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Egg Pasta — Determination of Cholesterol Used to Calculate the Egg Content

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Introduction

Determination of egg content in egg pasta is made either by cholesterol determination or by determination of total sterols. The latter method is however being abandoned because of its lack of specificity.

While the cholesterol determination, carried out by gas-liquid chromatography (GLC), presents no special problem, cholesterol extraction from the product is rather difficult. Many methods published (e. g. 1, 2, 3, 4) are indeed based on a liquid-liquid extraction procedure, which frequently leads to formation of emulsions, that often are very difficult to break. Sometimes foam even forms already during the initial boiling of the product.

The method described below removes these drawbacks by using another solvent system (suppression of emulsions) together with a continuous technique (time gain). Moreover, the initial treatment with hydrochloric acid becomes superfluous.

Choice of the solvent system

Since we intend to treat the test portion only with an alcoholic sodium hydroxide solution (which permits to release cholesterol without starch sticking), and alcohol being soluble in most solvents, we are compelled to choose a ternary system in order to extract the cholesterol. We have chosen the water/alcohol/hexane system.

Indeed, hexane dissolves cholesterol and can easily form a two-phase system with alcohol and water. Using the miscibility diagramme of these three components (5) we fix the following ratios:

— alcohol: about 55 Vol.%
— water: about 20 Vol.%
— hexane: about 25 Vol.%

These ratios ensure the formation of two phases.

In the course of work we have replaced pure water by water with 10% NaCl, since a slight emulsion may form at the interface of both liquids, which is strongly diminished in presence of NaCl.

Principle of the method

1. Saponification in alcoholic medium.
2. Continuous extraction.
3. Determination by gas-liquid chromatography (technique with internal standard).

Description of the method

Material

— Flat-bottomed flasks, long neck (8—10 cm), ST, 100 ml
— Round-bottomed flasks, ST, 25 and 250 ml
— Volumetric flasks, 100 ml
— Condensers with bulbs, min. length 15 cm
— Liquid-liquid extractor for light solvents, 250 ml
— Filtering funnels with sintered disc, porosity G2
— Miscellaneous glassware
— Gaschromatograph with a glass column 1/4″ O.D., 6′; 10% SE-52 on Chromosorb W.

Chemicals and solutions

— Hexane, GR
— Ethanol, GR
— NaCl, GR
— NaOH, GR
— 1 n NaOH solution in EtOH
— 10% aqueous NaCl solution
— Trimethylchlorosilane (TMCS)
— Hexamethyldisylazane (HMDS)
— Pyridine, GR
— Cholestane, GR
— Cholesterol, GR (to be dried before use).

Procedure

Preparation of the sample

Grind the pasta sample very finely (less than 300 μ).

Treatment of the sample

— Weigh to the nearest 0.1 mg, about 5 g of the finely ground sample into a 100 ml flat-bottomed flask.
— Add 40 ml 1 n NaOH in ethanol and boil under reflux for 1/2 h. Let cool.

— Filter quantitatively through a sintered disc of porosity G2 while rinsing the cake with ethanol (e. g. 4 x 10 ml).
— Transfer the filtrate quantitatively into the extractor and rinse the flask with ethanol. The final volume of the ethanolic solution should not exceed 135 ml. The filtration may also be carried out directly into the extractor.
— Fill up the extractor to 185 ml with 10% NaCl solution.
— Introduce 150 ml hexane into the distillation flask and extract in the continuous extractor for 1 hour.
— Evaporate the extract to about 5 ml and add the chosen amount of cholestane. Then transfer into a 25 ml flask and evaporate to dryness under vacuum (50—60°C).

The amount of cholestane is chosen so that the final extract contains:

about 1 mg cholestane if the presumed content is 1—2 eggs/kg
about 2 mg cholestane if the presumed content is 3—4 eggs/kg

Silylation of the sample

— Add to the dry extract the following reagents:
  — about 0.5 ml pyridine
  — about 0.3 ml HMDS
  — about 0.1 ml TMCS
— Close tightly, then heat at 60°C for 15 minutes. Inject into the chromatograph, previously calibrated with a standard.

