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Short communication

Quantification of cholesterol in foods using non-aqueous capillary electrophoresis

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Abstract

A simple method for the rapid quantification of cholesterol in egg yolk and milk by non-aqueous capillary electrophoresis (NACE) is described in this paper. The samples were treated with saponification and then quantified by NACE, in which 100 m*M* sodium acetate–acetic acid in methanol was employed as the running buffer. The correlation coefficient between the cholesterol concentration and the corresponding peak area was 0.999. The detection limit of cholesterol was 5 μg/ml (twice the signal-to-noise ratio). This method can be used as a routine method for the rapid and sensitive determination of cholesterol in foods.

Keywords: Cholesterol

1. Introduction

Cholesterol is important to good health at a reasonable level because it is a component of cell membranes and is converted into hormones. But a high cholesterol level is related to some diseases, such as atheromatosis and coronary heart disease [1]. Cholesterol comes from two sources: that produced in human body and that found in food, especially in animal products, such as eggs, dairy products, meat and poultry. An efficient and reproducible method for cholesterol determination in food is vital for the control of cholesterol uptake.

Various methods have been developed for cholesterol determination in foods, including spectrophotometry [2,3], gas–liquid chromatography [4,5], high-performance liquid chromatography (HPLC) [6–8] and others [9,10]. All these methods share the same procedures, a saponification extraction step and a multistage solvent extraction followed by purification and concentration. The chromatographic technique was a good method in cholesterol determination for the complete separation of the coexisting lipids. However, an improved resolution and a greater ease of operation can be expected with capillary electrophoresis (CE).

CE is powerful in the analytical field due to its improved resolution, smaller sample load and shorter operation time. But the water insoluble nature of cholesterol and coexisting lipids in foods [11] complicate the cholesterol determination by CE. Recently, non-aqueous capillary electrophoresis (NACE) has been widely applied to nonionic components [12] and lipoproteins [13] in a non-aqueous media, where it consists of a conductive electrolyte in either an organic solvent or a mixture of organic solvents. The advantages of NACE have been reported [14],

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including improved efficiency and enhanced detection sensitivity. Chicken egg and milk are highly favored foods for their nutritious and functional properties. In this study, the quantitative determination of cholesterol in chicken egg yolk and milk with NACE was investigated.

2. Experimental

2.1. Materials

The cholesterol standard was purchased from Sigma (St. Louis, MO, USA). All solvents used were of HPLC-grade or analytical-grade, and were purchased from Biological Science and Technology of Shanghai (Shanghai, China). The fresh milk was purchased from the Yili Group (Hohhot, China) and the fresh chicken eggs were purchased from a local market.

2.2. Sample preparation for capillary electrophoresis

The samples of egg yolk and milk were prepared with and without saponification before NACE analysis. A 1.0-ml aliquot of diluted egg yolk (0.1 g of egg yolk per ml of solution) or 1.0 g of milk was pipetted into a test tube with stopper. Then 3.0 ml of 95% ethanol was added and shaken for 3 min before further being subject to either direct solvent extraction or saponification.

Solvent extraction was carried out according to the method proposed by Zhang et al. [15], in which 2.5 ml of diethyl ether and 2.5 ml of petroleum ether were added while shaking. After the mixture was kept for 30 min to obtain phase separation, 0.5 ml of the organic phase was pipetted into an eppendorf tube and evaporated by vacuum drying. The residue was dissolved in 0.5 ml of the running buffer and applied to NACE for cholesterol determination.

The preparation of the saponification sample was conducted according to the method of Sim and Bragg [16]. The sample was added to 2.0 ml of potassium hydroxide solution (50:50, w/v) and incubated at 60°C for 30 min with periodic agitation. After cooling, the sample was added to 5.0 ml of hexane and vigorously shaken for 3 min. It was then added to 3.0 ml of deionized water, and the tube was capped and shaken for 3 min again. When a satisfactory phase separation was induced, 0.5 ml of the organic phase were pipetted into an eppendorf tube and evaporated by vacuum drying. The residue was dissolved in 0.5 ml of the running buffer and applied to NACE for the cholesterol determination of the sample.

