Food, Flavors, & Fragrances Article

Prevent Fraud in Egg Pasta with Simple Analysis of Cholesterol and Glycerides

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Eliminate the uncertainty of using cholesterol alone to authenticate egg content. Determine both glycerides and cholesterol in a single run using an Rtx®-65TG column and get definitive, fraud-identifying results.

Eggs enhance the nutritional and commercial value of pasta, and thus many countries have established minimum egg content levels (based on either counts or weights) for pasta and other egg-containing products. Although egg content standards have been established, methods are not usually specified and a number of procedures may be applied. Cholesterol methods are often used to authenticate products claimed on the label to be made with eggs; however, since cholesterol can be added using non-egg sources, its presence alone is not a reliable marker of egg content. Also, even if egg is the source of the cholesterol in the product, it is difficult to correlate quantitatively to egg content levels, because the levels of cholesterol found naturally in eggs are highly variable. The method presented here allows the use of glycerides, in addition to cholesterol, to assess egg content in pasta. This method provides chromatographic separation of cholesterol, diglycerides, and triglycerides, allowing fraudulent (non-egg) sources of cholesterol to be easily and accurately determined, so qualitative and quantitative comparisons can be made.

Simple Extraction Method

Current methods used for the extraction of fat from flour components generally involve either a 24-hour diethyl ether extraction or an 8-hour Soxhlet extraction. The extraction described here is rapid by comparison. In this simple procedure, fat is extracted from egg pasta dough and freeze-dried egg product by homogenizing the samples and pouring them into glass columns filled with sodium sulfate. The fat phase is eluted with 100mL diethyl ether and then evaporated with nitrogen. Approximately 50mg of the dried fat extract is then dissolved in 1mL internal standard solution (3,000 ppm squalene in diethyl ether). The extracted samples are analyzed by gas chromatography (GC) using an Rtx®-65TG column, which is specifically tested for triglyceride performance.

