J. Trop. Agric. and Fd. Sc. 36(2)(2008): 000–000

Determination of cholesterol in several types of eggs by gas chromatography

(Penentuan kolesterol di dalam beberapa jenis telur menggunakan kromatografi gas)

K. Madzlan*

Key words: cholesterol, gas chromatography, eggs

Abstract

A gas chromatographic method was used to determine the cholesterol in several types of eggs. The method consists of direct saponification of samples, extraction with hexane and injection on capillary gas chromatography column. Recovery studies of cholesterol in samples at level 300 ppm and 500 ppm gave percentage of recovery of 100.86 ± 0.68 and 99.68 ± 0.51% respectively. The average coefficient of variation (CV) for repeated analyses (n = 10) was 4.1%. Results showed that the amount of cholesterol in chicken eggs (small size), chicken omega eggs and quail eggs were 310.74 ± 3.90, 296.74 ± 1.33 and 410.27 ± 0.70% respectively.

Introduction

The nutritive value and functional properties of eggs are important in human diet. Egg contains protein of high biological value and other nutrients such as vitamins, minerals, phospholipids and other lipids. However, the egg yolk is extremely high in cholesterol and is a potential disadvantage in some human diets.

Cholesterol is an essential structural component of cell membranes and lipoprotein, and serves as the precursor for steroid hormones and bile acids (Maurice et al. 1994). There is an association between blood levels of cholesterol and the risk of coronary heart disease in humans (Stamler et al. 1986) and premature development of artherosclerosis (Oliver 1990). Many national and international organizations have recommended decreasing total intake of cholesterol in developed countries, with the aim of reducing the incidence of coronary heart disease (WHO 1982). The recommendation has led to cholesterophobia and consequently a clear decrease in consumption of egg in European Community (217 eggs/year in 1991) and in the United States (233 eggs/year in 1991) (Richardson 1992).

There are some conflicting views and results in relation to the analytical methods for cholesterol. Colorimetric determination of the cholesterol concentration in eggs and egg products has been questioned because the interfering compounds could lead to a significant overestimation (Beyer and Jensen 1989a). The chromatographic techniques are favoured because of their ability to separate and quantify cholesterol specifically, although Maurice et al. (1994) noted that reported levels obtained by gas chromatography (GC) are higher than those determined by high performance liquid chromatography (HPLC).

*Food Technology Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia
Author's full name: Madzlan Kasran
E-mail: madzlan@mardi.gov. my
©Malaysian Agricultural Research and Development Institute 2008

However, Jiang et al. (1991) reported no significant differences in egg cholesterol values obtained by enzymatic, GC and HPLC methods.

The aim of this study was to determine the cholesterol content in selected egg samples by a gas chromatographic method. These data could be of interest in the study of cholesterol intake. The analytical procedure was characterized to ensure its selectivity, accuracy and precision for the analysis.

Materials and methods

Chemical and reagent

Cholesterol and 5α-cholestane (>99% purity) reference standard were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hexane, methanol and potassium hydroxide (analytical grade) were purchased from Merck (Damstadt, Germany). Methanolic-potassium hydroxide solution (0.5 M) was prepared by dissolving with stirring 14 g potassium hydroxide into methanol and diluting to 500 ml with methanol.

Sample preparation and saponification

Sample preparation and saponification was based on the method developed by Botsoglou et al. (1998). Eggs were randomly collected from different supermarkets in Kuala Lumpur and Selangor. Eggs were boiled, left to cool and homogenized. Two grammes of homogenized egg was weighed in 15 ml sample preparation tube which contained 0.02 g 5α-cholestane as an internal standard. About 5 ml of methanolic-KOH was added and the tube was capped tightly before being vortexed for 20 s.

A lower half of sample tube was immersed in water bath at 80 °C for 15 min. The tube was removed and agitated on vortex mixer for 5 s every 5 min. The tube was then cooled and the cap was removed. About 1 ml of water and 5 ml of hexane were added into the tube and vortexed vigorously for 1 min. The tube was then centrifuged (Hettich Universal 32 R, Germany) at 7,000 rpm for 15 min. Then, 1 µl of upper phase was injected into GC for analysis.

