## FOOD COMPOSITION AND ADDITIVES

# Rapid Gas Chromatographic Method for Simultaneous Determination of Cholesterol and α-Tocopherol in Eggs

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**A new method was developed for simultaneous determination of cholesterol and α-tocopherol in eggs. It involves rapid and simple sample preparation accomplished in one tube and chromatographic separation that does not require derivatization of analytes. Total analysis time per sample is 40 min. Labor, cost, and use of hazardous chemicals are minimized. To ensure selectivity, accuracy, and precision, critical analytical parameters were investigated. Overall recoveries were 98.8 and 99.2% for cholesterol and α-tocopherol, respectively. Linearity was acceptable for both analytes (r = 0.9964 for cholesterol and 0.9996 for α-tocopherol) in the fortification range examined. Precision data based on within-day and between-days variation gave overall relative standard deviations of 2.0% for cholesterol and 7.0% for α-tocopherol. The method was applied successfully for quantitation of cholesterol and α-tocopherol in eggs.**

Cholesterol and α-tocopherol are among the nutritionally significant lipids in foods that increasingly require routine analysis. Cholesterol is a precursor of bile acids, steroid hormones, and vitamin D and is the principal steroid in foods of animal origin, occurring in both free and esterified form. Assessment of dietary cholesterol intake is of growing interest to consumers because cholesterol in serum has been implicated in atherosclerosis (1). α-Tocopherol is the prevalent congener of vitamin E. It is a natural antioxidant that extends the oxidative stability of dietary fat, showing also outstanding antioxidant activity in living cells. Accurate determination of these compounds is of great importance to the food industry and recently has been of special interest to poultry scientists who have placed considerable emphasis on altering, through changes in diet fed to chicken, the fatty acid and cholesterol composition of egg yolk (2).

The cholesterol and α-tocopherol contents of eggs are controversial. Data available from food composition tables and recent reports show wide variability, mainly because of differences in analytical methods used (3–7). Cholesterol and α-tocopherol usually are determined separately by methods including spectrophotometry (5, 6–9), liquid chromatography (LC; 5, 6, 10, 11), and gas chromatography (GC; 6, 12–19). When data for both analytes are required, it is possible to determine them in the same analysis because they are both found in the nonsaponifiable fraction and exhibit similar physicochemical properties.

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Simultaneous determination of cholesterol and α-tocopherol in eggs by LC has been reported (20). However, such a determination has not been achieved yet by GC. With its excellent precision, accuracy, and high degree of automation, GC has become the method of choice for cholesterol analysis, especially when many samples are to be analyzed (6, 21). The very few reports on simultaneous determination of cholesterol and α-tocopherol by GC either concern analysis of matrixes other than egg (22, 23) or are limited to issues relating to chromatographic behavior of analytes, which in many GC systems produce peaks with nearly identical retention times (10, 21).

Here, we describe a simple GC method for simultaneous determination of cholesterol and α-tocopherol in eggs. It involves rapid sample preparation accomplished in one tube and capillary GC analysis that does not require derivatization of analytes. To ensure selectivity, accuracy, and precision, critical analytical parameters were investigated.

## METHOD

### *Apparatus*

(a) *Capillary column GC system.*—Shimadzu Model GC-15A GC system equipped with Model AOC-17 autosampler, flame ionization detector, and Model Class-VP chromatography data system (Shimadzu Corp., Kyoto, Japan). Operating conditions: fused silica capillary column, 15 m × 0.32 mm id, coated with SPB-1 (Supelco, Inc., Bellefonte, PA) with film thickness of 1.0 μm; oven temperature, programmed from 250° to 275°C at 2°C/min and held there for 12 min; helium carrier gas, 2 mL/min; hydrogen, 30 mL/min; air, 300 mL/min; injection port temperature, 300°C; flame ionization detector temperature, 300°C; split ratio, 20:1; injection volume, 1 μL.

(b) *Sample preparation tubes.*—15 × 150 mm culture tubes with Teflon-lined screw cap suitable for sterilizing liquids (Corning, Inc., Corning, NY).

(c) *Water bath.*—Temperature regulated (±1°C; Model 3044, Kottermann, Hanigsen, Germany).

(d) *Vortex mixer.*—Model G-560E, Scientific Industries (Bohemia, NY).

