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Determination of Cholesterol in Pasta Products Using Gas–Liquid Chromatography

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A simple gas–liquid chromatographic method has been developed for the measurement of cholesterol and some plant sterols, such as campesterol, β-sitosterol and avenasterol, in pasta products. Samples were saponified, without prior extraction of lipids, and the concentrated unsaponifiables analysed directly by gas liquid chromatography. The results were evaluated statistically, and they showed that recoveries were superior to those with standard methods. The analysis is simple, accurate, and will probably be suitable for the monitoring and nutritional evaluation of pasta and other food products.

Introduction

Owing to concern over the role of dietary cholesterol in cardiovascular diseases, modification of diet will undoubtedly be widely used to reduce the incidence of heart attack[1,2].

Pasta, made from wheat semolina, is often fortified with a number of ingredients. Usually these are added to the dough during processing to improve the pasta's nutritional value and physical and organoleptic properties[3]. The most common and important ingredient is egg, either as whole egg or egg yolk[3]. While wheat contains no, or only traces, of cholesterol, egg has a high cholesterol content[4] which is of concern to consumers interested in maintaining a low cholesterol intake. In the United States, noodles must contain eggs in compliance with Standards of Identity of the Food and Drug Administration[4]. Food Standard Regulations set a minimum of 5·5% by weight of egg solids or egg yolk in the total solids of noodle products. A cholesterol content as high as 198 mg/100 g has been reported for noodles containing 5·56% yolk solids[5].

There are numerous published methods for determining cholesterol[6]. Different methods of analysis may give different results, however. The differences in results among methods probably arise from the possible structural involvement of cholesterol in the plant cell[7]. Cholesterol forms complexes with other molecules, mainly with phospholipids and proteins[15], thus changing their physical and chemical properties, and resulting in its inefficient extraction and poor recovery, the most critical step in the analyses[13]. Procedures in most cases call first for complete extraction of total lipids and unsaponifiables from total lipids: there is little uniformity in procedure. The currently accepted standard methods[11] differ even in extraction procedures, one of the most

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critical steps in the analysis[13], and one that is responsible for erratic results. In one of the official methods, cholesterol is extracted by precipitation as dibromide, by the addition of bromine in glacial acetic acid. Coprecipitation of cholesterol and unsaturated phytosterol dibromides, occurs if conditions are not closely controlled however. Digitonin, another common precipitant[5], varies in purity, and forms similar compounds with practically all naturally occurring sterols[12].

Among the different methods for determining cholesterol, the two most recent are high performance liquid chromatography (HPLC) and gas–liquid chromatography (GLC)[8–10]. Most of these methods, are time consuming, however.

The purpose of this investigation was to develop a simple and reliable method for the analysis of cholesterol and other plant sterols in pasta products using GLC.

Experimental

General procedure

Materials. Egg noodles were obtained from commercial sources or prepared in the laboratory from durum wheat semolina and fresh egg yolk, as follows: Semolina (50 g) was mixed with deionized water and egg yolk (33% absorption) in a Farinograph mixing bowl until a homogeneous dough was formed (5–7 min). The dough was then immediately sheeted between two rollers seven times, while gradually reducing the gap from 2 mm to 0·7 mm. The sheets (30–50 cm^2) were dried at room temperature. Sterol standards were purchased from Sigma Chemical Co.

Sample preparation. Samples (100 g) were prepared for analysis by grinding egg noodles in a Udy Cyclon grinder and passing through a 40-mesh sieve before weighing.

Gas–liquid chromatography. Gas–liquid chromatography was carried out with a Perkin–Elmer 3920B gas chromatograph equipped with a flame ionization detector. A glass column (2 m × 2 mm ID), packed with 3% (w/w) OV-17 on 80–100 mesh Gas-Chrom Q, was used isothermally at 240 °C. The carrier gas was helium at a flow rate of 40 ml/min. Injector and detector temperatures were 225 and 245 °C respectively. For quantification, 5α-cholestane was used as internal standard, and sterols were identified by cochromatography using authentic standards.

Analyses

Method A. Samples were analysed according to the method of AOAC, 1984*a*[11].

Method B. Samples were analysed according to the method of AOAC, 1984*b*[11].

Method C. About 0·5 g (±0·01 g) of well mixed sample was saponified in tightly capped (teflon lined) 15 ml centrifuge tubes containing 50% (w/v) KOH (1 ml), 95% (v/v) ethanol (4 ml) and cholestane (100 μg). The tubes were flushed with nitrogen, capped tightly and placed in a boiling water bath for 1 h or as otherwise stated. The tubes were shaken occasionally to wet the inside wall and wash the adherent particles down. After the saponification the tubes were cooled and water (2 ml) and *n*-hexane (3 ml) were added. The tubes were shaken vigorously and a portion (2 ml) of the upper hexane layer was concentrated under a stream of nitrogen, and analyzed for free sterols using GLC.