Recovery of standards from spiked sample

Recovery study was done by using milk powder as a sample. An amount of 2 g of homogenized milk was spiked with two levels of 300 ppm and 500 ppm of cholesterol. Samples were extracted, saponified and analyzed for cholesterol as described above.

Repeatability and reproducibility of cholesterol

Repeatability was measured using the variation of five consecutive analyses of the same sample. The precision of the method was determined by intralaboratory analysis of five repetitions of cholesterol determination on the same boiled egg sample for 10 days. The analyses were carried out by the same analyst in the same laboratory by using the same material and reagents.

GC analysis and quantification of cholesterol

Analysis of cholesterol was performed with a Hewlett-Packard (HP) 5890 GC equipped with an on-column capillary injector and a FID detector (Hewlett-Packard Co., Wilmington, Del., USA). A cross-linked methyl siloxane capillary column, 0.32 mm (i.d) x 25 m with 0.52 µm film thickness (HP Ultra 1) was used. A splitless inlet was used to inject samples into capillary column and ramped oven temperature was used.

Initial temperature was 250 °C and held for 2 min. The temperature was increased to 300 °C at 10 °C/min, and held for 2.5 min. Inlet temperature was 280 °C and the detector temperature was 300 °C. Helium was the carrier gas at constant flow of 1.0 ml/min. The area of each peak was integrated using the Chemstation software (Hewlett-Packard Co., Wilmington, Del., USA) and the amount of cholesterol was

calculated using an internal standard, 5α-cholestane (Du and Ahn 2002).

The calculation of cholesterol was as follows:
Calculation of response factor, Rf:

$$Rf = \frac{W_{is} \times A_{std}}{W_{std} \times A_{is}}$$

Where: R_f = Standard response factor
W_{is} = Wt. of internal standard, 5α-cholestane
A_{is} = Peak area of internal standard
W_{std} = Wt. of standard
A_{std} = Peak area of standard

$$\text{Cholesterol content Wmg/100 g sample)} = \frac{A_s \times W_{is} \times 100}{A_{is} \times W_s \times R_f}$$

where: A_s = Peak area of sample
A_{is} = Peak area of internal standard
W_s = Wt. of sample (g)
W_{is} = Wt. of internal standard
R_f = Response factor

Results and discussion

Chromatogram shows that cholesterol peak was sharp without tailing (*Figure 1*). Some fatty acid methyl esters are present just after the solvent peak without contaminating the cholesterol peak. Some workers (Guardiola et al. 1994; Du and Ahn 2002) include derivatisation of analytes prior to injection into GC. However, because of good chromatographic results, derivatisation step of analytes was not included in this study.

Derivatisation through trimethylsilation of analytes might further improve the peak shape, retention time and improve sensitivity. However, trimethylsilation not only adds an extra step but also could increase noise, lead to formation of artifacts, decrease recovery and result in poor linearity because of silicone deposits in the flame ionization detector. It can also raise safety concerns because many silylating agents are toxic, flammable and toxic (Botsoglou et al. 1998).

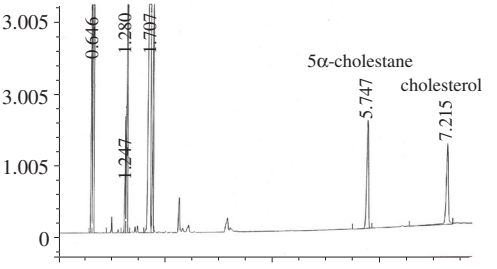

Figure 1. The chromatogram of cholesterol and 5α-cholestane

Determination of cholesterol in eggs has been studied extensively. The common methods used include extraction of total lipids, removal of solvents, hot saponification in alkaline medium, extraction of non-saponifiable material, repeated washes, concentration of extracts and derivatisation prior to analysis. These steps are time consuming as well as material intensive.

The direct saponification of the samples had eliminated some of the steps. Recently, the direct saponification method is employed frequently for the sample preparation of cholesterol (Botsoglou et al. 1998; King et al. 1998; Du and Ahn 2002). Bragagnolo and Amaya (2003) found that the cholesterol level obtained by direct saponification was higher than those obtained by the lipid extract.