**Table 1. Influence of temperature, heating time, and KOH strength on efficiency of saponification of egg yolk samples, as represented by recovered cholesterol (mg cholesterol/100 g yolk)**

| Saponification time, min | 0.5M KOH | | 2.0M KOH | | Saturated KOH | |
|---|---|---|---|---|---|---|
| | 60°C | 80°C | 60°C | 80°C | 60°C | 80°C |
| 5 | 946.0 | 986.2 | 995.8 | 1005.6 | 954.6 | 966.6 |
| 15 | 1144.2 | 1174.3 | 1135.2 | 1128.9 | 930.0 | 944.0 |
| 30 | 1175.0 | 1126.0 | 1054.8 | 1069.8 | 926.2 | 918.6 |
| 60 | 1154.8 | 1169.8 | 1035.6 | 1029.9 | 932.8 | 922.4 |
| 120 | 1164.2 | 1162.1 | 1018.0 | 1007.5 | 912.0 | 920.8 |

(e) *Centrifuge.*—IEC Model Centra-MP4, equipped with 6-position rotor with 15 mL carriers (Needman Heights, MA).

(f) *Solvent dispensers.*—5.0 mL (Model P5000), and 1.0 mL (Model P1000), precision pipettes (Gilson, Villiers-le-Bel, France) to conveniently dispense solvents.

(g) *Magnetic stirrer plate.*—With variable speed control (Fisher Scientific, Pittsburgh, PA).

(h) *Autosampler vials.*—Teflon-lined screw-cap vials with 1.5 mL capacity (Shimadzu).

### Reagents

(a) *Hexane, methanol, and potassium hydroxide (KOH).*—Analytical grade (Merck, Darmstadt, Germany).

(b) *Cholesterol and α-tocopherol standard solutions.*—Using cholesterol and α-tocopherol (>99% purity) reference standards (Sigma Chemical Co., St. Louis, MO), prepare individual 2 mg/mL stock solutions in hexane. Prepare individual standard intermediate solutions by diluting portions of the stock solutions with hexane. Prepare mixed standard working solutions by transferring appropriate volumes from each standard intermediate solution into 10 mL flasks and diluting to volume with hexane to cover the range 6–54 μg/mL for each analyte (6.7, 13.3, 20.0, 33.3, and 53.3 μg/mL). Protect solutions from light, and keep them at –20°C when not in use. Prepare fresh standard intermediate solutions every month for cholesterol and each working day for α-tocopherol.

(c) *Methanolic KOH solution (0.5M).*—Prepare by dissolving, with stirring, 14 g KOH into methanol and diluting to 500 mL with methanol.

(d) *Pyrocatechol solution (200 mg/mL).*—Prepare by dissolving 1 g pyrocatechol (Sigma) in 5 mL methanol. Protect from light, and keep in a refrigerator when not in use. Prepare fresh every day.

### Sample Preparation

Accurately weigh 0.2 g (±0.001) egg yolk into sample preparation tube. Add 100 μL pyrocatechol solution and 5 mL methanolic KOH solution, agitate immediately on Vortex mixer for 20 s, and cap tightly. Immerse lower half of tube in 80°C bath for 15 min; remove and agitate on Vortex mixer for 15 s every 5 min. Several tubes can be handled conveniently by placing them in a wire basket. Cool tube with tap water, remove cap, add 1 mL water and 5 mL hexane, and agitate vigorously on Vortex mixer for 1 min. Centrifuge 1 min at 2000 × *g*, transfer a portion of the upper phase to the autosampler vial, and close vial cap.

### Chromatography, Preparation of Calibration Curve, and Calculations

Generate calibration curve by injecting 1 μL from each mixed standard working solution; plotting peak areas vs mass of analytes injected; and computing slope, intercept, and least-square fit of standard curves. Use calibration curve slopes and intercept data to compute mass of analytes in injected (1 μL) unknown sample extracts. For cholesterol determination, appropriately dilute sample extracts with hexane and reinject. Calculate concentration of cholesterol and α-tocopherol in unknown samples as follows:

$$\text{Analyte concentration, mg/100 g} = M \times V \times 2.5$$

where $M$ is the mass (ng) of each analyte in injected sample extract (1 μL) according to corresponding calibration curve and $V$ is the dilution factor, if any, applied.

