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Selected ion monitoring of tert-butyldimethylsilyl cholesterol ethers for determination of total cholesterol content in foods

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A gas chromatography/mass spectrometry (GC/MS) method, using tert-butyldimethylsilyl (tBDMS) derivatives of sterols for detection in the selected ion monitoring (SIM) mode has been applied to the determination of total cholesterol in food (eggs, dairy products). Generally, comparison of results with literature data shows agreement with the amount of cholesterol determined by other chromatographic techniques, and slight underestimation if compared to the amount of cholesterol estimated by colorimetric techniques. The saponification and extraction procedure allowed for 98·6% recovery of spiked cholesterol in milk samples with a coefficient of variation of 2·1%. Amounts as low as 5 ng per 100 g food can be detected using external standards with 95% accuracy.

INTRODUCTION

A great number of methods, either gravimetric, colorimetric or chromatographic for the determination of cholesterol in foods have been developed over the years. This topic has been reviewed by Feely *et al.* (1972) on data prior to 1972 and Sweeney & Weiraugh (1976) for the period 1972–1976. The accumulated data, acquired mostly through colorimetric methods, contributed to the latest update of the US Department of Agriculture handbook no. 8–1 (Posati & Orr, 1976), and is still referred to by nutritionists and dieticians. Although colorimetric methods (Rudel & Morris, 1973; Pantulu *et al.*, 1975; Bachman *et al.*, 1976; Grossman *et al.*, 1976) are well suited for routine and automated analysis, there is a tendency to overestimate the cholesterol content of foods, primarily through other interfering chromogens present in the samples. More accurate determination of cholesterol in foods has been achieved by chromatographic techniques such as HPLC (Beyer & Jensen, 1989; Goh *et al.*, 1989) and GLC (Sheppard *et al.*, 1977; Kovacs *et al.*, 1979; Ryan & Gray, 1984) the latter being officialized by the AOAC (Punwar & Derse, 1978).

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Gas chromatography–mass spectrometry (GC/MS) is a powerful tool that can be used for qualitative and quantitative analysis of biological material. Enhancement of sensitivity and specificity of the ionization process can be attained through proper derivatization (Anderegg, 1988). The tBDMS ethers of sterols have been known to give intense $[M\text{-}C_4H_9]^+$ diagnostic ions that are suitable for selected ion monitoring (SIM) in quantitative analysis (Millington, 1975; Halket & Lisboa, 1980).

In this work, we investigated the application of a method for the determination of total cholesterol in foods by selected ion monitoring of tBDMS derivatives, the results being compared with literature data collected through colorimetric and chromatographic techniques.

MATERIALS AND METHODS

Reagents and chemicals

MTBSTFA from Regis Chemical Co.
Cholesterol 95% from Sigma Chemical Co.
Dimethylformamide (DMF), distilled in glass from Anachemia.
Chloroform, distilled in glass from Anachemia.
Hexane USP grade from Fisher.

Sample preparation

Food samples to be analysed, either raw or suspended in a proper volume of water were homogenized in a Waring blender for at least 3 min. A 0·5 g aliquot of the homogenate was then saponified and extracted according to the protocol of Gilliland *et al.* (1985). Briefly, 3 ml of 95% ethanol was added to the sample homogenate, followed by 2 ml of 50% potassium hydroxide. The tubes were vortexed thoroughly after addition of each component, heated for 10 min at 60°C, and cooled. Five ml of hexane were dispensed into each tube and vortexed. Three ml of water were then added and the mixing was repeated. Tubes were allowed to stand at room temperature for 15 min to permit phase separation. An aliquot of 50 μl of the hexane phase was transferred to a 250 μl chromatographic vial inlet and evaporated to dryness under nitrogen flow for the derivatization step.

Derivatization

A single derivatization was performed, using 40 μl of dimethylformamide (DMF) as solvent and 40 μl of MTBSTFA as alkylsilylating agent leading to the corresponding tertbutyldimethylsilyl (tBDMS) derivatives. The reaction was allowed to take place for 2 h at 65°C. The addition of 120 μl of chloroform as dilution solvent completed the process and the samples were then analysed.

Gas chromatography

A Hewlett-Packard 5890A gas chromatograph equipped with a split-splitless injection port was used. The capillary column was a Hewlett-Packard Ultra-1 cross-linked methyl silicone (12 m, 0·2 mm i.d. 0·33 μm film thickness). The carrier gas was helium with an inlet pressure of 83 kPa (12 psi), split-vent; 50 ml min^{-1}, μ: 40 cm s^{-1}. The initial oven temperature of 150°C was increased at a rate of 10°C min^{-1} during 15 min and remained 5 min for a total runtime of 20 min. Injections were made in a splitless mode for 0·2 min, followed by the split mode with a split flow of 50 ml min^{-1} and a septum purge of 2 ml min^{-1}. The injector temperature was maintained at 305°C. The gas chromatograph was also equipped with an automatic liquid sampler (Hewlett Packard 7673A).

