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Article

Cholesterol contents in raw and cooked chicken meat through a validated method

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Abstract: Reliable database of cholesterol content is limited in the diet of many countries of the world. The objective of this study was to determine the cholesterol content in raw and cooked chicken meat through an accurate, precise and validate method. The raw meat sample was collected from Department of Animal Science and Technology, Sunchon National University, South Korea. The cholesterol content was determined by gas chromatography (GC) using an internal standard (5α-cholestane) method for quantification.The method was validated with respect to specificity, chromatographic parameters, linearity, precision, accuracy and limits of detection. Standards Reference Material (SRM) sample, frozen diet composition 1544 was analyzed and the results indicated that the method was accurate with over 97% recovery. The coefficient of variation of repeatability 5.2% and reproducibility 2.4% were also indicated that the method was precise. The cholesterol contents were determined in thigh and breast meat with the values of 95.54 and 71.43 mg/100g, respectively. On the other hand, cholesterol content found in cooked chicken meat with the values of 64.36 mg/100g for boiled chicken soup with ginseng and 79.40 mg/100g for chicken skewer. The present findings suggest that the method was reliable for quantification of large number cholesterol value in food samples.

Keywords: cholesterol; raw and cooked chicken meat; GC; SRM

1. Introduction

Cholesterol is an essential molecule for humans as a component of cell membranes and as a precursor of steroid hormones and bile acids. It is naturally produced in liver of the body and also found in cholesterol rich foods such as beef, pork, chicken, milk, egg, and high fat content foods. The quantitative determination of cholesterol content in foods is very important in relation to nutritional and health aspects. Currently, the nutritional labeling of foods is obligatory due to legal regulations in many countries. In addition, there is a health concern about the role of excess plasma cholesterol on heart- and artery-related diseases (Brown, 1990; Ferguson, 2010; Kannel, 1971). The concept that dietary cholesterol contributes to hypercholesterolemia and cardiovascular disease(CVD) risk has been a fundamental part of public health policy. Dietary recommendations to reduce the risk of cardiovascular disease (CVD) include lowering the contribution to daily energy intakes of total fat and cholesterol in order to avoid their cholesterol raising effects (Gidding *et al.,* 2005). The World Health Organization (WHO) and Food and Agriculture Organization (FAO) of the United Nations have set recommendations for a maximum intake of 300mg cholesterol per day for the adult population.

Cholesterol analysis is usually done by several analytical methods including colorimetry, fluorimetry, gravimetry, enzymatic, and chromatographic methods (Allain *et al.,* 1974; Njeru *et al.*, 1995; Nogueira and Bragagnolo, 2002; Spiric *et al*., 2010; Wu *et al*., 2007). The chromatographic techniques are favored because of their ability to separate and quantify cholesterol. Among the different chromatographic techniques, gas chromatography (GC) (Ballesteros *et al*., 1996; Dinh *et al*., 2008; Fenton, 1992; Fernando and Bean, 1985;

Habib *et al*., 1986) and high-performance liquid chromatography (HPLC) (Beyer and Jensen, 1989; Casiraghi *et al*.,1994; Costa *et al.*, 2006; Csallany *et al*., 1989; Kim *et al.*, 2012) are the most commonly employed methods, with GC recognized as a more practical option with regard to the instrument operation, the chromatographic run speed, and costs (Souza *et al*., 2012). Many reports have also been published for cholesterol analysis using simultaneous saponification procedure which extracts fat soluble compounds such as cholesterol, tocopherols, tocotrienols, retinol, and β-carotene (Ballesteros *et al*., 1996; Chun *et al*., 2006; Katsanidis and Addis, 1999).

The present study aims to produce a reliable cholesterol content food using the simple saponification and extraction method used for fat-soluble vitamin analysis in the United States diet (Chun *et al*., 2006) coupled with GC analysis. Cholesterol content of representative samples composed of chicken skewer and boiled chicken soup with ginseng and raw chicken meat were determined using the said saponification, extraction and quantitation method. Method validation for this assay was performed by determining performance parameters: precision, accuracy, LOD, LOQ, and linearity.