## Results and Discussion

Determination of cholesterol in eggs has been studied extensively. Among commonly used GC methods, some require extraction of total lipids, removal of solvents, hot saponification in alkaline media, extraction of nonsaponifiable material, repeated washes, concentration of extracts, and derivatization prior to analysis (13, 17). These steps are time consuming, as well as labor and material intensive. Of at least equal importance is the interlaboratory variability reportedly inherent in such highly manipulative treatments (24). Other more recent methods based on direct saponification of sample (15, 16, 18) have eliminated some of the steps. However, cholesterol determinations remain laborious and costly, requiring hazardous reagents, the procurement, recovery, and disposal of which are becoming increasingly expensive. Our method minimizes time, labor, and expendable materials, with sample preparation essentially completed in a single tube. To keep the procedure as simple and reliable as possible, all analytical steps were thoroughly investigated.

**Table 2. Effect of vegetable fat on recovery of cholesterol and α-tocopherol[a]**

| Vegetable fat added, g/tube | Recovery of cholesterol, % | Recovery of α-tocopherol, % |
|---|---|---|
| 0.050 | 99.2 ± 1.2 | 101.0 ± 1.6 |
| 0.100 | 96.8 ± 1.8 | 99.2 ± 2.6 |
| 0.150 | 100.4 ± 1.0 | 97.4 ± 2.8 |
| 0.200 | 99.6 ± 1.8 | 97.0 ± 1.2 |
| 0.250 | 98.2 ± 1.6 | 99.0 ± 2.8 |
| 0.300 | 91.6 ± 2.4 | 93.8 ± 1.8 |

[a] Values are means ± standard deviations; $n = 3$.

### *Direct-Saponification Step*

Conditions for direct saponification of food samples are not well documented; various combinations of temperature, heating time, and KOH concentration are claimed to be suitable for converting esterified cholesterol to its free form (21). To establish optimum saponification conditions, 60 identical samples (0.2 g pooled egg yolks) were prepared and processed at various temperatures (60° or 80°C), heating times (5, 15, 30, 60, or 120 min), and strengths of methanolic KOH solution (saturated, 0.5M, or 2M). Two samples were processed for each set of conditions and then combined before hexane (10 mL) extraction and processing through the rest of the method.

When the methanolic KOH solution was 0.5M or 2.0M, cholesterol recovery increased as heating time increased from 5 to 15 min (Table 1). This result indicated that a 5 min heating time at either 60° or 80°C is not adequate for complete conversion of esterified cholesterol. Having attained its maximum value at 15 min, cholesterol concentration could remain essentially constant for heating times up to 120 min with 0.5M KOH. With 2.0M KOH, however, cholesterol recovery decreased gradually as heating time was increased. These results suggested that a 15 min saponification at 80°C with 0.5M KOH is sufficient for complete conversion of esterified cholesterol.

With saturated methanolic KOH, cholesterol recovery was very low no matter what temperature or heating time was applied (Table 1). Contrary to the findings in a recent report (18), these results unequivocally show that saponification with saturated methanolic KOH cannot proceed without loss of cholesterol content. Because auto oxidation of cholesterol has been observed in some rigorous hydrolysis schemes (9), the protective effect of antioxidants added before saponification was also evaluated. However, neither pyrogallol, pyrocatechol, nor butylated hydroxytoluene significantly reduced loss of cholesterol.

Unlike recovery of cholesterol, recovery of α-tocopherol was highly dependent on the level and type of added antioxi-