2.3. NACE of cholesterol

The NACE was performed on a Beckman P/ACE 5510 system with diode array detector and system gold software (Palo Alto, CA, USA). All the separations were carried out using a fused-silica capillary (Yongnian Optical Fiber Factory, China), 50 μm I.D.× 47 cm (40 cm to detector). The data were collected and the peak migration time and area were analyzed by Beckman system gold software.

To determine the optimum running conditions, a series of running buffers (100 m*M* sodium acetate–acid buffer) were prepared by mixing 100 m*M* sodium acetate in methanol with 100 m*M* acetic acid in methanol at different ratios from 2:1 to 19:1. Before each run, the capillary tube was rinsed with the running buffer for 5 min. The samples were applied to the capillary with pressure injection for 10 s at 0.5 p.s.i. The electrophoresis was carried out at a voltage of 23.5 kV (47 cm×500 V/cm), capillary temperature of 25°C, wavelength of 210 nm and electric current of ~18 μA. All buffers and solutions used in the study were filtered through a 0.22-μm membrane (Gelman, Michigan, CA, USA)

2.4. Calibration of the cholesterol standard

Cholesterol was dissolved in a running buffer as a stock standard solution (1 mg/ml) and stored at −20°C. A series of working standards (0, 0.05, 0.10, 0.20, 0.33, 0.50, 0.67 and 1.0 mg/ml) were obtained by diluting the stock standard solution with running buffer. The correlation between the cholesterol concentration and corresponding peak area was established under the given NACE conditions.

2.5. Cholesterol determination by colorimetry

The cholesterol contents of egg yolk and milk were also determined by colorimetry according to the method given in Ref. [17], in which cholesterol could be determined at 520 nm after reaction with phthalaldehyde in an acetic environment. The samples were pretreated according to the same saponification procedures as stated above except that a 100-μl sample (500-μl for the milk sample) rather than the 0.5-ml sample of the organic phase of the egg yolk sample was pipetted into a test tube for vacuum drying. Then 1 ml of a fresh solution of 50 mg of phthalaldehyde in 50 ml of glacial acetic acid was added and thoroughly mixed by a vortex mixer for 10 min before addition of 0.5 ml of concentrated sulfuric acid. After 15 min, the absorbance of the sample and a series of standards were determined at 520 nm, using a UV–Vis spectrometer Lambda Bio 10 (Perkin Elmer).

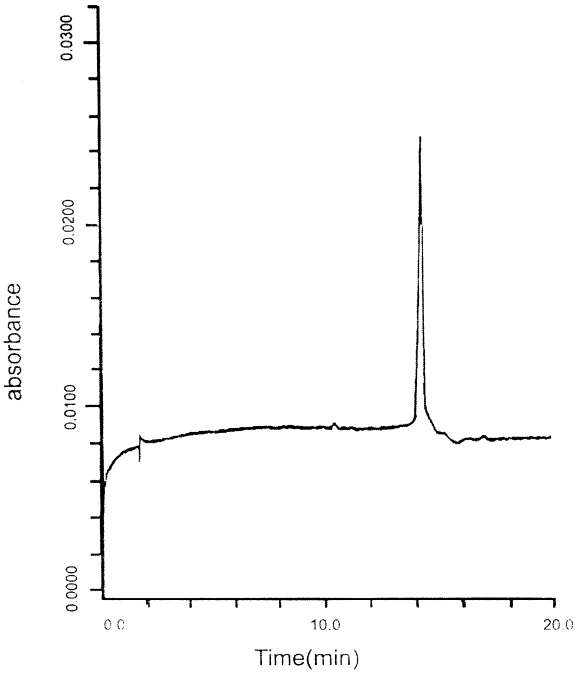

Fig. 1. The electropherogram of the cholesterol standard. Running buffer: 100 m*M* sodium acetate–acid (19:1, v/v) in methanol; capillary: 47 cm×50 μm I.D.; separation voltage: 23.5 kV; detection: 210 nm.

