AXELSEMRAU

Determination of the egg content of foodstuff
with the CHRONECT Workstation Cholesterol

Application note 1905

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Introduction

The determination of cholesterol is routinely performed in egg-containing foods. The aim is to determine the amount of the ingredient whole egg or egg yolk, as these are valuable components. In 2006, the Arbeitskreis Lebensmittelchemischer Sachverständiger (ALS) commented on the composition of an average shell egg taking into account various factors. According to this, an egg with 50 g mass and 24 % dry matter has a pure egg yolk content of 16 g. The percentage of pure yellow is 50 % dry matter, and the cholesterol content is 195 mg per 50 g whole egg. From this information, the cholesterol content of a sample can be used to determine the egg content.

Method and device setup

The CHRONECT Workstation Cholesterol is based on the CHRONECT Workstation Sterols. The system consists of the following components:

- CHRONECT Robotic XYZ robot
- HPLC pump (Nexera XR LC-20AD)
- UV/VIS detector (SPD-20AD)
- GC with FID (Nexis GC-2030)
- CHRONECT LC-GC interface
- CHRONOS software

Sample Preparation

The samples, starch-containing and starch-free, are homogenized and sieved if necessary. 200 to 300 mg of sample are weighed into a 10 mL vial and closed. The samples are then placed in the sample rack.

All following steps are performed automatically with the CHRONECT Robotic Autosampler. First, the internal standard (ISTD) 5β-Cholestan-3α-ol (trivial name: epicoprostanol, c = 1 mg/mL, V = 0.1 mL) is added. This is followed by an enzymatic starch hydrolysis for starch-containing samples by adding 1 mL of diluted α-amylase solution. Starch degradation takes 30 minutes. The option of carrying out the starch hydrolysis is possible during sequencing using the CHRONOS software. After starch degradation, the analysis procedure is identical for all starch-containing and starch-free samples. A saponification step follows after addition of 1.5 mL ethanolic potassium hydroxide solution, which takes 40 minutes. The unsaponifiables are then extracted with 4 mL *n*-hexane. In addition, 2.5 mL saturated citric acid solution is added to the neutralization solution. The sample extract is mixed by the agitator of the CHRONECT Robotic. A 200 µL aliquot of the organic phase is then transferred into a 2 mL vial containing dried Na_2SO_4 and 800 µL *n*-hexane. Thus, any water residues are bound, and the sample is diluted again. This sample preparation is based on the methods of the ASU according to §64 LFGB and was only adapted and optimized for the CHRONECT Workstation.

LC-GC analysis

10 µL of the diluted extract are injected into the LC-GC system. The LC run is used to fractionate the sample. A normal phase column (silica gel, 250 mm x 2.1 mm; 5 µm; 60 Å) is used as HPLC column. The eluents are *n*-hexane/2-propanol (98/2 (v/v)) and methyl-*tert*-butyl ether (MTBE). The separation is isocratic. The MTBE is used for backflushing the column.

The Δ5-4-desmethylsterol fraction, including the ISTD and cholesterol, elutes from the LC column after approx. 8 min. The start and end of elution of the fraction is regularly controlled by injecting a solution containing the ISTD and cholesterol. The control is performed by means of a UV detector at 205 nm. Normally, the fraction elutes within 2.5 min. At an eluent flow of 0.3 mL/min, 0.75 mL is transferred to the retention gap. The Retention Gap is installed inside the GC oven, which is set to +80 °C at the start of the transfer and keeps it constant for 5 min. Most of the transferred solvent is evaporated via a Solvent Vapor Exit Valve (SVE), which closes after the transfer. Gases and temperatures are controlled via the LC-GC interface. The internal standard and cholesterol have a significantly higher boiling point than the solvent (*n*-hexane/2-propanol, 98/2 (v/v)), which means that they initially remain on the retention gap. The GC oven program and gas flow have been optimized so that the ISTD and cholesterol are baseline-separated. It is also important that there is no coelution with any phytosterols that may also be present, for example sunflower oil in mayonnaise. The detection is done by FID.