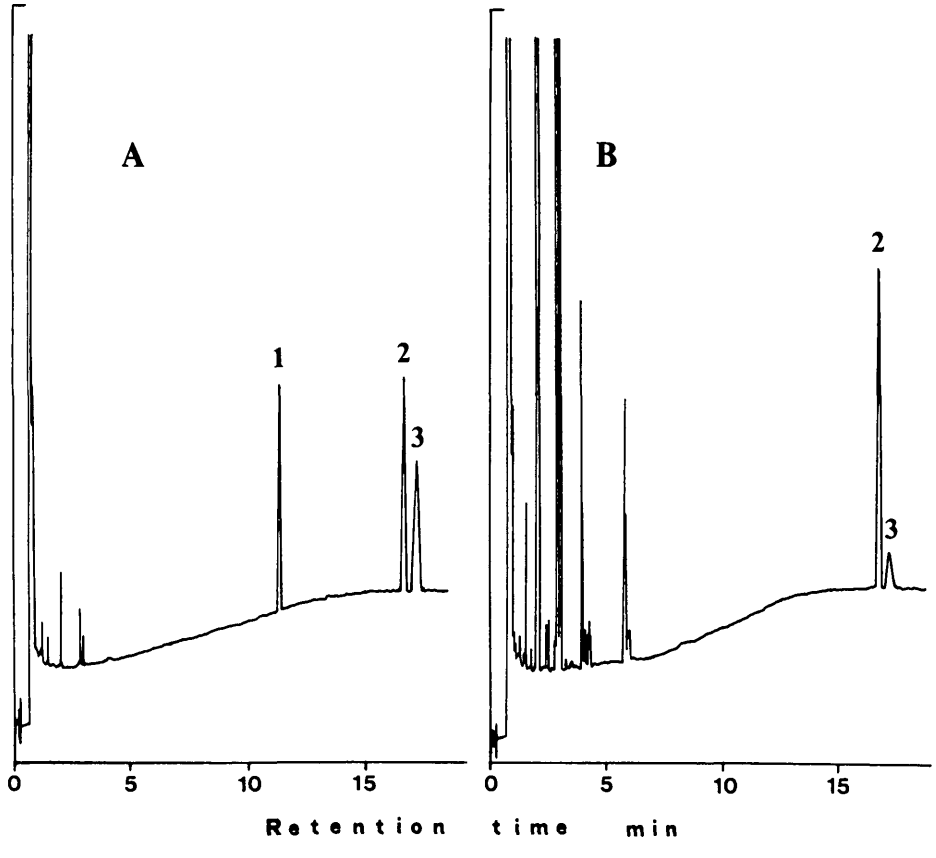


**Figure 1. Typical chromatograms of (A) a standard solution of cholesterol and α-tocopherol containing 5a-cholestane as internal standard and (B) an egg yolk extract. Peaks: 1, 5a-cholestane; 2, cholesterol; 3, α-tocopherol.**

**Table 3. Recovery of cholesterol and α-tocopherol standards added to egg yolk**

| | Analyte added ($n$ = 5), mg/100 g | | Mean concn found, mg/100 g ± SD | | Relative standard deviation, % | |
|---|---|---|---|---|---|---|
| Spiking level | Cholesterol | α-Tocopherol | Cholesterol | α-Tocopherol | Cholesterol | α-Tocopherol |
| 0 | 0 | 0 | 1089.9 ± 19.0 | 7.3 ± 0.3 | 1.7 | 4.1 |
| 1 | 285.7 | 66.8 | 1356.0 ± 32.8 | 74.6 ± 1.1 | 2.4 | 1.5 |
| 2 | 571.4 | 133.6 | 1645.8 ± 23.9 | 139.4 ± 2.4 | 1.5 | 1.7 |
| 3 | 857.1 | 200.3 | 1934.0 ± 26.2 | 206.6 ± 2.8 | 1.4 | 1.3 |

dants. In the absence of antioxidants, α-tocopherol recovery after 15 min saponification at 80°C with 0.5M KOH was only 38%. Addition of 20 mg butylated hydroxytoluene increased recovery to 57%. But addition of 20 mg of either pyrocatechol or pyrogallol totally protected α-tocopherol from oxidation. Pyrocatechol seemed more suitable for use in this method than pyrogallol, because it did not darken as rapidly as pyrogallol did in the alkaline medium.

### *Extraction Step*

When hexane or other nonpolar solvents are used for post-saponification extraction of cholesterol and α-tocopherol from foods and fats, addition of various amounts of water is, in most cases, a mandatory preliminary step (21). Our experiments to establish the amount of water needed for efficient extraction showed that when no water is added, extraction efficiency of hexane for both cholesterol and α-tocopherol is low, ranging from 30 to 40%. Addition of 0.5 mL water increases extraction efficiency to 85–93%. Further addition of 0.5 mL makes extraction quantitative, eliminating the need for a second extraction. Therefore, the minimum volume of water required for efficient extraction is 1 mL. These findings support most published procedures but disagree with a recent method (18) that claims quantitative extraction of cholesterol in the absence of water when saturated methanolic KOH is used. We checked this possibility, but the results did not differ significantly from those initially found.

