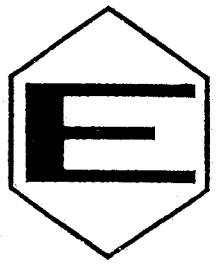
High performance liquid chromatographic determination of egg content in egg noodles

STRATEGIES FOR FOOD QUALITY CONTROL

AND

ANALYTICAL METHODS IN EUROPE



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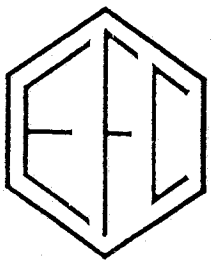

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HPLC DETERMINATION OF EGG CONTENT IN EGG NOODLES

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Abstract

The information about egg content in egg noodles is important from both a production and a regulatory point of view. The most frequently adopted analytical procedures are based on determination of cholesterol present in the unsaponifiable matter of the lipid fraction. Cholesterol can be determined either by gravimetric analysis, or by gas chromatography, or by high performance liquid chromatography, or by enzymatic methods. In this paper a reversed- phase high performance liquid chromatographic method is presented which allows for the determination of both the cholesterol/campesterol and the cholesterol/ β-sitosterol + campestanol ratios. These ratios can be used as an indication of egg content. The method involves recovery of sterols from the unsaponifiable matter by thin layer chromatography and subsequent direct injection into the high performance liquid chromatographic device. Standard samples of macaroni products added with 1,2,3,4,5,6 and 7 eggs/Kg of flour have been examined; for each sample the cholesterol/campesterol and cholesterol/β-sitosterol + campestanol ratios have been determined. These ratios have been utilized in the analysis of twenty real samples of egg noodles claimed to contain four eggs/Kg of flour.

Introduction

The information about egg content in egg noodles is important from both a production and a regulatory point of view[1].

The most frequently adopted analytical procedures are based on determination of cholesterol present in the unsaponifiable matter of the lipid fraction. Cholesterol can be determined either by gravimetric analysis[2-3], or by gas chromatography (GC)[4-7], or by high performance liquid chromatography (HPLC)[8], or by enzimatic methods[9].

In this paper a high performance liquid cromatographic (HPLC) method is presented which allows for the determination of the cholesterol/campesterol and cholesterol/β-sitosterol + campestanol ratios. These ratios can be used as an indication of egg content.

Materials and Methods

Apparatus

A Spectra-Physics 8700 high performance liquid chromatograph, equipped with a variable wavelength Knauer 8700 spectrophotometric detector and a 10 μl loop, was used. A Supelcosil LC-18 stainless steel column, 250 x 4.6 mm, 5 μm, was employed.

Analyses were carried out isocratically at room temperature by utilizing isopropanol as eluent system, 1 ml/min. flow rate. The spectrophotometric detector was set at 209 nm.

Peak areas were determined by means of a Spectra-Physics 4270 integrator.

Each sample was analyzed in duplicate; each determination was carried out in triplicate.

A Carlo Erba mod. 5360 gas chromatograph equipped with a split-splitless injector and FID was used. A fused silica capillary column, 25 m x 0.25 mm i.d., coated with SE 54, 0.10 μm film thickness (Supelco), was employed. Operating conditions were: split ratio 1:40 v/v, carrier gas (H_2) flow rate, 1.00 ml/min, injector and detector temperatures, 300°C, oven temperature, 265°C.

Standards and reagents

Cholesterol, β-sitosterol and campesterol were obtained from Aldrich Chemical Company. All reagents and solvents were of analytical grade and were purchased from C. Erba (Milano, Italy).

Samples of macaroni products and egg noodles

Standard samples of macaroni products added with 1,2,3,4,5,6 and 7 eggs/kg of flour were prepared industrially. Samples of egg noodles were obtained from commercial sources.

Analytical procedure

a) Extraction of sterols

Sample is finely ground in a Mouli grater and then screened to 25 mesh. 50 g of ground sample are extracted with acetone for 16 hours in a Soxhlet apparatus. 5 g of the extracted fat are saponified under reflux and continuous shaking with methanolic 2 M potassium hydroxide in a 250-ml round-bottomed flask. When a clear solution is obtained, the reflux is continued for 20 minutes. 50 ml of water are added through refrigerant, the solution is cooled and tranferred to a 500-ml separatory funnel. 150 ml of water and 100 ml of ethyl ether are added. The funnel is vigorously shaken and the ether layer is tranferred to another separatory funnel. Should an emulsion is formed, a few ml of methanol are added to obtain two well separated phases. The extraction is repeated twice again, by employing two 50-ml volumes of ethyl ether. The ether layers are combined in a separatory funnel. The ether solution is washed with water (50 ml x 4) until the basic reaction disappears. The solution is filtered over anhydrous sodium sulfate by using a pleated filter into an emery-cap Erlenmeyer flask. Both sodium sulfate and filter are previously saturated with ethyl ether. Then the solvent is removed on a rotary vacuum evaporator at 30°C. The unsaponifiable matter is dried with a stream of N_2 and weighed to constant weight. A 5% (w/v) chloroform solution of the unsaponifiable matter is prepared. The solution is transferred to a silica gel plate previously immersed in methanolic 0.2 M potassium hydroxide for 10 seconds and activated at 110°C for 15 minutes. The plate is developed with a 65:35 (v/v) hexane/ethyl ether mixture, dried with hot air and spraied with an alcoholic solution of 2,7-dichlorofluorescein. The plate is put in a stove at 110°C for a few minutes and then exposed to u.v. light to highlight the sterolic band (the slowest moving one). The sterols are recovered from silica gel by extraction with hot chloroform. The solution is filtered into a volumetric Erlenmeyer flask; the filter is washed over and over again with hot chloroform. The solvent is removed on a rotary vacuum evaporator at 30°C and finally on a stream of N_2. The sterols are dissolved in isopropanol and the solution is directly injected into the HPLC system.

b) Silylation

The fractions eluted from the HPLC system are dried on a stream of N2. The sterols are weighed and silylated with a solution of 9:3:1 (v/v/v) pyridine/hexamethyldisilazane/trimethylchlorosilane; then the solution is injected into the gas chromatograph.