Mass spectrometry

A Hewlett-Packard 5970B mass selective detector (MSD) was interfaced with the 5890A gas chromatograph, with the capillary column inserted directly into the ion source (70 eV electron energy). The GC/MS interface was maintained at 275°C. The MSD was automatically calibrated with the Autotune program at the beginning of each day to 502 D using perfluorotributylamine (PFTBA) as the calibration compound. The MSD was used in scan mode for preliminary assessment of ions and in selected ion monitoring (SIM) mode at low mass resolution for quantitation of compounds.

RESULTS

A recovery test was conducted to verify the performance of the extraction procedure and is summarized in Table 1. The extraction of added pure cholesterol was 98·6 ± 2·1% complete, the recovery being the difference between unspiked and cholesterol standard spiked duplicate samples of milk. Under the derivatization conditions, the reaction was complete, and derivatives were very stable to hydrolysis for several weeks as found by Corey & Venkateswarlu (1972). As little as 5 ng/100 g could be detected using the fragment ions $[(CH_3)_2SiOH]^+$ of 75·0 m/z^+ and $[M\text{-}C_4H_9]^+$ of 443·45 m/z^+ in a selected ion monitoring mode at a dwell time of 100 ms per ion and low mass resolution.

The linear dynamic range of cholesterol standard was 10^4 in magnitude ranging from concentrations of 20 pg/μl to 500 ng/μl. Relative retention time of tBDMS-cholesterol was 15·27 min and the external standard curve had a $R^2 = 0{\cdot}99$.

The total cholesterol content of a variety of dairy products and eggs was determined by GC/MS and summarized in Table 2. The values obtained are in concordance with the data from the AOAC obtained using a chromatographic method (GLC). Upon comparison with USDA data, there is an overestimation of the cholesterol content for samples that were analysed by colorimetric methods as expected.

DISCUSSION

Among the sample preparation steps, it is important to homogenize thoroughly the material to be analysed and to be careful in the manipulations in order to reduce variability between samples. Elimination of such errors can easily be effected through the use of internal stan-

Table 1. Recovery of cholesterol from whole milk

Amount added (mg)	Amount recovered[a] (mg)	Percentage recovery[b]
0	0·072	N/A
1·0	1·033	96·4
2·0	2·084	100·6
5·0	5·011	98·8

[a] $n = 2$.
[b] $X = 98{\cdot}6\%$.
$s = 2{\cdot}11\%$.

Table 2. Cholesterol content of selected foods obtained by GC/MS compared with colorimetric methods data reported by USDA (1976), and chromatographic (GLC) method data reported by AOAC (1978)

Food	Milk fat[a] (%)	Cholesterol content (mg/100 g)		
		GC/MS (SIM)	USDA[b] 1976	AOAC[c] 1978
Milk				
Skimmed	0·2	2	2	N/A
1%	1·0	4·8	4	N/A
2%	2·0	8·4	8	N/A
3·25%	3·25	14·4	14	10·4
Cheese				
Cheddar, mild	31	75·5	105	85·4
Cheddar, strong	31	77·8	105	85·4
Cheddar, lowfat	4	23	N/A	N/A
Cream, regular	31	50·2	N/A	N/A
Cream, lowfat	23	30·5	N/A	N/A
Yogurt				
Regular	3·8	10·5	13	N/A
Lowfat	0·1	4·9	N/A	N/A
Butter				
Regular	81·11[b]	180	219	190
Cultured	N/A	160	N/A	N/A
Eggs				
Whole		480	548	394
Whole 'healthy'[d]		388	N/A	N/A
Yolk		1358	N/A	N/A
Yolk 'healthy'		1120	N/A	N/A

[a] Milk fat percentage as reported on packaging label.
[b] From Posati & Orr (1976).
[c] From Punwar & Derse (1978).
[d] It is claimed that the cholesterol content of so-called 'healthy' eggs is 175 mg/egg, which translates to approximately 300 mg/100 g.
N/A: data not available.

dards structurally analogous to cholesterol, or better yet through the use of isotopic cholesterol standard.