2. Materials and Methods

2.1. Preparation of standards

Cholesterol (Tokyo chemical industry, Tokyo, Japan) and 5α-cholestane (Sigma-Aldrich, MO, USA) were used as external and internal standards, respectively. Cholesterol stock solution (1mg/mL) was prepared and properly diluted with *n*-hexane containing BHT (0.01%, w/v) in a volumetric flask before use. Standard curves were obtained by regression analyses using six concentrations of cholesterol standards (0.05, 0.10, 0.20, 0.40, 0.80, and 1.00 mg/mL) for cholesterol quantification. An internal standard, 5α-cholestane (1mg/mL) was properly diluted with *n*-hexane containing BHT (0.01%, w/v) in a volumetric flask before use. Aliquot of 0.5mL of solution was added with standard and sample as a correction factor to standardize injection errors.

2.2. Sampling and sample preparation

The raw chicken meat sample was collected from Department of Animal Science and Technology, Sunchon National University, South Korea. The cooked meat of chicken dish samples were collected from Korea with the equal population through KFDA (Korea Food and Drug Administration) sampling protocols. All samples were stored at -70°C until analysis. The fat contents in samples were analyzed by the AOAC method (AOAC, 1995). All analysis was completed in duplicate.

2.3. Saponification and extraction

Cholesterol was extracted according to fat-soluble vitamin analysis procedure by Chun et al. (2006) with minor modification. Cholesterol separation was conducted using a HP 5890 Series II (Hewlett-Packard Co., USA) GC system equipped with a HP-5 capillary column (30 mm X 0.32 mm X 0.25 μm, J &W Scientific, USA) and a flame ionizing detector (H_2 : 30 mL/min and air: 300 mL/min). Flow rate of carrier gas (nitrogen) was 3 mL/min. Both injection and detection temperatures were 300°C. Cholesterol was separated on column set to 290°C for 15 min and elevated to 320°C at the rate of 15°C/min to burn up remaining components. Column temperature was decreased to 290°C at the rate of 15°C/min and then maintained for 2 min before the next injection. Cholesterol peaks were identified by comparison of retention times to the standards. The content of cholesterol in samples was calculated using the following equation and expressed as mg/100 g of edible weight:

Cholesterol (mg/100 g) = $K \times P_1 / P_2 \times W$ **x** 100

P_1= Peak area of cholesterol, P_2 = Peak area of 5α-cholestane, W = Sample weight, K = Factor, K value was obtained from internal and external standard curves of peak area vs. concentration.

2.4. Precision and accuracy

Precision and accuracy of cholesterol analysis method were determined with commercial infant formula (Mother's organic-3, Namyang, Korea) used as a quality control (QC) sample in this study. Two different levels of precision were investigated: repeatability and reproducibility. Repeatability precision was determined by repeating analysis of cholesterol in QC sample five times on the same day. Reproducibility precision was determined by analyzing QC sample once a day in duplicates for 5 days. The coefficient of variation of repeatability and reproducibility were calculated.

Accuracy of cholesterol analysis was determined by analyzing cholesterol in SRM 1544 (frozen diet composite). Analytical cholesterol content in SRM samples was compared with the certified cholesterol values provided by NIST and then accuracy was calculated and expressed as recovery of cholesterol from SRM samples.

2.5. Linearity, LOD and LOQ

A linearity test of cholesterol standard curves was completed over the range of 3.9 to 500.0μg/mL. LOD was calculated based on the detector signal-to-noise (S/N) ratio (Food Chemical Codex, 1996). To obtain the LOD, the standard detection of S/N ratio was multiplied by 3 as a factor and then added to the average of the S/N ratio. LOQ was calculated by multiplying the standard deviation (SD) of the response by 10 and then added to the average of the S/N ratio.

3. Results and Discussion

3.1. Method validation of cholesterol analysis

Figure 1 show the GC chromatograms obtained from cholesterol analysis for standards (5α-cholestane and cholesterol) and SRM 1544. Accuracy of cholesterol analysis was evaluated in this study by analyzing SRMs (1544), which are shown in Table 1. Analytical cholesterol value of SRM 1544 was 14.7 ± 0.05 mg/100g. The value was fall within the range of certified value provided by NIST (National Institute of Standards and Technology). That value falls into the range of the respective certified values provided by the NIST, showing that accuracy of this assay is excellent.

