Trace Components in Milk Fat and Other Dairy Products
SUMMARY

1. Carbonyl compounds in cow and buffalo milk fats were studied over a period of one year. The range and average quantities of different carbonyl compounds observed in cow and buffalo milk fat were: total carbonyls, 3.6 - 6.0 (average 4.72) and 4.2 - 6.2 (average 5.0) µM; ketoglycerides 1.6 - 2.9 (average 1.94) and 1.71 - 3.40 (average 2.08) µM; monocarboxyls 0.33 - 0.53 (average 0.423) and 0.36 - 0.53 (average 0.453) µM/g respectively, indicating significantly higher monocarboxyl content in buffalo than in cow milk fat. A positive correlation was observed between the ketoglycerides and total carbonyl contents of milk fats.

2. The 2,4-DNP hydrazones of monocarboxyls were further separated into classes and individual compounds. The methyl ketone percentage was more in cow (87.5%) than in buffalo (81.7%) milk fat, while the quantities of aldehyde classes were more in buffalo than in cow milk fat.

Among the methyl ketones detected and identified tentatively, C₃ and C₁₁ - C₁₅ odd numbered methyl ketones were in large quantities whereas C₅, C₇, C₉ and C₁₄ were only in trace quantities. The C₂ - C₁₂ alkanals, C₄ and C₆ alk-2-enals and C₅ and C₆ alk-2,4 dienals were tentatively identified in both cow and buffalo milk fats.

3. Cow milk fat contained significantly higher amounts (nearly twice, 0.51 µM/g) β-ketoglycerides than buffalo milk fat (0.36 µM/g fat). The lower odd numbered methyl ketones...
(C₅ – C₉) which were present in trace quantities in fresh milk fat, were produced during heating of milk fat in presence of water; heptanone-2 was produced in greater extent in both cow and buffalo milk fats.

4. Monocarboxyls were found in significantly higher quantities in low melting fraction (LMF) than in high melting fractions (HMF) of milk fats. However, significant difference in the quantities of total carbonyls and ketoglycerides in these two fractions was not observed. The percentage proportions of methyl ketones and aldehydes were the same in both the fractions.

5. The average quantities of steam-volatile monocarboxyls (SVMC) in cow and buffalo skim milks were 0.64 μM and 0.55 μM/100 ml respectively. However the difference in the values were not statistically significant. Buffalo and cow whole milks contained 1.60 μM and 1.34 μM/100 ml SVMC, the average fat content being 3.03 and 4.43 respectively. But the SVMC on the basis of per g fat was higher in cow (0.16 μM) than in buffalo (0.13 μM) whole milk. This was attributed to the higher amounts of β-ketoglycerides in cow milk fat.

6. The SVMC isolated from skim milk and whole milk distillates were further separated into classes and individual compounds. In both skim and whole milk, the percentage of methyl ketones was more in cow than in buffalo milk and whole milk samples of both cow and buffalo contained higher proportions of methyl ketones than the skim milk.
Only acetone ($C_3$) and butanone ($C_4$) were detected and identified in the steam distillates of skin milks while all the odd numbered methyl ketones from $C_3$ - $C_{15}$ and butanone were detected in that of whole milks. The presence of large quantities of $C_{11}$ - $C_{15}$ odd numbered methyl ketones in milk fat and the absence of these in the steam distillates of skin milks indicated that these methyl ketones may play an important role in the flavour of whole milk. The absence of $C_5$ - $C_{15}$ odd numbered methyl ketones in the steam distillates of skin milk indicated that the methyl ketone precursors namely $\beta$-ketoglyceraldehydes were absent in skin milk.

Among the aldehyde classes, alkanals were in significant quantities and only small quantities of alk-2-ens were found while the alk-2,4 dienals were almost absent in the steam distillates of both skin and whole milks. Skin milk contained only lower alkanals ($C_2$ - $C_5$), whereas all the alkanals from $C_2$ - $C_{12}$ were detected and identified in the whole milk. Acetaldehyde was the predominant aldehyde found in both skin and whole milks.

7. Skin milk obtained from substandard commercial milk samples contained larger quantities of SWMC than that of fresh milk. There was no increase in SWMC till the milk had 0.35% developed acidity. The naturally curdled skin milk samples showed much higher quantities of SWMC than skin milk of substandard milks having about 0.35% developed acidity. This indicated that the production of SWMC in these milks were due to proteolysis or lactic acid fermentation. The percentage proportions of
aldehydes were more in these milk samples as compared to those of normal milk. In addition to all the carbonyls found in fresh skin milk, the skim milk from substandard milk showed the presence of C6, C7 and C8 alkanals, which appear to contribute significantly to the off-flavour noticed in these milks.

