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HPLC Determination of the Cholesterol Content of Egg Noodles as an Indicator of Egg Solids

W. Jeffrey Hurst,* M. D. Aleo, and Robert A. Martin, Jr.

An HPLC method is presented which allows for the determination of cholesterol content which serves as an indicator of egg solids. The method involves the extraction of the noodles with methanolic KOH and the removal of interfering substance by the use of a commercially available Sep-pak. The final HPLC determination uses a reversed-phase column with a mobile phase of hexane/isopropyl alcohol and detection at 205 nm. The method is accurate and precise and is suitable for use in both product monitoring and nutritional labeling schemes.

INTRODUCTION

Presently, the method for the determination of egg solids in noodles is based on the analysis of lipid phosphorus (AOAC, 1980) with a calculation to arrive at egg solids. The method employed is cumbersome, time-consuming, and sometimes yields sporadic results. Additionally, the amount of lipid extracted varies with the organic solvent used (Hubbard et al., 1976). On the other hand, cholesterol measurement itself is straightforward. When the cholesterol content of the egg used to make the noodles is known, it is simple to measure the cholesterol in noodles and calculate the amount of egg based on the fact. The information about egg content in noodles is important from both a production and regulatory viewpoint.

Cholesterol analysis can be accomplished by a variety of methods including GLC and enzymatic techniques (Shen et al., 1982; Newkirk and Sheppard, 1981; Henry et al., 1971). HPLC has been used (Henry et al., 1971; Heffmann and Hunter, 1979; Kiuchi et al., 1975; Orgen et al., 1980) in the analysis of cholesterol in foods with the use of benzoate derivatives (Newkirk and Sheppard, 1981) while in the biological area the analysis was accomplished with direct detection at 205 nm (Duncan et al., 1979). The use of enzymes is possible but the enzyme used attacks the common sterol site (Roschlau et al., 1981) causing all sterols to mimic cholesterol. Since plants contain other sterols such as stigmasterol and sitosterol a direct analysis of cholesterol is not possible by enzymatic means.

In this paper, a method is described for the analysis of cholesterol in egg noodles by using a nonaqueous reversed-phase (NARP) HPLC technique with detection at 205 nm.

MATERIALS AND METHODS

HPLC. The HPLC apparatus consisted of an M6000A solvent delivery system (Waters Assoc.), an Alltech C_{18} column (4.0 mm i.d. × 25 cm) (Alltech Assoc.), a variable wavelength detector, a data unit, and miscellaneous ancillary equipment. The detector used was a Model 100-40 variable wavelength at 205 nm (Altex Inst. Co.). Data acquisition was performed with a Shimadzu ITG-4A Data Unit. The HPLC mobile phase consisted of hexane: IPA (99.9/0.1) (v/v) at a flow rate of 2.0 mL/min. All solvents were LC grade. The mobile phase must be thoroughly degassed prior to use or an unstable base line results.

Samples and Standards. Samples of egg noodles and dried whole egg solids (DWES) were obtained from commercial sources. The cholesterol standard was obtained from Pfansteihl Laboratories, sitosterol from Aldrich Chemical Co., and stigmasterol from Sigma Chemical Co. The [^{14}C] cholesterol used in recovery studies was obtained from New England Nuclear (0.02 μCi). The scintillation cocktail reagent was J. T. Baker β-count.

Liquid Scintillation Counting. Two-milliliter portions of the impinging, wash, and elution solutions were collected and placed into 4-mL capacity scintillation vials containing 2 mL of scintillation cocktail. The combined vials were then counted for 2 min each with a Tracor Model 6811 liquid scintillation system. Recovery data were then calculated based on total counts obtained from each vial.

Preparation of Samples. Samples are prepared for analysis by grinding in a Mouli grater or equivalent to insure sample homogeneity. Samples are then passed through a 25 mesh sieve prior to initial weighing. For egg noodles accurately weigh 1.0 g of prepared sample while for dried whole egg solids weigh 100 mg to ± 0.1 mg.