Slover et al. (22) reported that fat in the saponification mixture also can affect the extraction efficiency. To investigate the matter, we prepared a series of 6 sample preparation tubes so that each one contained a fixed amount of both analytes but a variable amount of vegetable fat (0–0.3 g hydrogenated palm oil) free of either analyte. The content of each tube was saponified, extracted, and chromatographed as described, replicating each analysis 3 times. Results (Table 2) showed that extraction efficiency of hexane is not affected by fat, provided the amount in sample is ≤250 mg.

### *Chromatography*

Although thin-film columns are generally preferred for analysis of high-molecular-mass, high-boiling-point compounds to minimize bleeding from the column, we achieved good results for cholesterol and α-tocopherol with a short thick-film column. As Figure 1 shows, both peaks are sharp without tailing. The thick film covering the active silanol groups on the surface of the fused silica seems to prevent adsorption of underivatized analytes, and thus, peak distortion does not occur. Some fatty acid methyl ester peaks are sizable, but they did not present any separation or contamination problem on the capillary column, eluting very early, just after the solvent front. On the other hand, plant sterols eluted long after α-tocopherol and were well-resolved from each other.

Because chromatographic results were acceptable, we did not consider derivatization of analytes prior to injection onto the GC system, in accordance with other workers (15, 16, 18, 25, 26). Trimethylsilylation of analytes might further improve peak shape, reduce retention time, and improve sensitivity. However, trimethylsilylation not only adds an extra step in the

**Table 4. Precision of determination of cholesterol in egg yolk**

| Day | Concn of cholesterol found, mg/100 g | Mean value ± SD, mg/100 g | RSD, % |
|---|---|---|---|
| 1 | 1073.4, 1044.1, 1098.0, 1069.0, 1096.3 | 1076.2 ± 19.8 | 1.8 |
| 2 | 1118.2, 1101.6, 1070.4, 1122.8, 1090.0 | 1100.6 ± 19.1 | 1.7 |
| 3 | 1087.0, 1068.2, 1114.6, 1098.0, 1101.6 | 1093.9 ± 15.6 | 1.4 |
| | Overall mean | 1090.2 ± 21.0 | 1.9 |

| *Variance estimates* | |
|---|---|
| Source | RSD, % |
| Between-days | 2.6 |
| Within-day | 1.9 |
| Overall | 2.0 |

**Table 5. Precision of determination of α-tocopherol in egg yolk**

| Day | Concn of α-tocopherol found, mg/100 g | Mean value ± SD, mg/100 g | RSD, % |
|---|---|---|---|
| 1 | 7.5, 6.6, 7.0, 7.4, 6.4 | 7.0 ± 0.4 | 6.1 |
| 2 | 7.0, 7.2, 7.9, 7.5, 8.1 | 7.5 ± 0.4 | 5.5 |
| 3 | 7.0, 7.8, 7.2, 8.0, 6.8 | 7.4 ± 0.5 | 6.3 |
| | Overall mean | 7.3 ± 0.5 | 6.8 |
| | Variance estimates | | |
| | Source | | RSD, % |
| | Between-days | | 8.7 |
| | Within-day | | 6.7 |
| | Overall | | 7.0 |

procedure but also could increase noise; lead to formation of artifacts; decrease recovery; result in poor linearity because of silicone deposits in the flame ionization detector; and raise safety concerns because many silylating agents are toxic, flammable, and corrosive.

### *Calibration*

Both internal (15, 16, 18, 25) and external (10, 27, 28) standard calibration techniques have been proposed for analysis of cholesterol and α-tocopherol. Because delivery of sample volumes is quite precise with modern automatic sampling systems, the internal standard (IS) technique is most useful for assays that require extensive sample pretreatment including derivatization, where variable recoveries of the target analytes may occur. For an essentially manipulation-free and well-tested procedure such as the one we describe here, use of the IS technique may not be advantageous. On the contrary, it may actually increase precision error because of the frequent calibration needed for measuring 2 peak areas rather than one (29, 30).