Significant increase was noticed in acetone and acetaldehyde.

8. The quantities of SWMC produced in 'dahi' prepared from skim milk was found to depend on the cultures used. S. lactis and S. diacetylactis produced about 3.5 μM and 9.0 μM/100 ml, SWMC respectively. The percentage proportion of methylketones was more in S. diacetylactis cultured skim milk than in that of S. lactis cultured. Acetone and acetaldehyde were the major carbonyls produced by these cultures. Substantial quantities of butanone found in these cultured milks which was not observed in naturally curdled skim milk indicated that this compound may have some importance in the flavour of cultured milks.

9. The type of carbonyl compounds found in colostrum skin milk and fat were similar to those found in normal skin milk and fat respectively. However, the concentrations of the carbonyls were much less in the case of colostrum, especially in respect of content of methyl ketones.

All the carbonyls in general increased with stage of lactation, however, the increase was very marked during the first month of lactation period.

10. During storage, in buffalo milk fat, the peroxides developed early and also the rate of development of peroxides
and carbonyls was higher than in cow milk fat. The increase in all the aldehyde classes namely alkamals, alk-3 enals and alk-3,4 dienals was more in buffalo milk fat while that of methylketones was more in cow milk fat at the end of 9 months of storage period. Among the aldehydes, alkamals were produced in maximum quantities. The monocarboxyls and ketoglycerides produced were proportional to the peroxides developed during the storage. The rate of breakdown of peroxides was more in cow milk fat than in buffalo milk fat. The increase in monocarboxyl content was proportional to that of ketoglycerides and the proportion of ketoglycerides produced was more in cow milk fat than in buffalo milk fat.

The C5, C7 and C9 methylketones which were in trace quantities in fresh milk fat increased during the storage of milk fats. Maximum increase was noticed in the case of 2-heptanone, thus indicating that the pattern of formation of methylketones during the storage of milk fat was similar to that of heated milk fat in presence of water, through the hydrolysis and decarboxylation of diketoglycerides.

11. Both IMF and whole fat of buffalo milk fat autoxidized more quickly than those of cow milk fat, while the development of peroxide was slow in IMF of buffalo than in that of cow milk fat. However within the different fractions of the same milk fat the rate of development of peroxide was in the order, IMF > whole > IMF.
12. Autoxidation was also studied separately in milk fats which were made carbonyl free initially. The pattern of development of peroxides, monocarboxyls and ketoglycerides were similar to that observed in the case of storage of original fats. The ratio of the amount of monocarboxyls developed to that of ketoglycerides, increased with temperature of autoxidation. The average ratios observed in the case of autoxidation of carbonyl free buffalo milk fat were 2.36, 4.54 and 6.39 respectively at 37, 60 and 90 + 1°C indicating higher proportion of ketoglycerides produced at higher temperatures of autoxidation.

13. The higher methyl ketones (C9 - C15) found in original fats were not produced during autoxidation. Only the C3 - C8 methyl ketones were detected and identified tentatively, in the autoxidized carbonyl-free fats. The autoxidation of carbonyl-free LMF produced higher proportions of C3, C6, C7 and C8 methyl ketones while that of HMF produced higher quantities of C3, C5 and C6 methyl ketones.

All the aldehydes found in fresh milk fat were also observed to have been produced during autoxidation of carbonyl-free fats. Among the C2 - C12 alkanals identified, the C5-C8 members were predominant in the whole fat, while C7, C8 and C9 were pronounced in the LMF and pentanal (C5) and hexanal (C6) were in higher quantities in the IMF. In the LMF and whole fats C4 - C9 alk-2 enals were tentatively identified while the IMF did not contain the C8 and C9 members. All the alk-2,4 dienals C5, C6, C7 and C10 were tentatively identified in the autoxidized carbonyl free LMF, whole and IMF of buffalo milk fat.
The UV absorption spectra of antioxidized carbonyl free milk fat showed no peaks corresponding to those of conjugated polyene fatty acids indicating that these acids were oxidized to give rise to carbonyl compounds during autoxidation of milk fat.

14. The total carbonyls, and monocarbonyls in milk fat increased significantly, during spontaneous oxidation of cow and buffalo milk at 5 ± 1°C. The increase in the monocarbonyls corresponded well with the TBA values. There was significant increase in aldehydes, especially in alkanals indicating that these compounds may have some role in the off-flavour noticed in spontaneously oxidized milk.

15. Buffalo milk fat contained significantly higher amounts of dissolved oxygen than cow milk fat. The average quantity of dissolved oxygen observed in cow and buffalo milk fat were 2.1 ml/100 g and 3.2 ml/100 g respectively, indicating that the higher content of dissolved oxygen in buffalo milk fat may be responsible for quicker development of peroxides and hence the carbonyls, noticed during storage.