Choice of the calibration standard

Product to be analysed containing	Cholestane dissolved in hexane (mg)	Cholesterol dissolved in hexane (mg)
1—2 eggs/kg	about 1	about 1.5—2
3—4 eggs/kg	about 2	about 3

Each standard is to be silylised as above.

Calculation of the egg content

The calculation is made as indicated by *Dresselhaus* and *Acker (3)*.

Gas-liquid chromatography

Conditions recommended for a column of 1/4" O.D., 6'; SE-52 10% on Chromosorb W HP:

— Injector: 300°C
— Oven: 275°C
— Detector: 300°C
— Carrier gas: 20—30 ml/minute N_2
— F.I.D. — air: 200—300 ml/minute
— hydrogen: 20—30 ml/minute

Results

The method has been applied to different pasta samples, in parallel with that of *Dresselhaus* and *Acker* (3). The results obtained can be found in Table 1.

Table 1.

Determination of cholesterol in pasta. Results in % cholesterol on product

Sample	Method (3)	Method of this article
1	0.037	0.041
2	0.047	0.046
3	0.098	0.097
4	0.046	0.050
5	0.042	0.045
6	0.072	0.074
7	0.096	0.087
8	0.057	0.058
9	0.040 (emulsions)	0.067
10	0.002	0.002
11	0.054 (emulsions)	0.072
12	0.075 (emulsions)	0.090
13	0.096	0.100

Besides, we have tested the reproducibility and repeatability of the method when carried out by different analysts. The following results were obtained:

Reproducibility

— Same sample for analysts 1 and 2 ($\bar{x}$ = mg cholesterol/100 g pasta)

	n	$\bar{x}$	$S_{reprod.}$	C.V % reprod.
analyst no. 1	8	73.00	0.98	1.34
analyst no. 2	10	74.13	1.40	1.88

— Same sample for analysts 1 and 3 ($\bar{x}$ = mg cholesterol/100 g pasta)

	n	$\bar{x}$	$S_{reprod.}$	C.V % reprod.
analyst no. 1	5	105.84	0.72	0,7
analyst no. 3	10	105.60	0,84	0.8

Repeatability

— Same analyst, but different samples ($\bar{x}$ = mg cholesterol/100 g pasta)

Sample	n	$\bar{x}$	$S_{repeat.}$	C.V % repeat.
1	5	71.96	1.95	2.7
2	5	105.84	0.72	0.7
3	5	73.80	0.80	1.1
4	5	87.37	1.91	2.2

Conclusions

The observations made during all our tests have shown the following advantages over the existing methods:

— gain of time (10 samples in 2 days)
— no more emulsion problems
— good repeatability and reproducibility
— possibility to carry out easily series of analyses
— suppression of the initial treatment with hydrochloric acid (which can produce foam).

Summary

A method has been developed for cholesterol determination in pasta products, which has the following advantages over the existing methods:
— absence of emulsions thanks to the use of a liquid-liquid extractor and a ternary solvent system (salted water — alcohol — hexane)
— important gain of time (3 x more rapid)
— possibility to carry out large series of analyses.

Zusammenfassung

Die Versuche haben uns erlaubt, eine Methode zur Cholesterinbestimmung in Teigwaren auszuarbeiten, die folgende Vorteile gegenüber den vorhandenen Methoden aufweist:
— Abwesenheit von Emulsionen dank der Verwendung eines Flüssig-Flüssig-Extraktors und eines ternären Lösungsmittelsystems (Salzwasser — Alkohol — Hexan)
— Beachtlichen Zeitgewinn (3 x schneller)
— Möglichkeit große Analysenserien durchzuführen.

Résumé

Les essais ont permis de mettre au point une méthode de dosage du cholestérol dans les pâtes alimentaires, qui a les avantages suivants sur les méthodes existantes:
— absence d'émulsions grâce à l'emploi d'un extracteur liquide-liquide et d'un système ternaire de solvants (eau salée — alcool — hexane)
— gain appréciable de temps (3 x plus rapide)
— possibilité d'exécuter de grandes séries d'analyses.

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