Several extraction solvents were used for extracting unsaponifiables, including petroleum ether, hexane and ether acetate (Beyer et al. 1986; King et al. 1998). Hexane is the most commonly used extraction solvent for non-polar lipids. Therefore, direct saponification and hexane for solvent extraction were adopted in this study.

As shown in *Figure 1,* the internal standard 5α-cholestane was first eluted, followed by the cholesterol and the separation was good. The retention time of 5α-cholestane and cholesterol was 5.74 min and 7.21 min respectively which comparatively shorter than the previous study by Guardiola et al. (1994) who reported that the retention time of

Table 1. Recovery of cholesterol standard in milk powder

Spiking level	Cholesterol added (ppm)	Cholesterol found (ppm) (mean ± s.d, n = 8)	% Recovery (mean ± s.d, n = 8)
0	0	8.76 ± 1.37	–
1	300	310.95 ± 2.09	100.86a ± 0.68
2	500	507.42 ± 3.08	99.68a ± 0.51
Average recovery of the method (n = 16)			100.31 ± 0.84

Means with the same letter are not significantly different (p >0.05)

Table 2. Precision of method for the determination of cholesterol in egg

Day	Mean value of cholesterol ± s.d (mg/100 g samples)	CV (%)
1	448.10 ± 7.06	1.57
2	450.60 ± 13.45	2.98
3	440.07 ± 12.87	2.92
4	427.66 ± 9.16	2.14
5	461.02 ± 8.96	1.94
6	482.38 ± 3.23	0.7
7	454.49 ± 11.85	2.61
8	451.93 ± 12.91	2.86
9	446.26 ± 8.49	1.90
10	480.81 ± 10.22	2.13
Overall mean	454.33 ± 18.67	4.1

CV = Coefficient of variation

Table 3. Cholesterol content of chicken egg (small size), chicken omega egg and quail egg

	Cholesterol content (mg/100 g ± s.d. egg), n = 6
Chicken egg (small size)	310.74b ± 3.90
Chicken omega egg	296.74c ± 1.33
Quail egg	410.27a ± 0.70

Means with different letter are significantly different (p <0.05)

cholesterol was 23 min. Botsoglou et al. (1998) and Du and Ahn (2002) reported that the retention time for cholesterol analysis was 26 min and 15 min respectively. In terms of overall analysis which includes saponification, extraction and GC analysis, time of analysis was comparatively shorter than other studies. The differences in retention time was normally due to the different methods used in GC analysis.

The recovery of cholesterol standard spiked in milk powder samples at a level of 300 ppm and 500 ppm is shown in *Table 1*. Results showed very good recovery of cholesterol in spiked samples. The percentage of recovery at 300 ppm and 500 ppm spiked in the samples were 100.86% ± 0.68 and 99.68% ± 0.51 respectively. There were no significant differences (p >0.05) in the recoveries between the repetitions for each level of cholesterol. Botsoglou et al. (1998) reported that the overall recovery of cholesterol was 99.2%. Meanwhile, Du and Ahn (2002) reported that the recovery rate is 97.87%.

The precision of method for the determination of cholesterol in egg as well as the mean value and variability are shown in *Table 2*. The overall coefficient of variation, CV was 4.1%. As indicated by relatively low CV, the precision and reproducibility was good. Du and Ahn (2002) reported that the CV value of triplicate analysis on three different days was 1.11%. Botsoglou et al. (1998) reported that the within-day and between days variation gave overall relative standard deviation of 2.0% at three different days.

There was a significant difference in the cholesterol level of chicken egg (small size), chicken omega egg and quail egg (*Table 3*). Quail egg contained higher level of cholesterol, while chicken omega egg contained the lowest level of cholesterol. The data obtained, however, are much lower than the USDA (2000) values of 8.4 mg/g egg. Variation of cholesterol level in eggs was due to varying spesies, breed, hen's age, egg and yolk weight and diet (Al-Zubaidi and Al-Taha 1984; Beyer and Jensen 1989b; Jiang and Sim 1991; Maurice et al. 1994).

Conclusion

Recovery studies of cholesterol in samples at level 300 ppm and 500 ppm gave percentage of recovery of 100.86 ± 0.68 and 99.68 ± 0.51% respectively. The average coefficient of variation for repeated analyses (n = 10) was 4.1%. Results showed that the amount of cholesterol in egg of chicken eggs (small size), chicken omega egg and quail egg were 310.74 ± 3.90, 296.74 ± 1.33 and 410.27 ± 0.70% respectively.

