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Research article

Effects of derivatization coupled with GC-FID analysis of cholesterol in some bakery products

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ABSTRACT

Cholesterol analysis by derivatization technique is a time consuming, costly, and complex process while analyzing cholesterol without derivation is a simple, and quick method.Researchers analyzed cholesterol using both derivatization and non-derivatization techniques successfully. The objective of this study was to investigate the effect of derivatization in cholesterol analysis particularly on bakery goods.The retention time of non-derivatized cholesterol (11.62 min) and non-derivatized α-tocopherol standard (11.60 min) was very close in HP-5 capillary GC column andthey eluted together while injected as mixed standard. As a result, cholesterol content determined by non-derivatized technique could be overestimated due to the presence of α-tocopherol inbakery products. The peak resolution (R_s) between derivatized cholesterol and derivatized α-tocopherol standard using the appliedgradient GC condition was 3.1 which is well separated (>1.5) based on AOAC guidelines. The derivatized gas chromatographic cholesterol analysis method was verified by limit of detection (LOD; 0.03 mg/100 g), limit of quantification (LOQ; 0.08 mg/100 g), linearity (R^2; 0.999),precision (repeatability: relative standard deviation (RSD) 1.5 %; reproducibility: RSD 1.9 %), and accuracy (102.1 % recovery). The verified cholesterol analysis method was subsequently applied to determine cholesterol content in selected bakery items, yielding a range of 2.76 ± 0.06 mg/100 g (chrysanthemum bread) to 114.26 ± 4.72 mg/100 g (castella).

1. Introduction

Cholesterol, a sterol compound with multiple hydrocarbon rings in its structure, serves as a precursor for bile acids, steroid hormones, and provitamin D_3. It plays a vital role in forming the cell membrane structure in our body. However, cholesterol is necessary for proper bodily function, but excessive consumption of dietary cholesterol is not recommended. This is because a high intake of cholesterol leads to increased levels of low-density lipoprotein cholesterol in the blood, which is the primary cause of cardiovascular diseases and premature death [1,2]. Moreover, elevated cholesterol intake may induce cerebral inflammatory markers and contribute to the development of colorectal and breast cancer [3]. Presently, cardiovascular diseases are the leading cause of premature death worldwide. According to projections by the World Health Organization (WHO), cardiovascular diseases are expected to cause over 23 million annual deaths by the year 2030 [4]. Consequently, individuals are increasingly conscious of their cholesterol intake and are opting for low-cholesterol diets instead of cholesterol-rich foods. Thus, it is crucial to understand the cholesterol profiles of commonly

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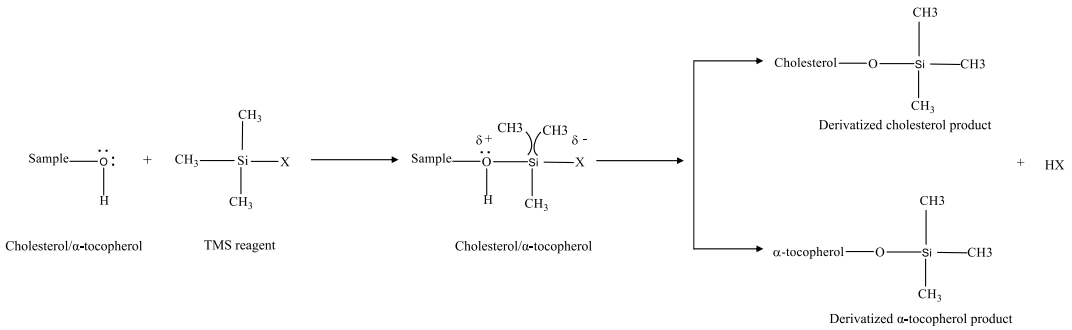

Fig. 1. A brief scheme of cholesterol and α-tocopherol derivatization.

consumed foods to assess personalized dietary choices.

