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n-Alkanols in cow and buffalo milk products

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ABSTRACT

n-Alkanols in cow and buffalo milk-fat were converted into 2, 6 dinitropyruvyl chloride phenyl hydrazone derivatives and estimated spectrophotometrically.

Milk-fat of buffaloes contained significantly lesser quantities of alcohols than of cows. The alcohol content of colostral-fat was less than that of normal milk-fat. Methanol, ethanol and butanol were tentatively identified in both cow and buffalo milk-fats. A significant increase in alcohol content was observed in milk-fats obtained from ripened cream. Alcohol content of cheddar 'cheese fats' increased during ripening of cheese. The increase was greater in buffalo milk cheese than in cow milk cheese though the flavour development was quicker in the latter. This indicated that alcohols may not be desirable in the cheese flavour.

Alcohols, though present in trace quantities, play a significant role in the flavour of dairy products, especially in the fermented ones. n-Alkanols are volatile and are usually isolated by low temperature and reduced pressure distillation of dairy products (Loney *et al.*, 1963; Scarpelino and Kossikowoski, 1961). Recently, alcohols were successfully converted into derivatives of pyruvyl chloride 2, 6 dinitrophenyl hydrazones (Schwartz and Brewington, 1966) for isolating them quantitatively from milk-fat. A study was made on alcohols in products of cow and buffalo milk like fresh milk-fat, ripened cream butter-fat and cheddar cheese to understand the significance of these alcohols in the flavour of such milk products.

MATERIALS AND METHODS

Milk-fat

The cream obtained from pooled cow and buffalo milk collected from the Institute's farm was churned to butter. The butter was melted at 100°C and filtered to get clear fat. In the case of ripened cream butter, the cream kept for natural ripening at 25°C for 48 hr was churned into butter, melted at 100°C and filtered to get clear fat.

Cheese-fat

Cheddar cheeses were prepared from pooled cow and buffalo milk using a mixed culture of *S. lactis*, *L. helveticus*, *S. thermophilus* and *L. casei* as recommended by Jagannath *et al.* (1973). Rennet was added @ 15 ml per 100 litres of milk. The maximum cooking temperature used was 38°C. The cheese obtained was stored for ripening at 8° to 10°C and humidity of 75 to 80%. Samples were drawn for analysis at intervals of 2 months. The samples were grated, filled in 50 ml tubes, warmed and centrifuged at 1,500 g. The clear fat was decanted.

Estimation of alcohols

Milk-fat samples were made cholesterol-free by passing a benzene solution through a degitonin : celite column (Schwartz *et al.*, 1967). The alcohols present in the cholesterol-free milk-fat were converted into 2, 6 dinitropyruvyl

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chloride phenyl hydrazone derivatives, and they were separated from other derivatives on acid alumina column (Timmen *et al.*, 1970). The unreacted lipids were removed by eluting the hexane solution through magnesium oxide : celite (1 : 1) column, and the alcohol derivatives eluted by chloroform were quantitated spectrophotometrically at 400 mμ on the basis of molar extinction coefficient E = 5950 (Schwartz and Brewington, 1966). The results obtained were corrected for 85% recovery by blank experiment. The alcohol derivatives were separated into individual compounds on silica gel-HF plates (Timmen *et al.*, 1970) developed with methyl cyclohexane-diethylamine (130 : 70 v/v) and tentatively identified on the basis of standard derivative.

RESULTS AND DISCUSSION

The alcohol content of pooled cow and buffalo milk-fat and colostral-fat are given in Table 1. Buffalo milk-fat contained significantly lesser quantities ($P < 0.05$) of alcohols than cow milk-fat. Colostral-fat of both cow and buffalo contained lesser quantities of alcohols than normal milk-fat. Methanol, ethanol and butanol were tentatively identified in the colostral and normal milk-fats. However, it was not possible to separate alcohol derivatives higher than butanol on the TLC plates.

Alcohols such as ethanol, methanol and n-propanol were identified in the distillates of raw and heated milk by several workers (Scarpellino and Kossikowski, 1961; Loney *et al.*, 1963). A large number of complex alcohols considered to be natural constituent of milk-fat were also identified by Schwartz (1970). In the present study only small quantities of ethanol and methanol could be detected on TLC plate by colour development in the presence of diethylamine vapour. The major portion of the derivatives appeared as a large band corresponding to butanol and other higher alcohols. The higher alcohol may comprise long-chain alcohols such as cetyl $CH_3(CH_2)_{14}CH_2OH$; stearyl $CH_3(CH_2)_{16}CH_2OH$ and myricyl $CH_3(CH_2)_{28}$—CH_2OH which are also found in depot fat of the animal. The microbial fermentation of carbohydrates in rumen may account for the lower n-alkanols found in milk-fat. The lower amounts of alcohols found in colostral-fat than in normal milk-fat may be attributed to the observation made by Smith (1973) that during production of colostrum less of depot fat is utilized for synthesis of milk-fat.