Parallel to the GC run, the LC column is backwashed and equilibrated. A complete analysis run, starting with the addition of the ISTD up to

the end of the GC run, takes about 150 minutes for samples containing starch. Due to the overlapping of individual sample preparation steps controlled by CHRONOS, two samples can be processed simultaneously within one sequence. While one sample is still being measured, the sample preparation of the next sample already starts. Thus, between 12 and 18 samples can be analyzed per day.

The cholesterol content is determined by calibration according to the internal standard method. The preparation of the individual calibration solutions or levels is carried out fully automatically by CHRONECT Robotic after the master solutions of the internal standard and cholesterol have been provided. The measurement is analogous to the method described above. The practical working range extends from 10 mg/100 g to 400 mg/100 g. The cholesterol determination limit is therefore 10 mg/100 g. The determined coefficient of determination is greater than 0.999.

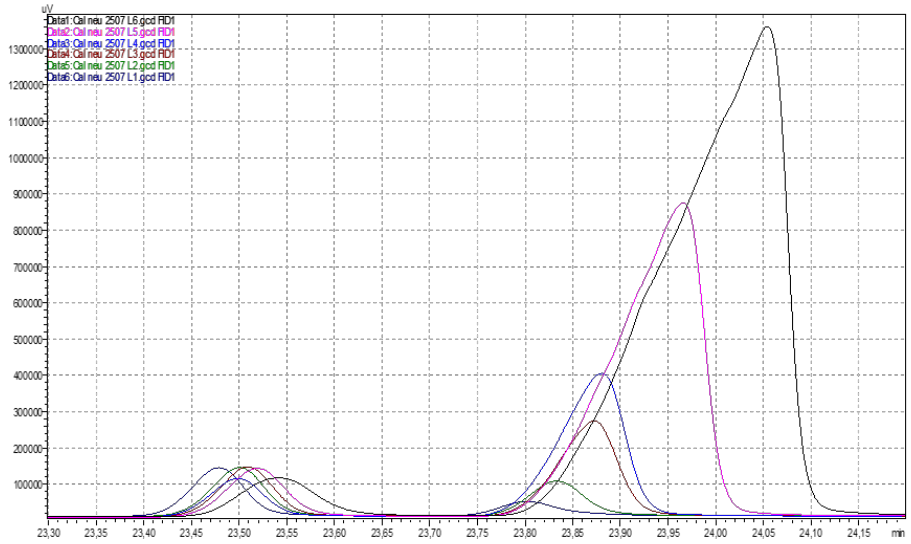

Figure 1: Calibrations from 10-400 mg/100 g.

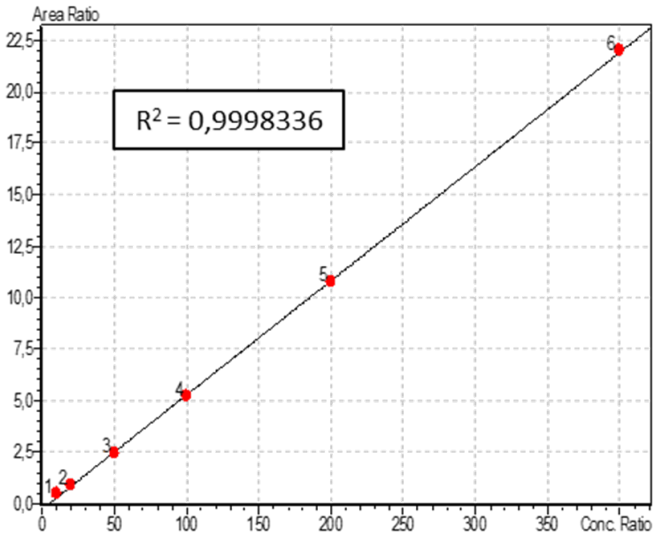

Figure 2: Regression line with coefficient of determination R^2.