To test the efficiency of external versus internal standard calibration in our system, we prepared 5 sample preparation tubes so that each one contained the same amount of 5a-cholestane (120 μg) as IS and a variable amount of cholesterol and α-tocopherol (33.5, 66.0, 100.0, 166.5, and 266.5 μg of each compound) in 5 mL hexane. Each mixture consisted of standards only, so they were analyzed by GC without saponification, with each analysis replicated 5 times. Cholesterol and α-tocopherol responses as either cholesterol/5a-cholestane and α-tocopherol/5a-cholestane peak area ratios or distinct cholesterol and α-tocopherol peak areas were plotted against amount of cholesterol or α-tocopherol. Regression analysis showed linear responses for both types of calibration and both analytes in the range examined: $y_{IC} = 0.003 + 0.0414x$, response factor = 1.001 ± 0.023, $r_{IC} = 0.99996$ for internal standard calibration of cholesterol; $y_{IT} = 0.001 + 0.0342x$, response factor = 0.838 ± 0.024, $r_{IT} = 0.99986$ for internal standard calibration α-tocopherol; $y_{EC} = 9.22 + 495.78x$, $r_{EC} = 0.99994$ for external standard calibration of cholesterol; and $y_{ET} = 0.50 + 409.63x$, $r_{ET} = 0.99992$ for external standard calibration of α-tocopherol, where $y_{IC}$, and $y_{IT}$ represent peak area ratios, $y_{EC}$ and $y_{ET}$ represent peak areas, and $x$ is the quantity (ng) of analyte injected. The excellent linearities suggested that both techniques are more than adequate for reliable quantitation of target analytes. We selected the external standard calibration technique, because it minimizes manipulations and analysis cost.

**Table 6. Cholesterol and α-tocopherol in commercial eggs (mg/100 g yolk)**

| | Mean concn ± SD ($n$ = 3) | | | Mean concn ± SD ($n$ = 3) | |
|---|---|---|---|---|---|
| Sample No. | Cholesterol | α-Tocopherol | Sample No. | Cholesterol | α-Tocopherol |
| 1 | 1120.3 ± 19.5 | 5.5 ± 0.4 | 11 | 1126.1 ± 21.0 | 7.0 ± 0.2 |
| 2 | 1096.0 ± 12.8 | 4.8 ± 0.3 | 12 | 1269.0 ± 25.4 | 6.8 ± 0.3 |
| 3 | 1077.0 ± 11.0 | 6.2 ± 0.4 | 13 | 1120.7 ± 26.3 | <2 |
| 4 | 1298.2 ± 18.4 | <2 | 14 | 1090.6 ± 15.8 | 2.8 ± 0.1 |
| 5 | 1164.2 ± 21.2 | 7.3 ± 0.3 | 15 | 1521.0 ± 21.1 | 3.0 ± 0.2 |
| 6 | 1196.7 ± 19.0 | 6.2 ± 0.4 | 16 | 1379.0 ± 10.1 | 26.4 ± 1.5 |
| 7 | 1084.8 ± 28.8 | 6.2 ± 0.3 | 17 | 1236.0 ± 13.6 | 112.8 ± 4.3 |
| 8 | 1095.8 ± 29.3 | 6.4 ± 0.4 | 18 | 1090.8 ± 18.2 | 5.2 ± 0.3 |
| 9 | 1110.0 ± 15.4 | 7.0 ± 0.4 | 19 | 1162.0 ± 20.4 | 4.8 ± 0.2 |
| 10 | 1089.8 ± 17.8 | 19.3 ± 1.0 | 20 | 1123.4 ± 17.6 | 5.0 ± 0.1 |

### Accuracy

The standard addition procedure was used to study method accuracy. Fifteen of 20 samples from an egg yolk were spiked with standard cholesterol and α-tocopherol at 3 levels (5 samples at each level) from a methanolic solution containing both analytes. Least-squares and regression analyses of the data (Table 3) based solely on the 3-level spiking showed that the relationship between "added" ($x$) and "found" ($y$) for each analyte was adequately described by a linear regression: $y = 1067.3 + 1.011x$, r = 0.9931 for cholesterol; $y = 8.1 + 0.989x$, r = 0.9991 for α-tocopherol. The intercepts of these regression lines, which represent the values (mg/100 g) predicted for unspiked samples, were not significantly different from the arithmetic means of the unspiked samples (1067.3 versus 1089.9 for cholesterol; 8.1 versus 7.3 for α-tocopherol), suggesting the absence of interference in extracted samples. The absence of interference permitted evaluation of accuracy based on data from both spiked and unspiked samples. Least-squares and regression analyses of these data gave acceptable linearities: $y = 1083.1 + 0.988x$, r = 0.9964 for cholesterol; $y = 7.6 + 0.992x$, r = 0.9996 for α-tocopherol. Therefore, the slopes (0.988 and 0.992 for cholesterol and α-tocopherol, respectively) of these regression lines could be used as estimates of overall recovery (98.8% for cholesterol; 99.2% for α-tocopherol) for the proposed method.