The dissolved oxygen in milk fat was found to have a direct correlation with the electrical potential of the fat solution in 7 per cent aqueous dioxan.

16. Cheddar cheese made from cow milk developed pronounced desired flavour at the end of 5 months of ripening while it took 7 months for buffalo milk cheese to get the same flavour.
The increase in monocarboxyls and total carbonyls during the ripening was slow and gradual and it was same at the end of 7 months of ripening, in both cow and buffalo milk 'cheese fats'. However, the ketoglyceride content did not show any change.

The quantitative nature of development of methyl ketones and aldehydes were similar in both cow and buffalo milk 'cheese fats'. The C₅ C₇ and C₉ methyl ketones which were present only in trace quantities and butanone (C₄) which was absent in fresh milk fat appeared significantly before the end of the first month itself and thereafter increased progressively. The lower aldehydes (C₁ - C₄) which were in trace quantities in milk fat increased significantly during ripening of cheese. The rate of increase in higher methyl ketones (C₁₁ - C₁₅) and the decrease in acetone (C₃) were the same in both cow and buffalo milk 'cheese fat', while the rate of development of lower methyl ketones (C₄ - C₉) was more in buffalo than in the 'Cheese fats' of cow milk. The development of methyl ketones in cheese of cow and buffalo milks could not be correlated with the flavour development in the respective cheeses. However, the production of lower aldehydes (C₁ - C₄) being higher in cow milk cheese, corresponded with the development of flavour, especially in the early stages of ripening.

17. Total free fatty acids (TFFA) and steam volatile free fatty acids (SVFFA) increased during the ripening of cheddar cheese. The TFFA as well as SVFFA were higher in the 'cheese fats' of cow than in that of buffalo milk of the same age. The rate of development of fatty acids became marked after 4 months in the case of TFFA and 2 months in the case of SVFFA. The
levels of free fatty acids especially of SVFFA in the 'cheese fats' of cow and buffalo milks during ripening appeared to have a good relationship with the flavour development in the respective cheeses. Fats from different lots of cheeses of both cow and buffalo milk having pronounced desired flavour had similar levels of TFFA (2.8 - 3.2 μM/g fat) and SVFFA (0.23 - 0.27 μM/g fat).

18. The monocarbonyl content of the sheep (0.30 μM/g) and pig (0.35 μM/g) body fats were significantly higher than those of cow (0.42 μM/g) and buffalo (0.46 μM/g) milk fats. Pig body fat contained higher amounts of total carbonyls and ketoglycerides than sheep body fat and milk fats. The lower odd numbered methyl ketones (C5 - C9) which were in trace quantities in milk fat, were present in much higher quantities in the body fats.

Sheep body fat contained two additional higher methyl ketones, tentatively identified as C17 and C19 which acted as tracer components in the detection of the presence of sheep body fat in milk fats.

19. The carbonyls found in the unsaponifiable matter of the fats were all monocarbonyls whose quantities were 1.40 μM, 1.60 μM and 0.75 μM/g fat in the case of sheep, pig and milk fats respectively. The main difference between the monocarbonyls of unsaponifiables and the original fats, was that lower alkanals (C2 - C5) were absent and the lower odd numbered methyl ketones (C5 - C9) were present in significant quantities in the unsaponifiables.
20. Buffalo milk fat (1.85 µM/g) contained significantly lesser quantities of alcohols than cow milk fat (2.29 µM/g fat). Methanol, ethanol and butanol were tentatively identified in both cow and buffalo milk fats. Colostrum milk fat of both cow (1.83 µM/g) and buffalo (1.30 µM/g) contained lesser amounts of alcohols than the normal milk fats. The alcohol content of milk fat increased progressively with the stage of lactation.

A significant increase in alcohol content of milk fat was observed in milk fats obtained from ripened cream indicating the possibility of contribution of these compounds to the rich flavour of desi ghee or milk fat prepared from such cream.

Alcohol content of cheddar 'cheese fats' increased during the ripening, the increase was more in buffalo than in cow milk 'cheese fats' although the flavour development was quicker in the cheese of cow milk. This indicated that alcohols may not contribute to the flavour of cheddar cheese.

21. Cow and buffalo milk fat contained about 0.90µM and 0.80 µM/g glyceryl ethers respectively. Colostrum milk fat of both cow and buffalo contained higher amounts (about 3-4 times) of glyceryl ethers than the normal milk fats. The glyceryl ether content of colostrum milk fat decreased progressively during the transformation to normal milk. The rate of decrease was more in buffalo colostrum milk fat than in that of cow.

The phospholipid fraction of buffalo cream contained only 12% of the glyceryl ether content of neutral lipids.