Recovery study. The relative effectiveness of the methods was evaluated by determining the

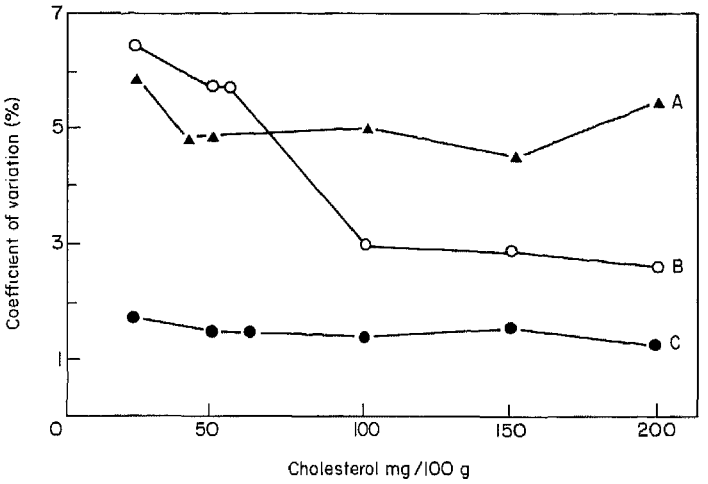

FIGURE 1. Effect of cholesterol concentration on the coefficient of variation of determination for methods A, B and C. For each point $n = 12$. The concentration of cholesterol in the samples was adjusted by diluting the cholesterol by the addition of pasta prepared without egg.

TABLE I. Reproducibility of cholesterol and phytosterol determinations in replicate samples of egg noodles as determined by three different methods (A, B, C) (mg/100 g samples)

	A	B	C				
			Fractions[a]				
Sample	I	I	I	II	III	IV	V
1	44·1	52·1	60·9	3·8	24·6	1·1	29·8
2	40·8	57·9	61·0	3·1	24·4	1·0	28·6
3	45·7	52·0	60·6	3·8	25·4	0·9	30·2
4	39·9	59·6	61·9	3·2	24·8	1·3	30·0
5	42·8	54·0	63·3	3·2	24·9	1·3	29·4
6	45·0	58·8	60·7	3·5	23·8	0·8	28·3
7	40·1	50·4	59·9	3·1	23·0	1·2	27·8
8	40·8	56·5	60·4	3·3	24·1	1·1	28·5
9	45·1	53·2	61·2	3·8	25·4	1·1	30·3
10	41·7	55·3	60·8	3·3	24·8	1·3	29·7
11	44·3	58·2	61·2	3·7	25·1	1·0	29·8
12	44·0	51·7	62·0	3·9	26·7	0·8	31·6
$\bar{X}$	42·8	55·0	61·2	3·5	24·7	1·1	29·5
SD	2·1	3·2	0·9	0·3	0·9	0·2	1·0
CV %	4·9	5·8	1·5	3·2	3·7	16·5	3·6

[a] I, cholesterol; II, campesterol; III, β-sitosterol; IV, avenasterol; V, total phytosterols (II–IV).

TABLE II. Recoveries of cholesterol from egg noodles[a]

Method	Sample[b]	Sample weight (g)	Cholesterol added (mg)	Cholesterol recovered	
				mg	%
A	S	0·5	—	0·43	—
	S+Ch	0·5	0·60	0·94	91
	S+ChP	0·5	0·62	0·98	93
B	S	0·5	—	0·55	—
	S+Ch	0·5	0·60	1·06	92
	S+ChP	0·5	0·62	1·04	88
C	S	0·5	—	0·61	—
	S+Ch	0·5	0·60	1·19	98
	S+ChP	0·5	0·62	1·27	103

[a] Values are the means of duplicate analyses. Cholesterol was added as free cholesterol or as cholesteryl palmitate ester.
[b] S = pasta sample; Ch = cholesterol; ChP = cholesteryl palmitate.

recoveries of added cholesterol and its palmitate ester. The compounds were dissolved in *n*-hexane added to the milled dry noodles, and the solvent was allowed to evaporate. Cholesterol was then determined in the samples by each of the three methods.

Effect of saponification. Samples were saponified as described for method C using time intervals as reported in Fig. 3.

Results and Discussion

The sterol contents of egg noodles, as determined by the three different methods, are presented in Table I. Methods A and B determine only cholesterol, whereas Method C determines cholesterol and the main phytosterols, campesterol, β-sitosterol and avenasterol. Method C gave higher values for cholesterol content than the official methods (a mean recovery of 61·2 mg per 100 g samples compared with 42·8 and 55·0 mg for Methods A and B, respectively). The standard deviation and the coefficient of variation percentage were significantly higher for the official methods. Method A, using digitonin to precipitate sterols, assumes 52 mg of plant sterols in 100 g of durum semolina or flour to derive the general formula used in the calculation of cholesterol content in egg noodles[11]. Method B, using bromide to precipitate sterols, assumes only 24 mg of plant sterols in the calculation, less than half that of Method A. Consequently, the concentration of cholesterol was underestimated using Method A compared with the results obtained by Method B.