Easy Identification of Fraudulent Product

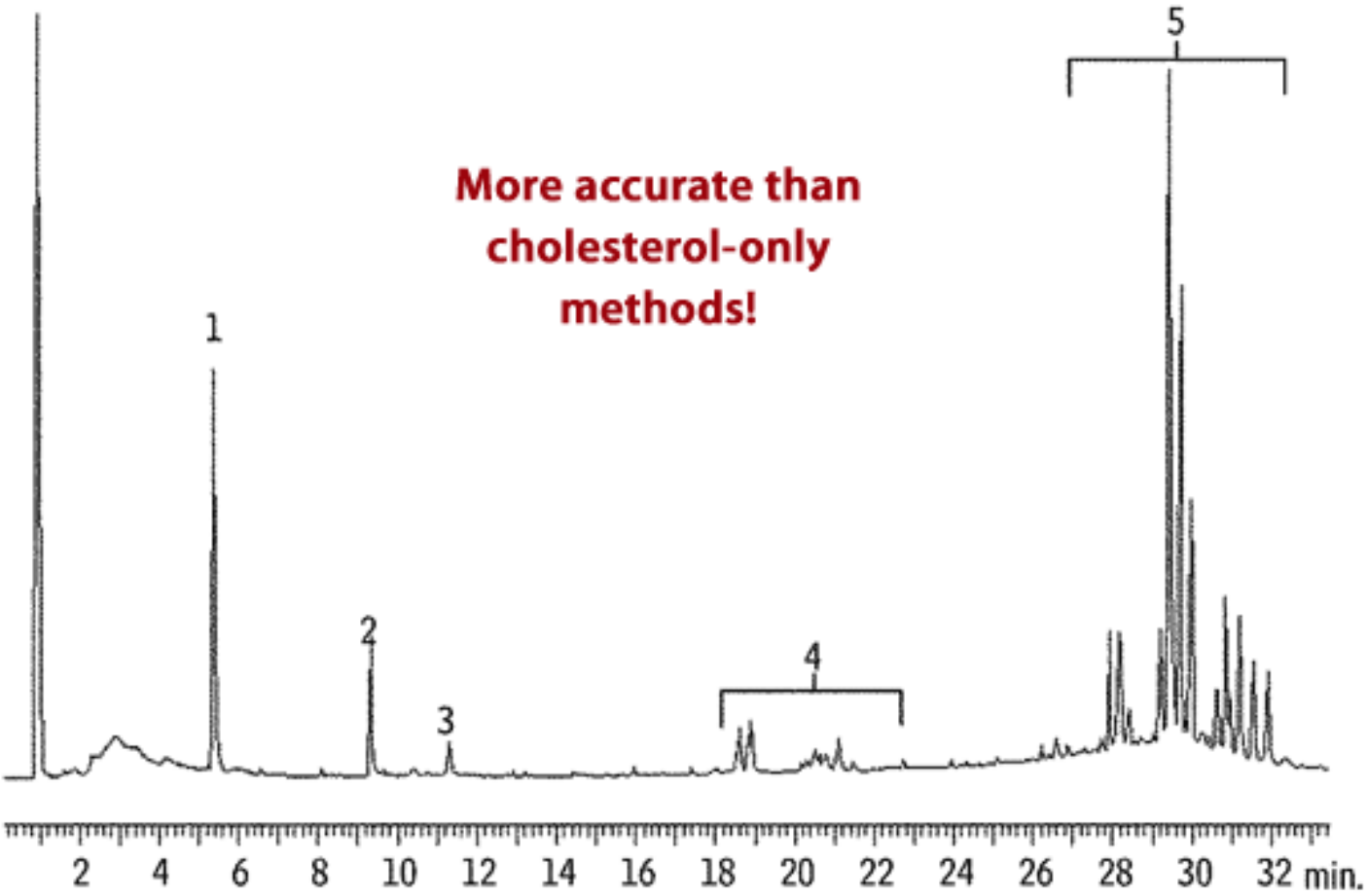
Excellent chromatographic separation of cholesterol, squalene, diglycerides, and triglycerides was obtained (Figure 1). Once separated, these fractions can be used to confirm the addition of egg fat by comparing the glyceride profiles of the egg pasta extract with those from the egg sample. Egg pasta products adulterated with non-egg sources of cholesterol will not show comparable patterns. Note, while cholestane often is used as an internal standard in cholesterol testing, the use of squalene instead in this method is advantageous as it allows both cholesterol and the glyceride profiles to be analyzed. Squalene is highly stable and similar to cholesterol, but the compounds are well-resolved on the Rtx®-65TG column. Cholestane is not sufficiently separated from cholesterol on this polar phase, however, for methods that recommend cholestane, separations can be accomplished on the less polar Rxi®-5ms column (Figure 2). In fact, for methods with a goal of high throughput cholesterol determination, rather than source authentication, using the Rxi®-5ms column under isocratic conditions can cut analysis time by nearly 50%.