Although red meat, poultry, fish, eggs, and dairy products are well-known sources of cholesterol [5].There are other sources of cholesterol that people may not always be aware of. Examples include bread, cake, and various types of cookies. While these foods contain relatively low levels of cholesterol individually, consuming them in large quantities can have adverse effects on human health. Bread (such as cream bread, buttercream bread, and croissant), cakes, and cookies are typically made with milk, butter, eggs, and wheat flour. Often, people consume more than two-thirds of a serving of these bakery products at once. The cholesterol content of milk, butter, and eggs are 8.2 %, 13.2 %, and 43.6 % respectively [6]. Thus, bread, cake, or cookies made with 3g of butter or 80g of egg would exceed the recommended daily intake of cholesterol (300 mg/day) as specified by the WHO [4]. Consequently, the cholesterol content of these bakery products can no longer be considered negligible, as it significantly affects blood serum cholesterol levels. However, information on the cholesterol content of highly consumed bakery products is limited.

Various methods have been employed to analyze cholesterol, including gravimetry, colorimetry, fluorimetry, and chromatography [2,7–9]. Among these, gas chromatography is considered the most suitable and reliable method to analyzecholesterol, as it allows for the separation of cholesterol from other similar phytosterol compounds [10]. Researchers used both derivatization and non-derivatization technique in gas chromatographic analysis of cholesterol and cholesterol oxidation products [7–14]. Several researchers have successfully developed and validated cholesterol analysis methods without derivatization [9–11]. But still now there is no such study that reveals the effect of derivatizations on the cholesterol content and analysisover non-derivatization.

Derivatization is the process of conversion of an analyte into another compound. Derivatization reaction eases the analytical ability by increasing the volatility, resolution and the specificity of the analyte. It also reduces the analyte absorption in the GC system and improves detector response. Therefore, cholesterol has been analyzed through derivatization process for many years. But derivatization makes cholesterol analysis more time-consuming, complex, and costly. Consequently, researchers are interested in shorter and simpler cholesterol analysis methods that do not require derivatization.Among different derivatization techniques silylation is the widely used derivatization for cholesterol analysis. In TMS derivatization, an active hydrogen (-OH, –COOH, –NH, $–NH_2$, –SH groups) is replaced by a trimethylsilyl leaving group. Silylation reaction is driven by that leaving group. The better the leaving group, the better the silylation.A representative TMS derivatization reaction scheme of cholesterol and α-tocopherol is presented in Fig. 1. Cholesterol and α-tocopherol both have an active hydrogen group (-OH) in their structure. Derivatization converts both cholesterol and α-tocopherol to corresponding identical compounds and helped them to be eluted separately rather than to be co-eluted (Fig. 1).

Therefore, it is important to carefully select the appropriate analytical procedure for cholesterol analysis. This study aimed to investigate cholesterol analysis using both derivatization and non-derivatization techniques to investigate the effect of derivatization for analyzing cholesterol in bakery products. Subsequently, the established technique underwent validation and assessment for its capacity to analyze the cholesterol composition within commonly consumed commercially manufactured bakery items such as bread, cookies, and cake.

2. Materials & methods

2.1. Reagents and materials

Butylated hydroxytoluene (BHT), heptane (99.0 %), cholesterol standard (99.0 %), 5α-cholestane (100 %), N, N-dimethyl formamide (99.8 %), hexamethyldisilazane (99.99 %), and cholrotrimethylsilane (99.98 %) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Potassium hydroxide (85.0 %) and pyrogallol were obtained from Daejung Chemicals & Metals Co. (Siheung-si, Korea) and Tokyo Chemical Industry Co. (Tokyo, Japan) respectively. All reagents used in this study were of GC grade. The National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) provided the standard reference material SRM-1849 (infant/adult nutritional formula) for this study.

2.2. Cholesterol extraction

The Ministry of Food and Drug Safety (MFDS) in the Republic of Korea provided various bakery items, including castella, croissant,

