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Estimation of Total Cholesterol in Ghee Prepared from Milk of Cows and Buffaloes*†

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Reinhold and Shiels' modification used in the estimation of cholesterol in blood has been successfully applied to estimate cholesterol in ghee. Twenty-six samples each of cow and buffalo ghee have been analysed for their total cholesterol content by this method and the data analysed statistically. Highly significant differences between the cholesterol levels of cow and buffalo ghee have been observed. Cow ghee contains higher levels (0.31%) of cholesterol than buffalo ghee (0.27%).

CHOLESTEROL, the major constituent of the unsaponifiable matter of milk fat, has, in recent years, attracted considerable attention due to its possible, though controversial, relationship to diseases like atherosclerosis[1]. Several reports[2–9] on the cholesterol contents of milk and milk products have appeared. The methods in these estimations have involved saponification of the milk fat followed by gravimetric estimation of cholesterol as digitonide or colorimetric estimation of cholesterol/cholesterol digitonide and are generally laborious. Reinhold and Shiels' modification[10], applied to the estimation of cholesterol in blood serum/plasma, avoided the saponification step and also obviated the use of digitonin. A survey of literature revealed that this promising method has not been extended to the estimation of cholesterol in milk fat. The present paper records our findings on the application of this method to determining cholesterol in ghee (milk fat).

Experimental

Materials :

1. *Ghee* was prepared essentially according to the *desi* method described by Srinivasan and Anantakrishnan[11], starting with the separate composite milk of cows and buffaloes maintained at the farm of the Institute. Temperature used to clarify butter was 110°C.

2. *Liebermann–Burchard reagent* was prepared fresh by adding slowly sulphuric and AnalaR (1 ml) to chilled acetic anhydride AnalaR (20 ml) and maintaining the mixture at 0°C for 27 min.

3. *Cholesterol* (E. Merck) was crystallised thrice from 95% ethanol, followed by preparative TLC over silica gel G plates activated for 2 hr at 110°C. The solvent system used was petroleum ether (60/80) : petroleum ether (40/60) : ether : acetic acid :: 25 : 12·5 : 15 : 0·6. The zone bearing the cholesterol band was scraped off, eluted with chloroform AnalaR and cholesterol recovered. Cholesterol, so obtained, was used in this study as pure cholesterol.

4. *Silica gel G* (acc. to Stahl for thin-layer chromatography) and *Digitonin* were of E. Merck quality.

Determination of total cholesterol in ghee by the direct colorimetric method (method I in Table I) : To ghee (0·2–0·25 g), weighed accurately, dissolved in chloroform 'AnalaR' (3 ml), Liebermann-Burchard reagent (4 ml) was added and the mixture allowed to stand at 25°C for 12 min. The optical density readings were then recorded at 650 nm on the Lumetron colorimeter, taking care that the measurements were completed within 15 min of the addition of the Liebermann-Burchard reagent. A blank was run simultaneously.

The standard curve (a straight line passing through the origin) was prepared by taking known amounts of pure cholesterol, developing the colour with the Liebermann-Burchard reagent as usual and recording the optical density readings against the corresponding amounts of cholesterol.

Estimation of 'total' cholesterol in ghee by gravimetric method (Method II in Table 1) : Ghee was analysed for the total cholesterol content by the Den Herder's method reported by Copius Peerboom[3].

Results and Discussion

Ten samples each of cow and buffalo ghee were analysed for their total cholesterol content by the direct colorimetric method (Method I in Table 1). Values obtained by the gravimetric method (Method II in Table 1) have also been included for comparison. Apparently, the values obtained in the direct colorimetric method (Method I) and the gravimetric method (Method II) are quite comparable. Experiments in which known amounts of pure cholesterol were added to ghee samples containing pre-determined cholesterol levels gave quite satisfactory

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recoveries of added cholesterol, thereby establishing the validity of the method reported here. However, the gravimetric method is inconvenient as a routine method for determination of cholesterol in milk fat because (i) it is extremely laborious and time consuming (It requires almost two days for one estimation), (ii) the saponification step uses fairly strong caustic alkalies and (iii) it requires a highly expensive and also a difficultly accessible reagent, digitonin.