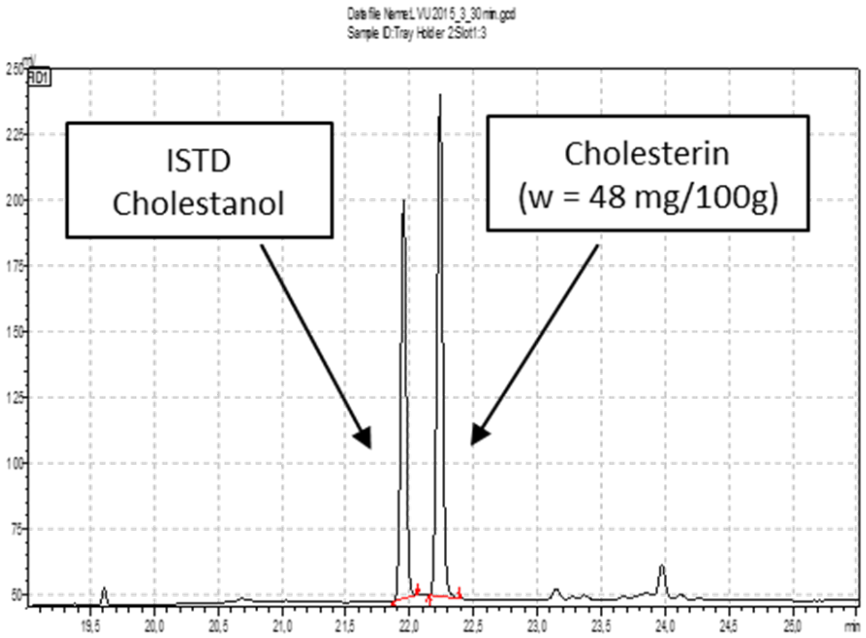

Figure 3: Chromatogram of a butter cookie samples. Cholesterin = Cholesterol.

Results

Validation and verification tests were performed to check the correctness and precision of the presented method. Four different sample materials were selected for validation: pyramid cake, butter cookie, spaetzle (each containing starch) and mayonnaise (starch-free). The validation characteristics are shown in Table 2. The relative repeatability standard deviation for a tenfold determination is less than 5 % for the matrices tested and thus within a normal range for gas chromatographic methods.

For the verification of the method, nine representative sample matrices were selected and their cholesterol content was determined in a triple determination using the methods ASU L 18.00-17 or ASU L20.01-13. These values served as reference values (= SET value) for comparison with the fully automatic egg content determination by means of LC-GC-FID (= ACTUAL value, also triple determination). Table 1 compares the values of both methods. The obtained contents are very well comparable. The quotient of ACTUAL/SET is between 90 and 110 % for all nine tested samples. The deviations between the results of the developed method and the reference methods are therefore extremely small

Table 1: Comparison with reference method.

[mg/100 g]	Procedure acc. to § 64 LFGB	Fully automated LC-GC method	ACTUAL/SET [%]
Sponge cake	171	162	95
Egg pasta	49	45	92
Swabian tagliatelle	107	100	93
Pyramid cake (Crumb)	238	236	99
Butter cookie	46	49	107
Spätzle	81	83	102
Salad mayonnaise	38	40	105
Mayonnaise	59	63	107
Egg liqueur	247	255	103

Table 2: Precision data.

[mg/100 g]	Mean value (n=10)	Repeat Standard Deviation	Relative Standard Deviation
Pyramid cake (Crumb)	236	5.3	5.0 %
Butter cookie	49	0.7	6,3 %
Spätzle	83	0.4	5.8 %
Mayonnaise	71	2.4	6.0 %

Summary

The CHRONECT Workstation Cholesterol allows a routine, fully automatic egg content determination by means of LC-GC-FID. The Workstation is suitable for the analysis of both starch-containing and starch-free samples. Compared to reference methods, the consumption of organic solvents is almost halved and the processing time can also be significantly reduced. With the reference methods according to § 64 of the LFGB, one employee needs up to eight hours for a sequence of eight samples. The subsequent measurement of the extracts can then be performed overnight, so that eight results are available after 24 hours.

When using the CHRONECT Workstation Cholesterol a coworker needs only one hour for the preparation of the weights and the measuring system and receives after 24 hours at least 12 and maximum 18 results. Extensive validation and verification tests have proven the excellent precision and accuracy of the new method. The CHRONECT Workstation Cholesterol is suitable therefore as optimal basis for the calculation of the value-giving ingredient egg in the respective food matrix.

The CHRONECT Workstation Cholesterol is a common development by Landeslabor Berlin-Brandenburg and Axel Semrau.

Subject to technical changes

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