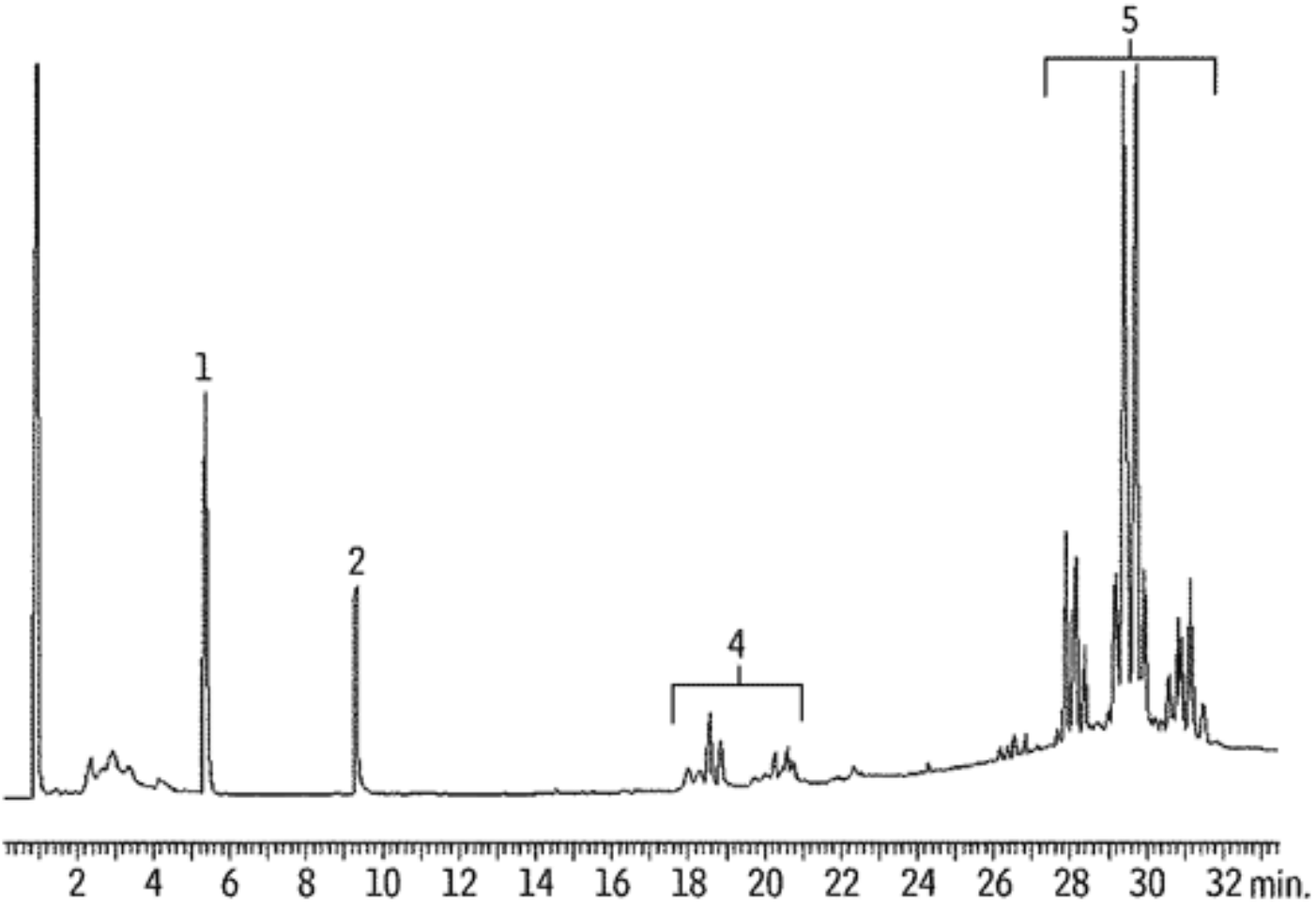
In summary, estimating cholesterol in food products is often part of the authentication testing of label claims regarding egg content. However, the presence of cholesterol in a product may be due to a non-egg source, and the natural variability of cholesterol levels in eggs further complicates quantitative conclusions. The method shown here simplfies fraud detection by incorporating glyceride testing. Easy comparision of the chromatographic profiles of egg and egg product (pasta) samples can be made using an Rtx®-65TG column, which is specifically tested to assure excellent separations and a reliable performance for glycerides.

1. squalene (IS)
2. cholesterol
3. β-sitosterol
4. diglycerides
5. triglycerids

Figure 1: Easily detect fraud by comparing cholesterol and glyceride profiles in one run on the Rtx®-65TG column.

A. Extracted egg pasta fats





Rtx®-65TG. 30m, 0.25mm ID, 0.1µm (cat.# 17008)

Column:	
Sample:	50µ/mL fat extract from egg in diethyl ether solution with 3,000ppm squalene (IS)
Inj.:	0.5µL, split (1:80), 70°C (hold 12 sec.) at 99°C up to 370°C (hold 5 min.)
Carrier gas:	hydrogen
Flow rate:	1.5mL/min.
Oven temp.:	A. 220°C (hold 2.0 min.) to 360°C @ 5°C/min. (hold 5 min.)
Det.:	FID @ 370°C