TABLE 1. TOTAL CHOLESTEROL LEVELS IN GHEE BY THE TWO METHODS

Type of ghee	Total cholesterol levels % (average values)*	
	Method I[b]	Method II[c]
Cow ghee	0.302	0.306
Buffalo ghee	0.284	0.292

* The values tabulated here are the average values of six ghee samples analysed in quadruplicate.
[b]: Method I is the direct colorimetric method applied to ghee.
[c]: Method II is the gravimetric method applied to ghee after saponification.

The direct colorimetric method reported in this paper is, obviously more convenient. It not only obviates the use of digitonin but it also eliminates the drastic saponification step. Thus, the direct colorimetric method gives not only results comparable to those obtained by the gravimetric method but it is also very rapid and convenient to use.

It was recognised that free and esterified cholesterol give different intensities of colour per unit weight. The final colour in the colorimetric procedure reported here is due to both free and esterified cholesterol present in milk fat, and obviously interpretation of results would be subject to serious error if there is an abnormal distribution of cholesterol between free and ester forms. It was observed by us[12] that cholesterol is present in milk fat mostly in 'free' form (80–85%). It can be seen, on the basis of overall distribution of cholesterol, between pure and ester forms, that the estimation of total cholesterol in ghee by the direct colorimetric method does not lack much in precision. Yet, the rapidity with which estimations by this method can be carried out gives this method a distinct advantage over the gravimetric method.

It is apparent from Table 1 that the cholesterol level in ghee is of the order of 300 mg%. This value is in broad agreement with the cholesterol values of milk fat reported by many workers[2], although Dam[13] had reported values as high as 640 mg% in milk fat.

Twenty-six samples each of cow and buffalo ghee were analyzed for the total cholesterol content by the direct colorimetric method. The data listed in Table 2 were analyzed statistically. It is evident that cow ghee contains higher levels of cholesterol (0·310%) than buffalo ghee (0·27%). The highly significant difference between the two species in

TABLE 2. LEVELS OF TOTAL CHOLESTEROL IN GHEE PREPARED FROM COW AND BUFFALO MILK

Sl. No.	Cow ghee %	Buffalo ghee %
1.	0.302	0.277
2.	0.304	0.265
3.	0.310	0.254
4.	0.326	0.292
5.	0.310	0.253
6.	0.300	0.241
7.	0.336	0.285
8.	0.336	0.272
9.	0.324	0.302
10.	0.321	0.291
11.	0.323	0.295
12.	0.315	0.261
13.	0.327	0.271
14.	0.332	0.264
15.	0.321	0.261
16.	0.320	0.262
17.	0.280	0.264
18.	0.297	0.277
19.	0.296	0.257
20.	0.300	0.268
21.	0.294	0.267
22.	0.289	0.264
23.	0.290	0.268
24.	0.300	0.265
25.	0.291	0.256
26.	0.287	0.252
Average	0.3100	0.2668

regard to the total cholesterol content are evident from Table 3 listing the 'analysis of variance' data. There is little information in literature about the comparative cholesterol levels of cow and buffalo milk fat. Gulvady *et al.*[8] had given extraordinarily low values for the cholesterol content of cow and buffalo milk. More recently, Pantulu *et al.*[14] have reported 396·2 mg% and 334·4mg% respectively as cholesterol values in the milk fats of cow and buffalo. These values appear to be somewhat higher than those being reported by us, but the trend between cow and buffalo milk fats is similar.

TABLE 3. ANALYSIS OF VARIANCE FOR 'TOTAL CHOLESTEROL' LEVEL IN DESI GHEE PREPARED FROM COW AND BUFFALO MILK

Source of variation	D.F.	Mean sum of square
Between species	1	0.024252*
Within species	28	0.008119
Total	29	

* significant at 1% level.

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