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DAIRY PRODUCTS

Comparison of Two Extraction Procedures for the Determination of Cholesterol in Dairy Products

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Both the Roese-Gottlieb and silicic acid methods have been used for the extraction of lipids from dairy products. However, the silicic acid method is widely used for the extraction of fatty acids for determination by gas-liquid chromatography. Since it would be advantageous to use this extract for cholesterol determinations, a comparison of the extraction procedures for dairy products was carried out to determine if they gave equivalent values. Cholesterol values were determined by gas-liquid chromatography of both extracts. Twenty samples of 6 dairy foods having a milkfat content ranging from 3 to 38% were compared. In every instance the Roese-Gottlieb extracts had higher values for cholesterol than did those from the silicic acid method. With very low fat products the Roese-Gottlieb value was high in 6 of 8 comparisons, but the range among samples was much greater. The Roese-Gottlieb or similar multiple solvent extract methods should be used for all dairy products and any other food product in which part of the cholesterol may be bound to a lipoprotein or otherwise not free in the fat.

The increasing concern about the cholesterol content of diets of selected individuals and population groups has intensified the need for reliable data on the cholesterol content of different types of dairy products. Except for fluid whole milk and butter oil, few dairy products have been analyzed (1, 2), and even these have shown variations in content of cholesterol and in the relationships between the cholesterol and lipid content. Procedures used for obtaining the lipid extracts as well as the methods for determining cholesterol may have accounted for much of this variation.

Studies by Homer and Virtanen (3) have shown that the cholesterol content of milkfat fluctuated with the stage of lactation and was subject to seasonal variation caused by changes in the diet. Thompson *et al.* (4) fractionated various classes of fat membrane lipids and found that cholesterol and cholesterol esters formed about 6% of these lipoproteins. Mulder and Zuidhof (5) found that 15% of the total milk cholesterol is not present in the fat phase. Therefore, preparation of fat samples for cholesterol analyses must be able to recover not only the cholesterol in the fat but also the cholesterol bound to the lipoprotein fraction.

This investigation was planned to compare the Roese-Gottlieb and silicic acid column methods for extracting lipids. Subsequent cholesterol determinations on the lipid extracts were made by using a gas-liquid chromatographic method.

Experimental

Apparatus and Reagents

(a) *Diatomaceous earth.*—Celite 545, or equivalent

(b) *Digitonin.*—Fisher Scientific Co., Fairlawn, N.J.

(c) *Cholesterol.* — Primary standard, purified through the dibromide (Eastman Organic Chemicals, Rochester, N.Y.).

(d) *Silicic acid.*—100 mesh (Mallinckrodt Chemical Works, St. Louis, Mo.).

Selection and Preparation of Samples

The dairy products selected for analysis consisted of 28 market samples purchased in the Washington, D.C. area. The samples included 4 brands each of whole fluid milk, nonfat dry milk, whipping cream, and uncreamed cottage cheese, and 3 brands each of Cheddar cheese, Swiss cheese, cream cheese, and low moisture, part skim mozzarella cheese.

The fluid whole milk, whipping cream, and nonfat dry milk samples were mixed by stirring. The hard cheese samples, namely, Cheddar, Swiss, and low moisture, part skim mozzarella, were finely shredded using a hand grater, while the soft cheeses, cream and uncreamed cottage cheese, were mixed in a blender (6). Each of the 28 samples was divided into 2 lots, transferred into polyethylene screw-cap bottles, and retained for the preparation of the lipid extracts by the 2 methods. Milk and cream samples

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in their fresh fluid forms were used directly for preparation of the lipid extracts. The nonfat dry milk and cheese samples were frozen and held below freezing for a few days until the lipid extracts from these products could be prepared.

Determination of Fat Content

The fat from the samples was extracted by the Roese-Gottlieb method, **16.052** (6), and by the silicic acid column method as it is used for obtaining fat to be analyzed for fatty acid composition (7). For the latter method, the sample was transferred to a mortar and mixed thoroughly with 20% (v/v) H_2SO_4. Dry silicic acid was added and mixed in, using a ratio of 5 g silicic acid to 3 ml or 3 g dairy product. Sample-silicic acid mixture was added to a 600 × 34 mm id column containing 5 g silicic acid, and $CHCl_3$-methanol (9 + 1) was used for elution at ca 50 ml/min. The eluate was dried with anhydrous Na_2SO_4 and the extract was concentrated in a flash evaporator (8).