The intra-day and inter-day precisions were evaluated by analyses of quality control sample. The coefficient of variation of reproducibility and repeatability were in the range 2.4 to 5.2%, respectively. The estimated results are shown in Table 2. A linearity test of cholesterol standard curves was completed over the range of 3.9 to 500.0 μg/mL. Excellent linear relationship between the peak areas and the amount of injected cholesterol was observed (r^2 = 0.998; graph not shown). LOD indicates the minimum amount or concentration of analyte (cholesterol) that can be consistently distinguished from zero (USFDA, 2015). On the other hand, LOQ is the minimum concentration of analyte (cholesterol) in the test sample that can be quantified with acceptable accuracy and precision (USFDA, 2015). The LOD and LOQ of the saponification coupled with GC method employed for cholesterol analysis in this study were 0.05 and 0.15 mg/100g, respectively.

Table 3 shows the cholesterol and fat content of raw and cooked chicken meat. Cholesterol content was found higher in thigh meat than breast meat due to more fat percent in thigh meat. Moreover, boiled chicken meat showed reduced cholesterol value than raw meat which is agreed with the findings of Talat *et al*., 2000. The two Korean side dish having chicken meat sample showed cholesterol 64.36 mg/100g in boiled chicken soup with ginseng and 79.40 mg/100g in chicken skewer. These analytical data of cholesterol are said to be authentic because the same procedure and machine was used to analyze the SRM sample for cholesterol content.

The saponification technique was performed with KOH in either water (AOAC, 1996a, b; Klatt *et al.,* 1995), or alcohol (Fenton, 1992; Hwang *et al*., 2003). In this study, the saponification using KOH in water with addition of 0.1% BHT was found to be a very effective mean to remove all fatty acids in the form of soaps in the aqueous phase, making them separable during extraction and purification. It has been reported that saponification or hydrolyzation is a very critical step when determining total cholesterol (Hwang *et al.,* 2003; Rodriguez-Palmero *et al.,* 1994; Kaneda *et al*., 1980; Naeemi *et al*., 1995; Klatt *et al*., 1995) in fat content sample.

Table 1. Accuracy of cholesterol analysis for SRM.

SRM[a]	Certified value (mg/100 g)	Analytical value (mg/100 g)	Recovery [b](%)	CV [c](%)
SRM 1544	0.0148 ± 0.0009	0.0147±0.0005	99.1	3.2

[a]SRM indicates standard reference material.SRM1544: frozen diet composite
[b]% Recovery = 100×(analytical value/ certified value)
[c]Coefficient of variation

Table 2. Precision of cholesterol analysis for commercial infant formula.

Sample	Parameters	Repeatability[c]	Reproducibility[d]
[a]Commercial infant formula	Mean±SD	36.1 ± 1.90	37.2 ± 0.90
	CV (%)[b]	5.2	2.4

[a]Commercial infant formula(Mother's organic-3,Namyang, Korea)
[b]Coefficient of variation (%) = (SD/mean) x100
[c]Determined by repeating analysis of cholesterol in QC sample five times on the same day
[d]Determined by analyzing QC sample once a day in duplicates for 5 days

Table 3. Cholesterol and fat contents in raw and cooked chicken meat (n=4*).

Meat sample	Fat (%)	Cholesterol (mg/100g)
Raw		
Thigh meat	0.59 ± 0.05	92.22 ± 7.48
Breast meat	0.34 ± 0.11	74.58 ± 0.89
Cooked Korean food		
Boiled chicken soup with ginseng	3.34 ± 0.19	64.36 ± 3.06
Chicken skewer	3.75 ± 0.23	79.40 ± 4.15