### Precision

Method precision was evaluated by assaying on each of 3 different days 5 egg yolk samples. To estimate overall precision, raw data were subjected to analysis of variance and expected mean squares for one-way classification-balanced design (31). Tables 4 and 5 show that the within-day precision was better than between-days precision for both analytes. Overall precisions were 2.0% for cholesterol and 7.0% for α-tocopherol in egg yolk.

### Cholesterol and α-Tocopherol in Egg Samples

Results of analysis of eggs from various local markets (Table 6) demonstrate method applicability. Cholesterol concentrations in the egg yolks ranged from 1077 to 1521 mg/100 g. This variability cannot be attributed to analytical errors, because all values are means of triplicate analyses. Extensive studies on the modification of egg composition have shown that genetics (32), diet (33), and management (34) can influence cholesterol level in eggs. Nevertheless, cholesterol values found in this study are comparable with data obtained by other workers (5, 6, 15, 20), although higher than some recently reported results (18).

Unlike cholesterol levels, α-tocopherol levels show enormous variation. Although most samples contained α-tocopherol in the range 2.8–7.3 mg/100 g egg yolk, 2 samples did not contain α-tocopherol (limit of detection corresponded to 2 mg/100 g egg yolk for a peak-to-noise ratio of 3:1), and 3 samples contained 19.3, 26.4, and 112.8 mg/100 g egg yolk. Slover (35) reported an α-tocopherol level of 1.2 mg; McLaughlin et al. (36), 2.05 mg; and Syvaoja et al. (37), 5.5 mg/100 g egg yolk. Feeding vitamin E supplements to hens may be the reason for the higher α-tocopherol levels found in this study compared with earlier studies. Surai et al. (38) showed that vitamin E transfer from the diet to the egg yolk takes place very rapidly. An increase of vitamin E supplementation in the hen diet of up to 320 mg/kg feed results in eggs with a vitamin E level in the yolk of about 70 mg/100 g (39). Lower levels of vitamin E (about 40 mg/100 g) in egg yolk have been found after supplementation of the hen diet with α-tocopherol acetate at 100 mg/kg (40).

Most GC methods for determining cholesterol in eggs cannot discriminate between cholesterol and α-tocopherol, because the 2 compounds exhibit similar physicochemical properties. Therefore, faulty results may be obtained by these earlier methods when eggs contain the high levels of α-tocopherol found in this study or even higher.

## Conclusions

The method has satisfactory analytical characteristics with respect to recovery, selectivity, and reproducibility. It is very rapid and simple, offering considerable savings in solvent, materials, sample manipulation, and analysis time. For analysis of 16 samples, sample preparation can be completed by a single analyst in about 1 h. The GC determinative procedure requires about 20 min for each sample but automation can extend analytical capacity. The method may be particularly suitable for laboratories where large throughput of compliance samples is obligatory.

## References

(1) Renaud, S., & De Longeril, M. (1994) *Repr. Nutr. Dev.* **34**, 599–607

(2) Hargis, P.S. (1988) *World's Poultry Sci. J.* **44**, 17–29

(3) U.S. Department of Agriculture (1976) *USDA Agriculture Handbook*, 8-1, USDA, Washington, DC

(4) U.S. Department of Agriculture (1989) *USDA Agriculture Handbook*, 8-1, USDA, Washington, DC

(5) Beyer, R.S., & Jensen, L.S. (1989) *J. Agric. Food Chem.* **37**, 917–920

(6) Jiang, Z., Fenton, M., & Sim, J.S. (1991) *Poultry Sci.* **70**, 1015–1019

(7) Marshall, M.W., Clevidence, B.A., Thompson, R.H., & Judd, J.T. (1989) *J. Food Compos. Anal.* **2**, 228–237

(8) Tsen, C.C. (1961) *Anal. Chem.* **33**, 849–851

(9) Bohac, C.E., Rhee, K.S., Cross, H.R., & Ono, K. (1988) *J. Food Sci.* **53**, 1642–1644

(10) Sheppard, A.J., Newkirk, D.R., Hubbard, W.D., & Osgood, T. (1977) *J. Assoc. Off. Anal. Chem.* **60**, 1302–1306

(11) Ball, G.F.M. (1996) in *Handbook of Food Analysis*, L.M.L. Nollet (Ed.), Marcel Dekker, New York, NY, pp 601–647