Acknowledgement

The author wishes to thank to Ms Zaharah Ismail and Ms Harnidah Datu Derin for technical assistance. The project was funded by Ministry of Agriculture and Agro-based Industry and MARDI.

References

Al-Zubaidi, S.S. and Al-Taha, T.J. (1984). Cholesterol content and some physical and chemical components of eggs from chicken of five different eggs. *Iragui Journal of Agricultual Science Zanco 2:* 7–12

Bragagnolo, N. and Amaya, D.R.B. (2003). Comparison of the cholesterol content of Brazilian chicken and quail eggs. *J. Food Comp. Anal. 16:* 147–153

Beyer, J.D., Sullivan, D.M., Smith, R.L. and Richter, E.F. (1986). Gas chromatographic determination of cholesterol in eggs products. *JAOAC Int. 72(5):* 746–748

Beyer, R.S. and Jensen, L.S. (1989a). Cholesterol content of commercially produced eggs in Georgia. *Poultry Science 68:* 1703–1706

—— (1989b). Overestimation of the cholesterol content of eggs. *Journal of Agricultural Food Chemistry 37:* 917–920

Botsoglou, N., Fletouris, D, Psomas, I. and Mantis, A. (1998). Rapid gas chromatographic method for simultaneous determination of cholesterol and α-tocopherol in eggs. *JAOAC Int. 81(6):* 1177–1183

Du, M. and Ahn, D.U. (2002). Simultaneous analysis of tocopherols, cholesterol and phytosterols using gas chromatography. *J. Fd. Sci. 67(5):* 1696–1700

Guardiola, F., Codony, R. Rafecas. M and Boatela. J. (1994). Selective gas chromatographic determination of cholesterol in eggs. *JAOCS 71(8):* 867–871

Jiang, Z., Fenton, M. and Sim, J.S. (1991). Comparison of four different method for egg cholesterol determination. *Poultry Science 70:* 1015–1019

Jiang, Z. and Sim, J.S. (1991). Egg cholesterol values in relation to egg age of laying hens and to egg and yolk weights. *Poutry Science 70:* 1838–1841

King, A.J., Paniangvait, P. and German, J.B. (1998). Rapid method for quantification of cholesterol in turkey meat and products. *J. Fd. Sci. 63(3):* 382–385

Maurice, D.V., Lightsey, S.F., Hsu, K.T., Gaylord, T.G. and Reddy, R.V. (1994). Cholesterol in eggs from different species of poultry determined by capillary GLC. *Food Chemistry 50:* 367–372

Oliver, M.F. (1990). Lipids and coronary disease-resolved and unresolved problem. *Brit. Med. Bull. 46:* 865–872

Richardson, D.I.S. (1992). *Int. Egg Market Rev. 49(1):* 26

Stamler, J., Wentworth, D. and Neaton, J.D. (1986). Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? *JAMA 256:* 2823–2828

USDA (2000). *Nutrient Database for Standard Reference,* Release 13, NDB No. 10199 (United States Department of Agriculture)

WHO (1982). *Prevention of coronary heart disease.* World Health Organization Technical Report Series. WHO, p. 678, Geneva

Abstrak

Kaedah kromatografi gas untuk menentukan kolesterol di dalam beberapa jenis telur kuning telah dijalankan. Kaedah tersebut melibatkan saponifikasi sampel secara terus, pengekstrakan dengan pelarut heksana dan penyuntikan sampel ke dalam kolum kapilari kromatografi gas. Kajian perolehan semula kolesterol ke atas sampel pada tahap 300 ppm dan 500 ppm telah memberikan peratus perolehan masing-masing sebanyak 100.86 ± 0.68 dan 99.68 ± 0.51%. Nilai purata pekali variasi (CV) pada analisis secara ulangan (n = 10) ialah 4.1%. Kandungan kolesterol di dalam kuning telur ayam (saiz kecil), telur ayam omega dan telur burung puyuh masing-masing ialah 310.74 ± 3.90, 296.74 ± 1.33 dan 410.27 ± 0.70%.

Accepted for publication on 3 August 2008