Results and Discussion

The determination of egg content in egg noodles is usually based on an absolute evaluation, specifically, the amount of cholesterol is determined as a function of the egg number. In this paper a relative evaluation is described, specifically, the ratio between the sterol of animal origin (cholesterol from eggs) and the sterols of vegetable origin (campesterol, β-sitosterol and campestanol from flour) are determined.

Under the experimental conditions described, a good separation is obtained for the mixture of sterols. As an example, figure 1 shows a typical chromatogram relative to a sample of egg noodle.

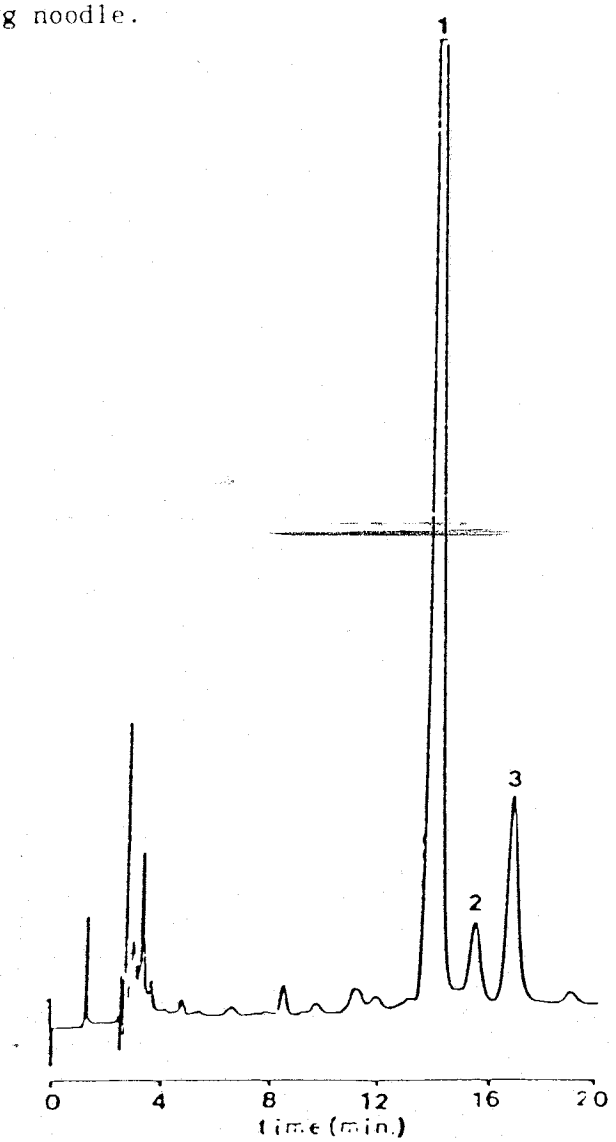

Fig.1. High performance liquid chromatographic separation of the sterolic fraction from an egg noodle. 1: cholesterol; 2: campesterol; 3: β-sitosterol + campestanol.

The identity and purity of the single compounds was checked by collection of the fractions eluted from the HPLC system, silylation and injection into the GC. The peaks of interest were identified as cholesterol, campesterol and β-sitosterol + campestanol rispectively by comparison of retention times and co-injection with pure standards. No minor peak appeared in the GC trace. Analyses carried out on standard samples allowed for both the cholesterol/campesterol (A) and the cholesterol/β-sitosterol + campestanol (B) ratios to be determined as a function of the egg number/Kg of flour.

Obtained results are reported in Table I. As may be seen, both ratios increase linearly when increasing the egg number of the sample.

A paired Student t-test was used to perform a statistical treatment of the data. Differences between obtained results were in all cases significant at the 0.001 level. An accurate evaluation of the egg number present in real samples is therefore possible.

Table 1. Cholesterol/campesterol (A) and cholesterol/β-sitosterol + campestanol (B) ratios obtained for examined standard samples.

egg number/Kg of flour	A	B
1	4.71 + 0.34	1.40 + 0.21
2	9.86 + 0.68	2.78 + 0.12
3	14.74 + 0.43	4.51 + 0.74
4	18.73 + 0.44	5.84 + 0.61
5	22.90 + 0.39	7.00 + 0.25
6	28.91 + 0.33	8.55 + 0.41
7	31.80 + 0.49	9.81 + 0.35

The method was applied to the analysis of 20 real samples of egg noodles claimed to contain 4 eggs/Kg of flour, as stated by present regulations. A mean A value of 18.54 and a mean B value of 5.96 were obtained; standard deviations were 0.30 and 0.13 respectively, and coefficients of variation were 1.61 % and 2.10 % respectively.

Differences in respect of both A and B values determined on standard samples were not statistically different at the 0.05 level.

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