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-cholestan (Sigma-Aldrich, MO, USA) were used as external and internal standards, respectively. Cholesterol stock solution (1 mg/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-cholestan (1 mg/mL) was properly diluted with n-hexane containing BHT (0.01%, w/v) in a volumetric flask before use. Aliquot of 0.5 mL 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 (H2: 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×P1/ P2× W x 100

P1 = Peak area of cholesterol, P2 = Peak area of 5α-cholestan, 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 500.0 μg/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 shows the GC chromatograms obtained from cholesterol analysis for standards (5α-cholestan-3-one 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 (r² = 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.

<table>
<thead>
<tr>
<th>SRM</th>
<th>Certified value (mg/100 g)</th>
<th>Analytical value (mg/100 g)</th>
<th>Recovery (%)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRM 1544</td>
<td>0.0148 ± 0.0009</td>
<td>0.0147±0.0005</td>
<td>99.1</td>
<td>3.2</td>
</tr>
</tbody>
</table>

*aSRM indicates standard reference material.*
*SRM1544: frozen diet composite*
*b% Recovery = 100×(analytical value/ certified value)*
*cCoefficient of variation*

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Parameters</th>
<th>Repeatability</th>
<th>Reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCommercial infant formula</td>
<td><em>Mean±SD</em></td>
<td>36.1 ± 1.90</td>
<td>37.2 ± 0.90</td>
</tr>
<tr>
<td>CV (%)</td>
<td>5.2</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

*aCommercial infant formula(Mother’s organic-3,Namyang, Korea)*
*bCoefficient of variation (%) = (SD/mean) x100*
*cDetermined by repeating analysis of cholesterol in QC sample five times on the same day*
*dDetermined 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*).

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

The values are mean ± standard deviation

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