Gas-Liquid Chromatography

A Barber-Colman Model 5000 gas chromatograph equipped with hydrogen flame ionization detector was used. GLC parameters: 6′ × 4 mm id stainless steel coiled column containing 3% JXR silicone on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories, Inc., State College, Pa.). Operating conditions: column 275°C; injection port and flame ionization detector 290°C; nitrogen carrier gas flow 90 ml/min.

The cholesterol analyses were performed on both fat extracts, using the digitonin-Celite method of LaCroix, **28.075–28.080** (6, 9). Daily standard curves were obtained over 2–12 μg, using a stock solution of 2 μg cholesterol/μl $CHCl_3$, with 8 μg cholesterol giving 50% full-scale deflection. The area under the GLC peak was calculated by triangulation. The area of cholesterol was plotted against μg cholesterol. A straight line relationship between peak area and mass was obtained under these conditions (2–8 μl extract injected). Chromatograms of samples obtained by both extraction methods gave single cholesterol peaks.

Results and Discussion

Table 1 presents data on the cholesterol content of several dairy products as determined on extracts obtained by using the Roese-Gottlieb and the silicic acid column methods. Values for mg cholesterol/g fat for dairy products extracted by the silicic acid method were considerably lower than values for extracts from the Roese-Gottlieb method. With whole milk and relatively high fat products, the range of cholesterol values with different samples of the same products varied less than 25% within each method. With low fat products a wider range of values was exhibited among the samples in both methods; thus, the comparisons shown may not be valid with low fat products (Table 1). Sample standard deviation in Table 1 is the variance of the cholesterol values obtained from market samples of dairy products and does not reflect method variance.

Since cholesterol is found in several forms, analyses for cholesterol content would be a function of the method of extraction from the dairy product. The Roese-Gottlieb method uses a multiple extraction procedure involving ether and alcohol which is necessary for removal of membrane material (3, 10). Therefore, both the fat globule- and protein-bound cholesterol should be recovered. The lower values obtained with the silicic acid procedure suggest that it is unable to completely remove the various bound forms of cholesterol from the whole food. Thus, the Roese-Gottlieb extraction or similar multiple solvent extraction procedure should be used for all dairy products and on any other food product in which part of the cholesterol may be bound to a lipoprotein or otherwise not free in the fat.

Recommendation

The Associate Referee recommends that further study be made on the effect of extraction methods on cholesterol values in dairy foods.

Acknowledgment

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Trade names are mentioned for identification only; no endorsement is implied.

The recommendation of the Associate Referee was approved by the General Referee and by Subcommittee C and was accepted by the Association; *see* (1972) *JAOAC* **55**, 349.

Table 1. Comparison of Roese-Gottlieb and silicic acid methods for the extraction of cholesterol from milk and milk products

Product	Fat, g/100 g	Cholesterol, mg/g Fat			
		Roese-Gottlieb		Silicic Acid	
		Mean[a]	Range	Mean	Range
Milk, whole fluid[b]	3.48	3.96 ±0.37	3.68– 4.49	2.99 ±0.19	2.75– 3.[illegible]
Cream, whipping[b]	37.85	3.63 ±0.14	3.45– 3.78	2.46 ±0.23	2.23– 2.[illegible]
Cheddar cheese, milk[c]	32.54	3.01 ±0.07	2.94– 3.08	2.61 ±0.19	2.63– 2.[illegible]
Swiss cheese[c]	24.12	3.66 ±0.19	3.52– 3.89	3.07 ±0.14	2.94– 3.[illegible]
Cream cheese[c]	35.52	3.16 ±0.20	2.94– 3.33	2.58 ±0.13	2.43– 2.[illegible]
Mozzarella cheese, low moisture, part skim[c]	16.98	3.91 ±0.14	3.82– 4.07	2.86 ±0.12	2.79– 2.[illegible]
Milk, nonfat dry[b]	0.664	29.88 ±6.60	22.40–37.20	29.00 ±4.71	24.40–35.[illegible]
Cottage cheese, uncreamed[b]	0.511	15.60 ±6.23	11.76–24.90	14.60 ±5.91	10.70–23.[illegible]

[a] Standard deviation is sample variance, not method variance.
[b] Based on 4 market samples examined in duplicate.
[c] Based on 3 market samples examined in duplicate.

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