The values are mean ± standard deviation

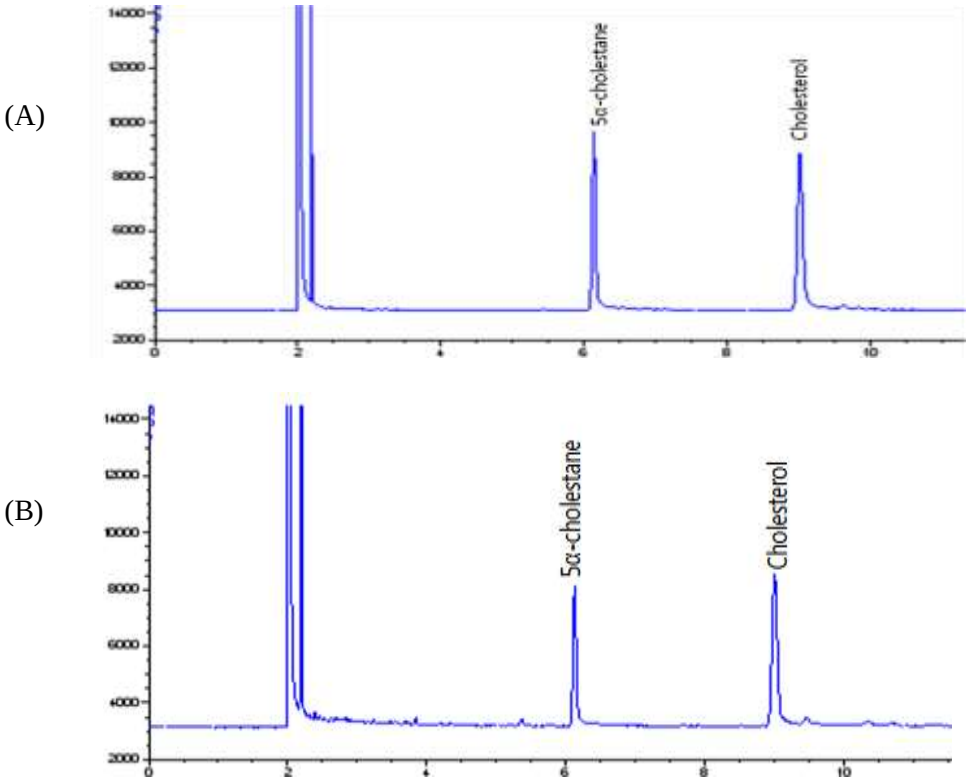

Figure 1. GC chromatograms of standards (5α cholestane and cholesterol) (A) and QC sample (B).

4. Conclusions

The cholesterol values of raw chicken thigh and breast meat were 95.54 and 71.43 mg/100 g respectively. The values for boiled chicken soup with ginseng were 64.36 mg/100 g and 79.40 mg/100 g for chicken skewer. The recovery of SRM 1544 sample was obtained over 99%. So, the results of cholesterol in raw and cooked chicken meat in this study are accurate. In this work, 5α-cholestane and SRM sample were found to be appropriate for the quantification of cholesterol in food sample by GC. The procedure developed in this work can be used for cholesterol analysis.

Conflict of interest

None to declare.

References

Allain CC, LS Poon, CSG Chan, W Richmond and PC Fu, 1974. Enzymatic determination of total serum cholesterol. Clinical Chemistry, 20: 470–475.

AOAC International, 1996a. AOAC Official Method 994.10: Cholesterol in Foods—Direct Saponification—Gas Chromatographic Method. Association of Official Analytical Chemists, Arlington, VA.

AOAC International, 1996b. AOAC Official Method 976.26: Cholesterol in Multi-component Foods—Gas Chromatographic Method. Association of Official Analytical Chemists, Arlington, VA.

Ballesteros E, M Gallego and M Valcarcel, 1996. Gas chromatographic determination of cholesterol and tocopherols in edible oils and fats with automatic removal of interfering triglycerides.Journal of Chromatography A, 719:221–227.

Ballesteros E, M Gallego and M Valcarcel, 1996.Gas chromatographic determination of cholesterol and tocopherols in edible oils and fats with automatic removal of interfering triglycerides.Journal of Chromatography A, 719: 221–227.

Beyer RS and LS Jensen, 1989.Overestimation of the cholesterol content of eggs. Journal of Agricultural and Food Chemistry, 37: 917–920.

Brown WV, 1990. Dietary recommendation to prevent coronary heart disease. Annals of New York Academy of Science 598:376–388.

Casiraghi E, M Lucisano, C Pompei and C Dellea, 1994.Cholesterol determination in butter by high performance chromatography.Milchwissenschaft 49:194–197.

Chun JY, JS Lee, Y Lin, J Exler and RR Eitemmiller, 2006. Tocopherol and tocotrienol contents of raw and processed fruits and vegetables in the United States diet. Journal of Food Composition and Analysis, 19:196–204.

Costa P, LC Roseiro, APartida´rio, V Alves, RJB Bessa, CR Calkins and C Santos, 2006. Influence of slaughter season and sex on fatty acid composition, cholesterol and α-tocopherol contents on different muscles of Barrosa˜-PDO veal. Meat Science, 72:130–139.

Csallany AS, SE Kindom, PB Addis and JH Lee, 1989. HPLC method for quantitation of cholesterol and four of its major oxidation products in muscle and liver tissues. Lipids Jul., 24:645–51.