mocha bread, dacquoise, soboro bread, doughnut, pizza bread, butter bread, macaron, cream puff, cheese ball, mammoth bread, chocolate conch bread, apple pie, jam bread, plain bread, and chrysanthemum bread. All the samples were obtained in a uniform form and placed in screw-capped plastic containers. They were transported in an icebox and stored at −70 °C until further analysis. Cholesterol was extracted and measured using the method described by Chun et al. with minor adjustments [14]. Initially, approximately 5 g of each sample was placed in a glass tube with a glass stopper, and then 10 mL of ethanol containing pyrogallol (6 %, w/v) was added. The mixture was thoroughly mixed using a Vortex-Genie 2 vortexer for 10 s and sonicated for 10 min. Next, 8 mL of 60 % (w/v) potassium hydroxide was added and vortexed. The glass tube was then purged with nitrogen gas, sealed with an air condenser, and placed in a shaking water bath at 75 °C and 100 rpm for 60 min. Subsequently, the samples were rapidly cooled with tap water and mixed with 20 mL of a 2 % (w/v) sodium chloride solution. Each sample was vigorously mixed with 15 mL of extraction solution (hexane: ethyl acetate = 85:15, 0.01 % BHT) for 2 min. The solution was allowed to separate into distinct layers for 10 min. The upper unsaponifiable layer was carefully gathered in a 50 mL-volumetric flask by passing it through an anhydrous sodium sulfate layer. This extraction process was repeated two more times, and all the upper layers were combined in the volumetric flask. The resulting sample extract (unsaponifiable layer) was adjusted to 50 mL with the extraction solvent and thoroughly mixed. A 10 mL aliquot of the extract was dried using N_2 gas, and the residue was dissolved in 3 mL of acetone and dried under N_2 gas. All the samples were extracted triplicates.

2.3. Trimethylsilyl derivatization

The extracted bakery samples and standards(cholesterol and α-tocopherol) underwent derivatization. The concentration of the cholesterol and α-tocopherol standard stock solution were 0.025 mg/mL and 0.005 mg/mL respectively.To accomplish this, the dried residues were dissolved in three mL of N, N-dimethyl formamide (DMF). Then, one mL of DMF was mixed with 200 μL of HMDS (Hexamethyldisilazane) and 100 μL of trimethylchlorosilane (TMCS) at room temperature. The mixture was thoroughly mixed and left undisturbed for 15 min. Subsequently, 1 mL of a 0.1 mg/mL 5α-cholestane (internal standard) solution and 10 mL of deionized distilled water were added to the derivatized products and vigorously vortexed. Within 10 min, phase separation occurred, and the upper layer was collected in a GC vial using anhydrous sodium sulfate salt for gas chromatographic separation.

2.4. Without derivatization

Cholesterol extraction was preformed according to section 2.2. Then 1 mL of aliquot were quantitatively taken out from the 50 mL extract and dried using N_2 gas. The dried residues were then redissolved in hexane and filterinto a GC vial through anhydrous sodium sulfate salt for gas chromatographic separation.Cholesterol and α-tocopherol standards were mixedtogether and filteredfor gas chromatographic separation.

2.5. Cholesterol analysis by GC

The cholesterol derivatives were separated using a Hewlett Packard 5890 Series II GC system (Palo Alto, CA, USA) with a 7673A auto-sampler, a Hewlett Packard-5 capillary column (30 m × 0.32 mm × 0.25 μm; Agilent J&W Scientific Inc., Technologies, Folsom, CA, USA), and a flame ionizing detector (FID). The temperatures for injection and detection were both set to 300 °C. Initially, the oven temperature was adjusted to 250 °C. Then, it gradually increased at a rate of 15 °C/min in two stages. The first stage involved ramping up the temperature to 285 °C and maintaining it for 18 min. Subsequently, the temperature was further increased to 300 °C at the same rate and held for 2 min. Afterwards, the oven temperature was decreased to 15 °C/min, resulting in a total run time of 32.47 min for the gas chromatographic method. Hydrogen and air were utilized as fuel gases (H2: 30 mL/min and O2: 300 mL/min), while nitrogen served as the carrier gas (N2: 3 mL/min). The split ratio was 50:1. All analyses were conducted in triplicate.

2.6. Peak identification and quantification

Cholesterol peak identification was performed by using retention time (RT) and relative retention time of the cholesterol standard (RRT). RRT is the ratio of the retention time of internal standard and the retention time of cholesterol standard. The retention time and relative retention time of the derivatized cholesterol standard were12.15 min and 0.68 respectively. RRT is determined by using the following equation (i).

RRT = retention time of internal standard/retention time of cholesterol … … (i)

RRT has no unit, asit is the ratio of two retention times. The identified peak was then quantified by using equation (ii)

$$\text{Cholesterol content (mg/100 g)} = 100\{X(V_3/V_4)/[W(V_2/V_1)]\} \ldots \ldots \quad \text{(ii)}$$

Where, X = cholesterol concentration in a sample.

V_1 = total extract volume.