The increase in alcohol content of milk-fats during ripening of cream is shown in Table 2. The milk-fats obtained from cream naturally ripened at 25°C for 48 hr of cow and buffalo milk contained significantly higher amounts ($P < 0.01$) of alcohols than those obtained from respective fresh cream. The band corresponding to ethanol on TLC plate was more intense in milk-fat from ripened cream.

Production of ethanol (Habaj *et al.*, 1974) and butanol (Scarpellino and Kossikowoski, 1962) during lactic acid fermentation has been shown earlier. These lower alcohols though highly soluble in aqueous phase may also be soluble to some extent in the fat phase, thus contributing to the significantly higher amounts of alcohols observed in milk-fat prepared from ripened cream. Since alcohols like ethanol contribute to the flavour of fermented products, study on the role of alcohols in the flavour of *desi* ghee or milk-fat prepared from ripened cream may be of interest.

Production of alcohols in cheddar cheeses made from cow and buffalo milks during ripening at regular intervals of 2 months is shown in Table 3. The alcohol content in cheese-fat increased considerably with period of ripening. The increase was more in buffalo milk cheese than in cow milk cheese.

Alcohols such as ethanol, butanol, propanol, pentanol, heptanol and nonanol were identified in flavour volatiles of blue cheese in much lower concentration (Anderson and Day, 1966). Increase in these alcohols, especially butanol content, was noticed during ripening of cheddar cheese (Scarpellino, 1961; Bills *et al.*, 1966). These alcohols were present in

Table 1. Amounts of alcohols found in cow and buffalo milk-fats

	Cow		Buffalo	
	Milk-fat	Colostral-fat	Milk-fat	Colostral-fat
	μM/g fat			
1	1.98		1.65	
2	2.20	1.86	1.85	1.17
3	2.30		2.10	
4	2.25	1.84	1.86	1.40
5	2.20		1.70	
6	2.68	1.78	1.80	1.32
7	2.52		2.10	
8	2.21	1.82	1.75	1.35
Average	2.29	1.825	1.85	1.31

Table 2. Development of alcohols in milk-fats during ripening of cow and buffalo cream

Sample	Cow		Buffalo	
	Fat from fresh cream	Fat from ripened cream	Fat from fresh cream	Fat from ripened cream
	μM/g fat			
1	2.02	3.52	1.84	2.65
2	2.14	2.68	1.78	2.78
3	2.82	4.28	1.82	3.23
4	2.27	3.54	2.12	3.52
5	2.23	2.78	2.15	3.20
6	2.24	3.05	1.65	2.25
"t" value	5.47**		8.03**	

**Significant at 1% level.

Table 3. Development of alcohols during ripening of cheddar cheese made from cow and buffalo milk

Period of ripening in months	Cow		Buffalo	
	Sample 1	Sample 2	Sample 1	Sample 2
	μM/g fat			
0	2.82	2.50	2.20	1.80
1	3.20	2.95	2.65	2.50
2	3.45	3.62	3.70	3.60
4	3.80	4.00	4.25	4.15
6	4.35	4.55	4.85	5.15
8	4.85	5.10	6.25	5.60

approximately same ratio as of methyl ketones. During ripening of cheddar cheese made from cow and buffalo milks the increase in lower methyl ketones (C_4 to C_9) were higher in buffalo milk cheese than in cow milk cheese (Bhat *et al.*, 1978). Similarly, the quantities of alcohols estimated were higher in the buffalo milk cheese than in the cow milk cheese in the present study.

L. brevis reduced the butanone produced by *S. diacetilactis* and *Lactobacillus plantarum* to butanol during ripening of cheddar cheese (Keen *et al.*, 1974). In the present study also *Lactobacillus casei*, which resembles *L. brevis* used by Keen *et al.* (1974) may reduce the butanone formed during ripening of cheddar cheese made from cow and buffalo milks (Bhat *et al.*, 1978) to butanol and hence account for the increase in alcohol content of cheddar cheese-fats. Scarpellino (1961) showed that alcohols such as butanol is not desirable in cheese flavour since it contributes towards off-flavour of cheese. In the present study also changes in alcohol content of cheeses did not correspond with flavour development during ripening. Cheese made from buffalo milk which contained higher quantities of alcohols lacked in desired flavour as compared with cow milk cheese (Bhat *et al.*, 1978) indicating that production of alcohols during ripening of cheese may not be desirable in cheddar cheese flavour.

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