Dinh TTN, JR Blanton, JC Brooks, MF Miller, and LD Thompson, 2008. A simplified method for cholesterol determination in meat and meat products. Journal of Food Composition and Analysis, 21:306–314.

Fenton M, 1992. Review: chromatographic separation of cholesterol in foods. Journal of Chromatography A, 624:369–388.

Ferguson L, 2010. Meat and cancer.Meat Science 84:308–313.

Fernando T and G Bean, 1985. A comparison of the fatty acids and sterols of seeds of weedy and vegetable species of *Amaranthus spp.* Journal of the American Oil Chemists' Society, 62:89–91.

Gidding SS, BA Dennison, LL Birch, SR Daniels, MW Gillman, and AH Lichtenstein, 2005. Dietary recommendations for children and adolescents: A guide for practitioners: Consensus statement from the American Heart Association. Circulation, 112: 2061–2075.

Habib MA, MA Hammam, AA Saka and YA Ashoush, 1986. Chemical evaluation of Egyptian citrus seeds as potential sources of vegetable oils.Journal of the American Oil Chemists' Society, 63:1192–1197.

Hwang BS, JT Wang and YM Choong, 2003. A simplified method for the quantification of total cholesterol in lipids using gas chromatography. Journal of Food Composition and Analysis, 16:169–178.

Kaneda T, A Nakajima, K Fujimoto, T Kobayashi, S Kiriyama, K Ebihara, T Innami, K Tsuji, E Tsuji, T Kinumaki, H Shimma and S Yoneyama, 1980. Quantitative analysis of cholesterol in foods by gas–liquid chromatography. Journal of Nutrition Science and Vtaminology, 26:497–505.

Kannel WB, WP Castell, T Gordon and PM Mcnamara,1971.Serum cholesterol in lipoprotein and the risk of coronary heart disease. Annals of Internal Medicine, 74:1–12.

Katsanidis E and PB Addis, 1999. Novel HPLC analysis of tocopherols, tocotrienols, and cholesterol in tissue.Free Radical Biology and Medicine, 27:1137–40.

Kim JM, JH Ahn, IS Jeong, BM Kwak, D Leem, T Yoon, C Yoon, J Jeong and JM Park, 2012. Rapid determination of cholesterol in milk containing emulsified foods. Food Chemistry, 135:2411–2417.

Klatt LV, BA Mitchell and RL Smith, 1995. Cholesterol analysis in foods by direct saponification: gas chromatographic method—collaborative study. Journal of AOAC International, 78:75–79.

Naeemi ED, N Ahmad, TK Al-Sharrah and M Behbahani, 1995. Rapid and simple method for determination of cholesterol in processed food. Journal of AOAC International, 78:1522–1525.

Njeru CA, LR McDowell, RM Shireman, NS Wilkinson, LX Rojas and S Williams, 1995.Assessment of vitamin E nutritional status in yearling beef heifers. Journal of Animal Science, 73:1440–1448.

Nogueira GC and N Bragagnolo, 2002. Assessment of methodology for the enzymatic assay of cholesterol in egg noodles. Food Chemistry,79: 267–270.

Rodriguez-Palmero M, S de la Presa-Owens, A Castellote-Bargallo, SMC Lopez, M Rivero-Urgell and MC de la Torre-Boronat, 1994. Determination of sterol content in different food samples by capillary gas chromatography. Journal of Chromatography A, 672: 267–272.

Souza NE, GL Stroher, AC Rodrigues, LF Dias, MR Pedrão, LN Paula and JV Visentainer, 2012. Comparative Analysis and Validation Methodologies of GC and HPLC for Analysis of Cholesterol in Meat Products.American. Journal of Analytical Chemistry, 3: 306–311.

Spiric A, D Trbovic, D Vranic, J Djinovic, R Petronijevic and VM Sverak, 2010.Statistical evaluation of fatty acid profile and cholesterol content in fish (common carp) lipids obtained by different sample preparation procedures.Analytical Chemical Acta, 672:66–71.

Talat T, BM Bhatti and A Rehman, 2000. Estimation of cholesterol content in breast and thigh muscles of different strains of chicken (*Gallus domesticus*). Pakistan Vetenary Journal, 20(4): 206-208.

Wu HL, YT Lin, and SS Wu, 2007. Highly sensitive analysis of cholesterol and sitosterol in foods and human biosamples by liquid chromatography with fluorescence detection. Journal of Chromatography A, 1156:280–287.