3. Results and discussion

3.1. Calibration of cholesterol standard

The optimum separation and electropherogram for the cholesterol standard sample was obtained in a running buffer which was prepared by mixing 100 m*M* sodium acetate in methanol with 100 m*M* acetic acid in methanol at a ratio of 19:1, as shown in Fig. 1. The retention time for cholesterol was 14.8 min, while that of the electro-osmotic-flow (EOF) was 11.2 min, as determined by using hexane and acetone as markers. It is apparent that the migration of cholesterol was the result of electrophoresis rather than simply a movement caused by the effect of EOF. It is not exactly clear why and how an uncharged molecule like cholesterol can migrate in an electric field, but the fact that cholesterol did migrate in capillary electrophoresis indicates a new possibility for cholesterol separation.

A calibration curve was obtained between the cholesterol concentration and the corresponding peak area. The regression equation of the calibration curve was calculated to be $Y = 3.392X + 0.029$ with a correlation coefficient of 0.999 within the cholesterol concentration range 0–1.00 mg/ml. The calibration curve was repeated three times under the same conditions. The standard deviation of regression lines' slope and intercept were 0.020 ($N=3$) and 0.007 ($N=3$), respectively, indicating clearly an excellent reproducibility and accuracy of the method for cholesterol determination within the concentration range. The detection limit was calculated to be 5 μg/ml (twice signal-to-noise ratio) using the cholesterol standard.

3.2. Quantification of cholesterol in egg yolk and milk

The influence of saponification the egg yolk and milk samples on cholesterol determination was investigated. There was no discernible difference in the electropherograms of the standard cholesterol samples with or without saponification (data not shown).

