these variations were highly significant (P≤ 0.01). In case of buffalo, however, no significant difference was noticeable between 'desi' ghee (263mg%) and 'DC' ghee (275mg%) although 'desi' ghee and 'DC' ghee samples showed significant differences in cholesterol content when compared with butter fat (294mg%).
8.5.3 The fat isolated from butter milk contained significantly higher level of cholesterol \( (P \leq 0.01) \) as compared with 'desi' ghee. Cholesterol levels of 'desi' ghee and of the fats isolated from top and bottom layers of butter milk were of the order of 336, 503 and 627mg% respectively for cow and 254, 374 and 516mg% respectively for buffalo.

8.6 Correlation studies.

8.6.1 The correlation coefficients between 'total' cholesterol and unsaponifiable matter contents were highly significant \( (P \leq 0.01) \) in 'desi' ghee \( r=+0.8883 \) and 'DC' ghee \( r=+0.9602 \) prepared from cow milk and in 'desi' ghee \( r=+0.8950 \) prepared from buffalo milk. However, the correlation coefficients of 'DC' ghee prepared from buffalo milk and butter fats prepared from both cow and buffalo milk were not significant.

8.6.2 The correlation coefficients between the levels of unsaponifiable matter and 'total' cholesterol were statistically significant \( (P \leq 0.01) \) both in cow ghee \( r=+0.8309 \) and in buffalo ghee \( r=+0.8365 \).

8.7 'Free' and 'esterified' cholesterol in ghee.

8.7.1 A new TLC-cum-colorimetric method had been developed for the direct and independent estimation of 'free' and 'esterified' cholesterol in milk fat (ghee). The veracity of the method was further established through recovery experiments.
8.7.2 Cow ghee contained significantly higher levels (233mg%) of 'free' cholesterol than buffalo ghee (212mg%). In contrast, 'esterified' cholesterol level of buffalo ghee (63mg%) was significantly higher than that of cow ghee (47mg%). In other words, cow ghee contained about 15% of the 'total' cholesterol in 'esterified' form, whereas this level for buffalo was about 20%. These variations, in the levels of 'free' and 'esterified' cholesterol, both in cow and buffalo ghee were statistically significant (P< 0.01).

8.7.3 Variations due to season in case of 'free' cholesterol content were not significant both in cow and buffalo ghee but in case of 'esterified' cholesterol content, these variations were highly significant (P< 0.01). The average level of 'free' cholesterol for cow ghee was of the order of 298, 234, 276 and 274mg%, respectively during winter, autumn, summer and spring. The corresponding figures for buffalo ghee were 218, 210, 208 and 211mg% respectively. Seasonal variations in the level of 'esterified' cholesterol were highly significant (P< 0.01). Peak values of 'esterified' cholesterol were noticed during spring both in cow ghee (69mg%) and buffalo ghee (77mg%) and lowest in winter (39mg% for cow ghee and 54mg% for buffalo ghee).

8.7.4 The distribution pattern of 'free' and 'esterified' cholesterol was abnormal in colostral
fat. The ratio of 'esterified' to 'free' cholesterol on first day's postpartum secretion was 1.02 in cow and 1.25 in buffalo. This ratio had a tendency to decrease with the progress of lactation and fell to 0.29 and 0.30 respectively in cow and buffalo ghee on ninth day since parturition which was the usual pattern for normal milk fat.

8.8 **Fatty acid composition of cholesteryl esters of ghee.** The cholesteryl esters of buffalo ghee, in contrast to those of cow ghee, contained no C\textsubscript{9} (saturated as well as unsaturated) acids and only trace amounts of C\textsubscript{10:0} acid. The level of C\textsubscript{12:0} and C\textsubscript{18:1} acids was higher in buffalo ghee cholesteryl esters. Cow ghee cholesteryl esters contained relatively higher level of C\textsubscript{18:2} and C\textsubscript{18:3} acids. The level of C\textsubscript{18} acids, in general, and C\textsubscript{18:1} acid in particular was higher in buffalo ghee cholesteryl esters. In other words, buffalo ghee cholesteryl esters were relatively more unsaturated.

8.9 **Lanosterol in ghee.** The presence of lanosterol in ghee had been established by TLC technique. A new and simple TLC-cum-colorimetric method had been developed for the estimation of lanosterol in milk fat (ghee). This method was not only rapid but it avoided also the use of digitonin and pyruvyl chloride-2, 6-dinitrophenylhydrazone. The veracity of the method was further confirmed through recovery experiments.
The average level of lanosterol in cow and buffalo ghee was 9.3 and 8.2 mg% respectively. These variations due to species were, however, not statistically significant.

8.10 Carotenoids in ghee.

8.10.1 $\beta$-carotene and lutein had been shown as the components of cow ghee pigments. $\beta$-carotene appeared to exist in two forms, viz. 'free' and 'bound' forms. The 'free' form was the predominant one. Saponification process affected ghee pigments adversely. Buffalo ghee pigments, on the other hand, were ill-defined.

8.10.2 Cow ghee contained significantly higher levels (4.2 $\mu$g/g fat) of lutein than buffalo ghee (3.1 $\mu$g/g fat). Variations due to season were highly significant ($P<0.01$). Peak values were obtained during spring in cow ghee (4.9 $\mu$g/g fat) and during summer in buffalo ghee (4.4 $\mu$g/g fat). The minimum values were obtained during winter both in cow ghee (2.0 $\mu$g/g fat) and in buffalo ghee (1.1 $\mu$g/g fat).

8.11 Vitamin A in ghee. Cow ghee contained significantly lower level (23.3 I.U./100 g) of Vitamin A alcohol than buffalo ghee (39.8 I.U./100 g). However, season appeared to have no effect on the Vitamin A level of ghee.

8.12 Squalene in ghee. The level of squalene in cow ghee (52.3 $\mu$g/g fat) was significantly lower than that in buffalo ghee (62.4 $\mu$g/g fat).
8.13 Ubiquinone in ghee. The ubiquinone content of cow ghee was lower (5.03 µg/g fat) than that of buffalo ghee (6.51 µg/g fat). These variations were highly significant (P < 0.01).