The sample is weighed into a 250-mL round-bottom flask. Samples are refluxed for 30 min with 50 mL of 2.0 N methanolic KOH. The resulting suspension is transferred while hot into a 250-mL separatory funnel. The round-bottom flask was washed with two 25-mL portions of distilled water, which are then added to the separatory funnel; the solution is allowed to cool to room temperature. Ten milliliters of 10% (w/v) NaCl is added and the resulting solution is extracted with two 100-mL portions of

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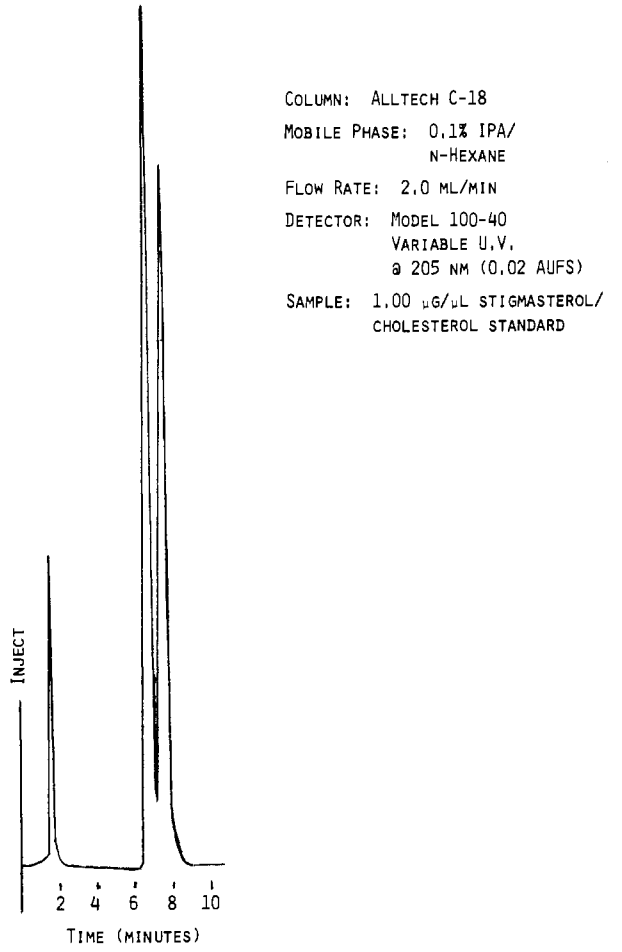

Figure 1. Chromatogram of stigmasterol/cholesterol standard.

Table I. Precision Study for Samples and Standard

sample	n	concn	%Cv
cholesterol standard	12	20 µg/inj	2.0
egg noodle	5	0.74 mg/g	3.5
dried whole egg solids	5	15.7 mg/g	1.1

1/1 (v/v) diethyl ether–petroleum ether. (For egg noodles, use three 100-mL washes.) The ether fractions are pooled and solvent removed by rotary evaporation at 30 °C to dryness. Samples are then diluted to 50 mL with petroleum ether. Ten milliliters are withdrawn and run through a silica Sep-pak and the eluent discarded. The Sep-pak is washed with 10 mL of 7% diethyl ether in petroleum ether and the cholesterol fraction is eluted with 10 mL of 75% diethyl ether–25% petroleum ether (v/v). The eluent is evaporated to dryness and brought up to volume (~5 mL) in mobile phase for analysis.

Analysis. Samples and standards were injected onto the column in duplicates. Injection volumes used were between 20 and 50 µL. Calculation of results were obtained by comparing peak areas obtained by injection of samples and standard. Figure 1 and 3 show sample chromatograms of cholesterol standard, egg noodle, and dried whole egg solids. Figure 1 shows the fine separation achieved between cholesterol and another plant sterol, stigmasterol, while Figure 2 shows a chromatogram of an extract of dried whole egg solids. Figure 3 shows a chromatogram of an egg noodle extract.

RESULTS

The previously described method was evaluated for accuracy and precision. Additional recovery was checked through the use of ^{14}C labeled cholesterol. Table I summarizes precision data for standards and samples.

When the HPLC conditions described were used, linearity was checked over a range from 0.052 µg/injection to 20.72 µg/injection by using varying concentrations and injection sizes of cholesterol; the correlation was $R = 0.99$. The method exhibited a lower limit of less than 1% calculated egg solids with the conditions outlined in this paper. Recovery studies for egg noodles and dried whole egg solids are listed in Tables II and III.