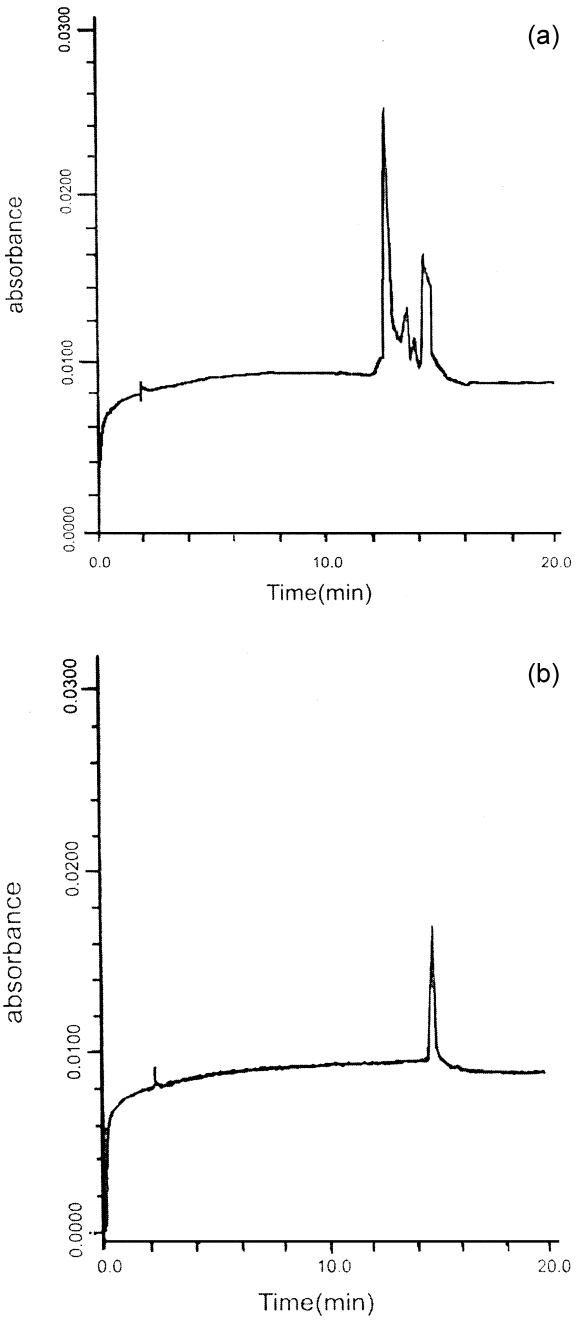

Fig. 2. (a) The electropherogram of egg yolk without saponification. (b) The electropherogram of egg yolk with saponification. Running buffer: 100 m*M* sodium acetate–acid (19:1, v/v) in methanol; capillary: 47 cm×50 μm I.D.; separation voltage: 23.5 kV; detection: 210 nm.

However, the electropherograms of the egg yolk samples with saponification (Fig. 2b) were significantly different from those without saponification (Fig. 2a). Only a cholesterol peak was obtained in the electropherograms of the egg yolk samples with saponification. In the electropherograms of the without-saponification egg yolk samples, interfering peaks become much more prominent than cholesterol peak whose shape was somewhat distorted while retaining a similar peak area with the saponification sample. Those interfering peaks could be derivatives of cholesterol, such as cholesterol ester or other lipids in egg yolk [11], which would also be extracted into the organic phase. Zhang et al. [15] also observed the similar differences in reversed phase chromatographic patterns of egg yolk samples with and without saponification. As a result of saponification, cholesterol esters and other lipids in egg yolk were hydrolyzed into their constituent acids and cholesterol or alcohols. The resultant acids and alcohols were so different chemically from cholesterol that they might be discarded during organic solvent extraction. In the reversed-phase HPLC determination of the cholesterol content in egg yolk, no differences were observed between the results of samples with and without saponification because of the baseline separation of cholesterol from the other components, accordingly saponification of samples is not considered necessary [15]. In the present work, however, the interference from other components in the samples without saponification could not be completely eliminated, which affected the calculation of the peak area of cholesterol. The same results were obtained with milk regarding the influence of the saponification samples (data not shown). Therefore, it is considered necessary to have egg yolk samples saponified to secure reliable results.

The results of the cholesterol determination in egg yolk and milk samples with saponification by non-aqueous capillary electrophoresis are listed in Table 1. The cholesterol content is expressed as milligrams per gram for egg yolk and milligrams per 100 g for milk. As shown in Table 1, the cholesterol content of chicken egg yolk and milk determined by NACE is similar to that of the colorimetric method within the reported values 9.21~22.8 mg/g for egg yolk [18] and ~14 mg/100 g for milk [19]. Moreover, the

Table 1
Cholesterol determination in egg yolk and milk by colorimetry and NACE ($N=3$)

	Egg yolk sample		Milk sample	
	Yolk (mg/g)	RSD (%)	Milk (mg/100 g)	RSD (%)
Colorimetry	19.97±0.27	1.4	13.41±0.85	6.3
CE	20.58±0.09	0.4	13.55±0.30	2.2

relative standard deviation of cholesterol for egg yolk and milk by NACE was 0.4 and 2.2%, respectively, which is far better than 1.4% for egg yolk and 6.3% for milk by the colorimetric method. Statistical analysis using the *t*-test showed that the two methods are not significantly different ($P<0.05$). This method has proved to be an excellent alternative for a precise, accurate and reproducible determination of cholesterol in egg yolk and milk.

4. Conclusion

A rapid, accurate and sensitive method has been developed for the quantitative determination of cholesterol using non-aqueous capillary electrophoresis (NACE). In comparison with the colorimetric method, the NACE method is more rapid with greater ease of operation for quantitative cholesterol determination in egg yolk and milk, rendering it a potent tool for cholesterol determination in foods.

Acknowledgements

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