When GLC is used for cholesterol determination, the estimation of total plant sterols is not required because of its specificity. Knowing the level of plant sterols, can be of interest also in certain diets, however[14].

Table II shows the recovery of cholesterol from pasta. It is evident that direct saponification of the samples and gas–liquid chromatographic analysis gives better recoveries than conventional methods for both, free and esterified cholesterol.

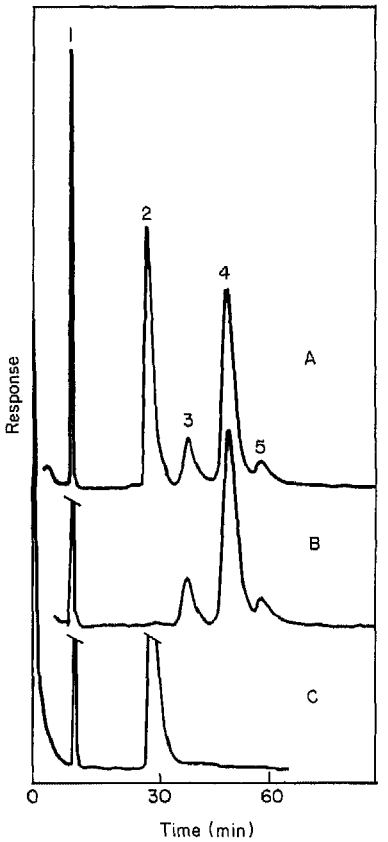

FIGURE 2. Gas–liquid chromatogram of sterols in egg noodles (A), noodles without egg (B) and egg yolk (C). Cholestane (1), cholesterol (2), campesterol (3), β-sitosterol (4), avenasterol (5).

The coefficient of variation was used to compare the precision of the methods at different concentrations of cholesterol (Fig. 1). Method C, using direct saponification and GLC consistently gave a lower coefficient of variation than the official methods within the range of cholesterol concentration shown (25–200 mg per 100 g of egg noodles).

Figure 2 shows a typical GLC chromatogram of the sterols in egg noodles, noodles without egg, and egg yolk using the direct saponification method. It can be seen that there was no interfering material for the estimation of concentrations of individual sterols. Moreover, when recovery of added egg cholesterol was tested, there was a linear relationship between the amount of egg added and the cholesterol recovery, as shown in Fig. 3.

It has been reported that cholesterol is readily oxidized during saponification by hot alkali[16]. From Fig. 4 it is apparent that, even after saponification for 4 h, there was no significant loss of cholesterol. A significant loss was evident after 16 h, however.

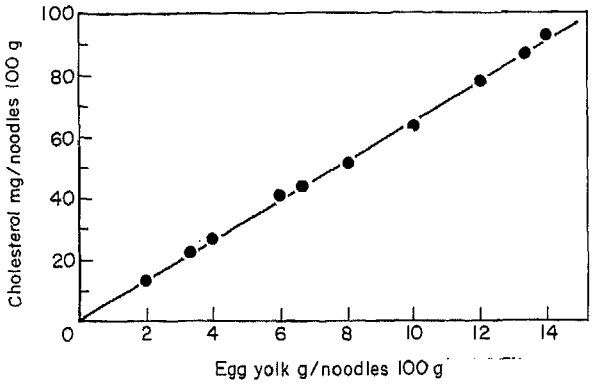

FIGURE 3. Recovery of cholesterol from egg noodles containing different amounts of egg yolk. Samples were prepared using fresh egg yolk and each point represents a single analysis.

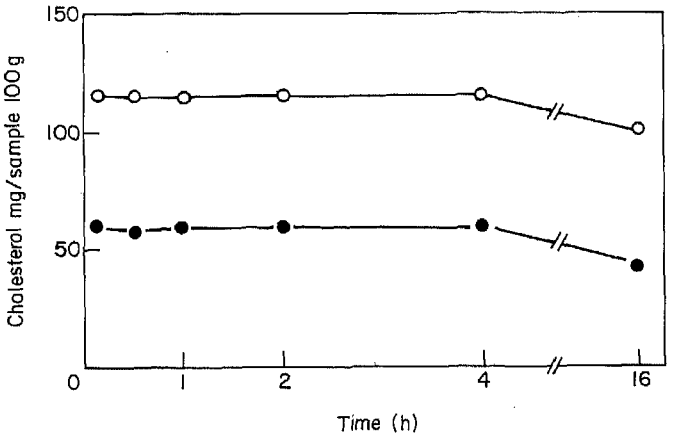

FIGURE 4. Effect of saponification time on the determination of cholesterol in egg noodles (●) and egg noodles with 60 mg cholesterol per 100 g sample (○) added as cholesteryl palmitate.

The official methods advocated by the AOAC require relatively large volumes (several hundred ml) of organic solvents per sample, while this direct saponification method, using GLC, requires only 20–25 ml. Preparation of samples for GLC determination is simple, with saponification and extraction of sterols being performed in the same small centrifuge tube without the need for extraction of total lipids. The proposed method thus requires less labor, is more rapid, and is more accurate than the current official methods.

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