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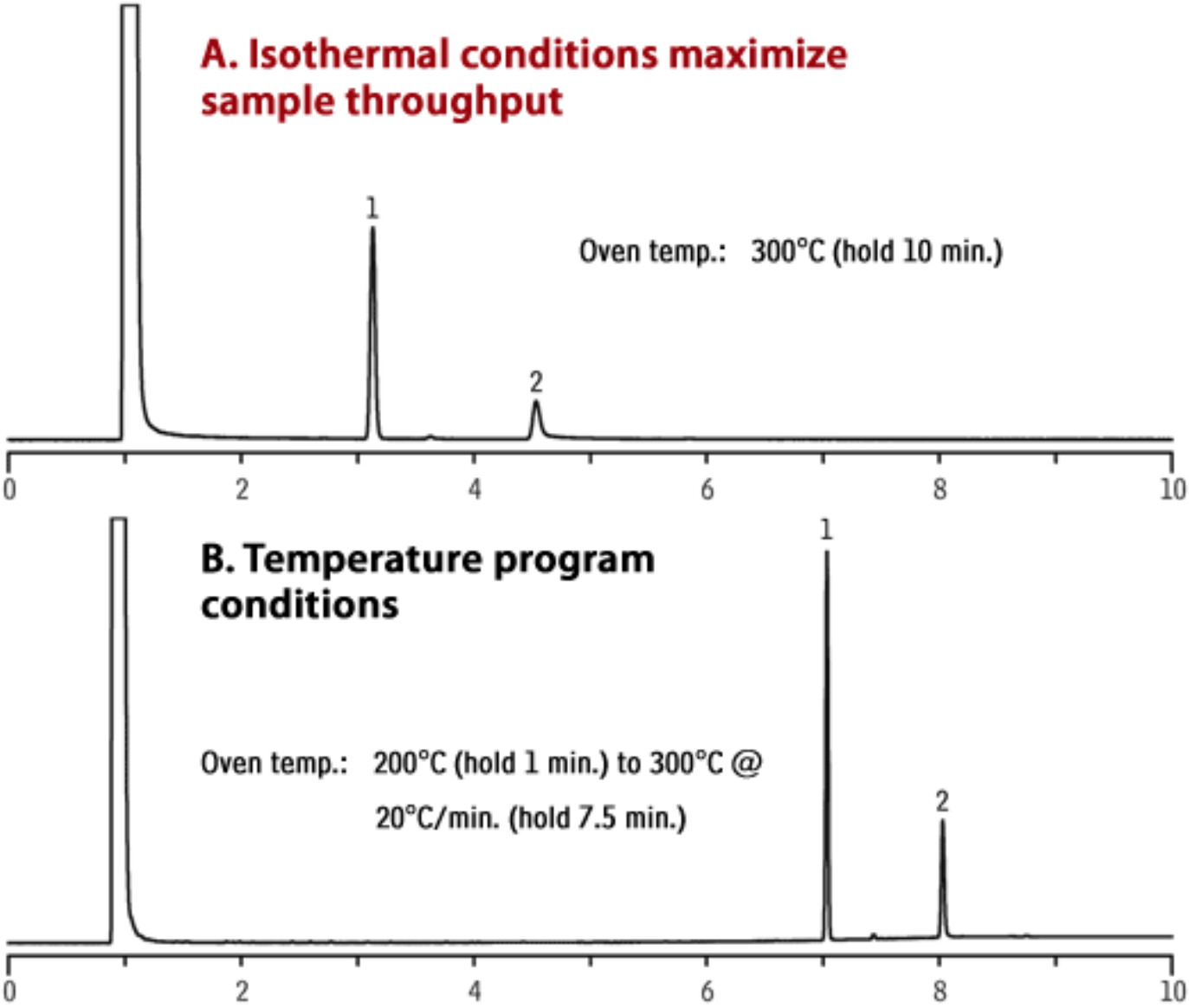
Figure 2: 5-minute run times benefit cholesterol methods requiring high sample throughput instead of source confirmation.

1. 5-α-cholestane (IS)
2. cholesterol



Column:	Rxi®-5ms, 15m, 0.25mm ID, 0.25µm (cat.# 13420)
Sample:	1,000µg/mL cholesterol in DMF, 1,000µg/mL 5-α-cholestane in hexane; 25ng cholesterol, 150ng 5-α-cholestane on column
Inj.:	1.0µL, split (20:1), single gooseneck inlet liner w/ wool (cat.# 22406)
Inj. Temp.:	250°C
Carrier gas:	helium, constant pressure (9.7psi @ 200°C)
Linear velocity:	24cm/sec.
Oven temp.:	See above
Det.:	FID @ 340°C

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