Table II. Recovery of Cholesterol from Egg Noodles[a]

mg added	mg recovered	% recovery
no add	0.74	
1.0	1.70	95.5
3.0	3.94	106.5
5.0	5.85	102.1
		x = 101.4

[a] n = 2. Sample size 1.00 g.

Table III. Recovery Study of Cholesterol from Dried Whole Egg Solids (DWES)[a]

mg added	mg recovered	% recovery
no add	1.57	
1.00	2.44	87.0
3.00	4.26	89.8
5.00	6.07	90.0
		x = 88.9

[a] n = 2. Sample size 100.0 mg.

Table IV. Cholesterol Analysis of Egg Noodles[a]

sample	n	concn, mg/g
1	3	0.82
2	4	0.77
3	4	0.72
4	2	0.70
5	2	0.70
6	2	0.80
7	4	0.79
		x = 0.77
		%Cv = 6.15%

[a] Sample size 1.0 g.

Table V. Cholesterol Analysis of Liquid Egg Yolks[a]

sample	n	concn, mg/g
1	2	12.90
2	2	14.75
3	2	15.76
4	2	14.34
5	2	14.94
6	2	11.79
7	2	12.92
		x = 13.69
		%Cv = 9.45%

[a] Sample size 1.0 g.

Table VI. Calculated Egg Solids in Noodles

sample	% egg solids
1	5.99
2	5.62
3	5.26
4	5.11
5	5.84
6	5.77
	x = 5.59

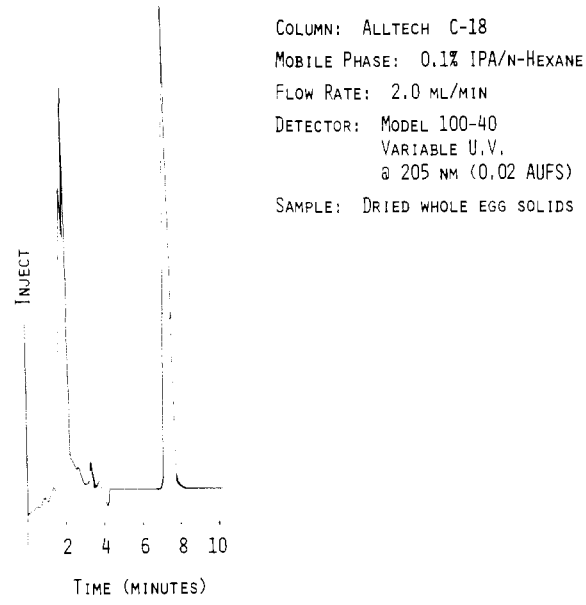

Figure 2. Chromatogram of dried whole egg solids extract.

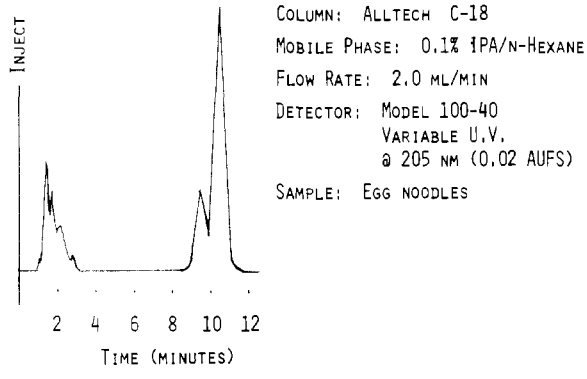

Figure 3. Chromatogram of egg noodle extract.

Additionally, recovery was checked by using ^{14}C-labeled cholesterol in all phases of the extraction. The cholesterol was added at the beginning of the analysis procedure and carried through the entire process with 99.4% of the added cholesterol recovered.

The method was then used to analyze a series of egg noodles and egg solids and then calculate the egg solids of the egg noodles based on this data. This is presented in Tables IV–VI.

In the calculations of the cholesterol content of liquid egg samples, all were corrected for moisture variances.

Assuming a mean value of 13.69 mg/g of cholesterol in commercial liquid egg, the percentage of egg noodles in Table I were calculated (Table VI). Samples in Table VI were formulated to 5.5% egg solids.

Based on the data generated in this study, this method of cholesterol analysis would permit an estimation of egg solids by the determination of cholesterol. The method described in this report is accurate and precise and presents an attractive alternative to the current lipid phosphorus methodology.

Registry No. Cholesterol, 57-88-5.

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