(12) Newkirk, D.R., & Sheppard, A.J. (1981) *J. Assoc. Off. Anal. Chem.* **64**, 54–57

(13) *Official Methods of Analysis* (1990) 15th Ed., AOAC, Arlington, VA, **sec 976.26**

(14) Van Elswyk, M.E., Schake, L.S., & Hargis, P.S. (1990) *Poultry Sci.* **70**, 1258–1260

(15) Fenton, M., & Sim, J.S. (1991) *J. Chromatogr.* **540**, 323–329
(16) Al-Hasani, S.M., Hlavac, J., & Carpenter, M.W. (1993) *J. AOAC Int.* **76**, 902–906
(17) Guardiola, F., Codony, R., Rafecas, M., & Boatella, J. (1994) *J. Am. Oil Chem. Soc.* **71**, 867–871
(18) Naeemi, E.D., Ahmad, N., Al-Sharrah, T.K., & Behbahani, M. (1995) *J. AOAC Int.* **78**, 1522–1525
(19) Sempore, B.G., & Bezard, J.A. (1996) in *Handbook of Food Analysis*, L.M.L. Nollet (Ed.), Marcel Dekker, New York, NY, pp 491–506
(20) Indyk, H.E. (1990) *Analyst* **115**, 1525–1530
(21) Fenton, M. (1992) *J. Chromatogr.* **624**, 369–388
(22) Slover, H.T., Thompson, R.H., & Merola, G.V. (1983) *J. Am Oil Chem. Soc.* **60**, 1524–1528
(23) Ballesteros, E., Gallego, M., & Valcarcel, M. (1996) *J. Chromatogr.* **719**, 221–227
(24) Lognay, G., Marlier, M., Dreze, P., Severin, M., & Wagstaffe, P.J. (1989) *Analyst* **114**, 1287–1291
(25) Beyer, J.D., Milani, F.X., Dutelle, M.J., & Bradley, R.L.J. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 746–748
(26) Tvrzicka, E., Mares, P., Pisarikova, A., Novakovic, J., & Hrabak, P. (1991) *J. Chromatogr.* **563**, 188–192
(27) Punwar, J.K. (1975) *J. Assoc. Off. Anal. Chem.* **58**, 804–810
(28) Oles, P., Gates, G., Kensiger, S., Patchell, J., Schumacher, D., Showers, T., & Silcox, A. (1990) *J. Assoc. Off. Anal. Chem.* **73**, 724–728
(29) Snyder, L.R., & Kirkland, J.J. (1979) *Introduction to Modern Liquid Chromatography*, John Wiley, New York, NY, pp 246–248
(30) Haefelfinger, P. (1981) *J. Chromatogr.* **218**, 73–81
(31) Wernimont, G.T. (1987) in *Use of Statistics to Evaluate Analytical Methods*, W. Spendley (Ed.), Association of Official Analytical Chemists, Arlington, VA
(32) Washburn, B.A. (1979) *Poultry Sci.* **58**, 529–535
(33) Codony, R., Barroeta, A., & Grobas, S. (1995) in *Proceedings of the VI European Symposium on the Quality of Eggs and Egg Products*, R.C. Briz (Ed.), R.C. Briz, Zaragoza, Spain, pp 361–373
(34) Jiang, Z., & Sim, J.S. (1991) *Poultry Sci.* **70**, 1838–1841
(35) Slover, H.T. (1971) *Lipids* **6**, 291–296
(36) McLaughlin, P.J., & Weihrauch, J.L. (1979) *J. Am. Diet. Assoc.* **75**, 647–665
(37) Syvaoja, E.L., Piironen, V., Varo, P., Koivistoinen, P., & Salminen, K. (1985) *Milchwissenschaft* **40**, 467–469
(38) Surai, P., Ionov, I., Buzhin, A., & Buzhima, N. (1995) in *Proceedings of the VI European Symposium on the Quality of Eggs and Egg Products*, R.C. Briz (Ed.), R.C. Briz, Zaragoza, Spain, pp 387–391
(39) Frigg, M., Whitehead, C.C., & Weber, S. (1992) *Br. Poultry Sci.* **33**, 347–353
(40) Jiang, Y.H., McGeachin, R.B., & Bailey, C.A. (1994) *Poultry Sci.* **73**, 1137–1143