The cholesterol content of dairy products seems fairly well correlated to the amount of milk fat present in the products, as was found in the literature (LaCroix *et al.*, 1973). The fact that the mild (less matured) and strong (longer matured) cheddar show the same quantity of cholesterol indicates that the bacteria involved in the cheese maturation process do not transform cholesterol into any other metabolite. In fact, another study has shown that even during the earlier stages of maturation (starting from fresh curd) there is no transformation of cholesterol. As for the eggs, the much higher content of cholesterol than advertised (+30%) in the so-called healthy eggs should trigger closer attention from regulatory agencies.

The general outcome of this work has been to verify the usefulness of GC/MS for quantitation of total cholesterol in foods. The method described here is highly sensitive and specific, although not requiring a sophisticated mass spectrometer. It is also possible to automate the analysis all the way from injection to production of a written report without any operator assistance through the use of macrocommands of the operating system software. It also outlines the need for a revision of the current tables on the composition of cholesterol in foods to reflect the technological improvements in analytical food chemistry during the last 15 years.

REFERENCES

Anderegg, R. J. (1988). Derivatization in mass spectrometry: Strategies for controlling fragmentation. *Mass Spectrom. Rev.*, **7**, 395–424.

Bachman, K. C., Lin, J. & Wilcox, C. J. (1976). Sensitive colorimetric determination of cholesterol in dairy products. *J. Ass. Off. Anal. Chem.*, **59**(5), 1146–9.

Beyer, R. S. & Jensen, L. S. (1989). Overestimation of the cholesterol content of eggs. *J. Agric. Food Chem.*, **37**, 917–20.

Corey, E. J. & Venkateswarlu, A. (1972). Protection of hydroxyl groups as tert-butyldimethylsilyl derivatives. *J. Am. Chem. Soc.*, **94**, 6190–1.

Feeley, R. M., Criner, P. E. & Watt, B. K. (1972). Cholesterol content of foods. *J. Am. Diet. Assoc.*, **61**, 134–48.

Gilliland, S. E., Nelson, C. R. & Maxwell, C. (1985). Assimilation of cholesterol by *Lactobacillus acidophilus*. *Appl. Environm. Microbiol.*, **49**(2), 377–81.

Goh, E. H., Colles, S. M. & Otte, K. D. (1989). HPLC analysis of desmosterol, 7-dehydrocholesterol, and cholesterol. *Lipids*, **24**(7), 652–5.

Grossmann, A., Timmen, H. & Klostermeyer, H. (1976). Enzymatic estimation of cholesterol in milk fat—an alternative to the methods in current use. *Milchwissenschaft*, **31**(12), 721–4.

Halket, J. M. & Lisboa, B. P. (1980). Simple mass chromatographic procedure for the detection and identification of sterols using derivative correlations. *J. Chromat.*, **189**, 267–71.

Kovacs, M. I. P., Anderson, W. E. & Ackman, R. G. (1979). A simple method for the determination of cholesterol and some plant sterols in fishery-based food products. *J. Food Sci.* **44**, 1299–301.

LaCroix, D. E., Mattingly, W. A., Wong, N. P. & Alford, J. A. (1973). Cholesterol, fat and protein in dairy products. *J. Am. Diet. Assoc.*, **62**, 275–93.

Millington, D. S. (1975). Determination of hormonal steroid concentrations on biological extracts by high resolution mass fragmentography. *J. Steroid Biochem.*, **6**, 239–45.

Pantulu, P. C., Bhimasena, M., Sethu Rao, D. & Anantakrishnan, C. P. (1975). Application of Liebermann-Burchard reaction to the unsaponifiable portion of milk lipids as a criterion for the quantitative determination of cholesterol in milk and milk products. *Milchwissenschaft*, **30**(12), 735–8.

Posati, L. P. & Orr, M. L. (1976). Composition of foods, dairy and egg products, raw-processed-prepared. *USDA Agric. Hand.*, **8**(1), 1–167.

Punwar, J. K. & Derse, P. H. (1978). Application of the official AOAV cholesterol method to a wide variety of food products. *J. Assoc. Off. Anal. Chem.*, **61**(3), 727–30.

Rudel, L. L. & Morris, M. D. (1973). Determination of

cholesterol using *o*-phthalaldehyde. *J. Lipid Res.*, **14**, 364–6.
Ryan, T. C. & Gray, J. I. (1984) Distribution of cholesterol in fractionated beef tallow. *J. Food Sci.*, **49**, 1390–3.
Sheppard, A. J., Newkirk, D. R., Hubbard, W. D. & Osgood, T. (1977). Gas-liquid chromatographic determination of cholesterol and other sterols in foods. *J. Ass. Off. Anal. Chem.*, **60**(6), 1302–6.
Sweeney, J. & Weiraugh, J. (1976). Summary of available data of cholesterol in foods and methods for its determination. *CRC Crit. Rev. Food Sci. Nutr.*, **8**(2), 131–59.