V_2 = taken extract volume to dry under nitrogen.

V_3 = added total N, N-dimethyl formamide (DMF) volume.

V_4 = taken DMF volume for derivatization.

Cholesterol concentration was determined from a standard curve equation.The verticalaxis of that standard curve represented the ratio of the peak area of cholesterol and internal standard, while the horizontal axis of the standard curve representedcholesterol concentration.Six different cholesterols to internal standard ratios were plotted against six different cholesterol standard concentrations to obtain a standard curve. The standard equation used in this study was$y = 10.3467x+0.0150$where; 'y' is the ratio of cholesterol and internal standard peak area. Cholesterol concentration was represented by 'x'and 0.0150 was the intercept.The R^2 value of the standard curve was 1.0000.

2.7. Method validation

Various method validation parameters were employed to validate the applied methodology, encompassing limits of detection (LOD), limits of quantification (LOQ), linearity, accuracy, and precision.LOD and LOQ for cholesterol were established using a signal-to-noise ratio of 3:1 and 10:1, respectively [14,15].The linearity of the cholesterol standard curve was assessed across six distinct concentrations, ranging from 0.0025 to 0.2000 mg/mL. Cholesterol analysis was validated according to the AOAC single laboratory method validation guideline [16].

The precision of the cholesterol assay was assessed by examining the standard reference material SRM-1869 (infant/adult nutritional formula II). Two distinct levels of precision, namely repeatability and reproducibility, were examined. Repeatability was determined by repeated cholesterol analysis of a commercially produced milk powder five times a day. On the other hand, reproducibility precision was determined by analyzing the milk powder sample once a day for 5 days. In contemporary times, the HorRat value has evolved into an accepted criterion for numerous modern chemical analysis methods endorsed by various international bodies, including AOAC International, the European Union, and other European organizations dedicated to food analysis such as the European Committee for Standardization and the Nordic Analytical Committee. The calculation of HorRat follows the formula devised by William Horwitz and Richard Albert [17].

2.8. Analytical quality control

In this study, a commercially available infant formula (Maeil, Seoul, Korea) was purchased from local market (Suncheon city, South Korea) and employed as the quality control (QC) sample.Obtained from a nearby market in Seoul, Korea, the QC sample was carefully sealed within an airtight poly bag and maintained at a temperature of −20 °C until the analysis phase. Cholesterol contents of commercial infant formula were analyzed during every assay to develop an analytical quality controlchart. The control chart employed mean±2SD and ±3SD lines, referred to as control and action lines, to manage the cholesterol analysis method.

3. Results and discussion

3.1. Problem in cholesterol analysis

We have been analyzing cholesterol of Korean local foods by non-derivatization technique for more than 10 years toestablish national nutritional database to increase awareness among consumers.While analyzing cholesterol content of animal free food products (plant-based food menu), sometimes (unreported peak) we obtained cholesterol similar peak at the time of cholesterol retention time.But cholesterol must not be present in plant-based sample.During cholesterol extraction, fat soluble other compound also extracted together with cholesterol in our analytical method. So, there is a high chance of eluting other cholesterol similar fat-solublecompound at cholesterol retention time. Fenton also demonstrated that cholesterol might overlap with other sterol/α-tocopherol in gas chromatographic analysis [18].

3.2. Cholesterol analysis by non-derivatization

There are also a group of researchers who successfully quantified cholesterol without derivatization from wide range of samples which includes eggs, milk, vegetable oil, human serum and Korean dishes [9–11,13,18,19]. Among them Choong et al. reported high level of cholesterol (193.4–3166.1 μg/g) in vegetable oil which may be the result of overestimation [19]. Cholesterol should not be present in vegetable oil. The absence of a derivatization process and potential failure to distinguish cholesterol from other sterols or α-tocopherols could account for the observed presence of cholesterol in vegetable oil. A study conducted by Fenton suggests the likelihood of co-elution of other sterols and α-tocopherols alongside cholesterol [18]. Consequently, it is our contention that the cholesterol content in vegetable oil, as reported by Choong et al. may not truly represent cholesterol, but rather could possibly be sterols or α-tocopherols.

3.3. Mechanism of derivatization reaction

3.3.1. Peak elution of derivatized-/non-derivatized-cholesterol and α-tocopherol

The derivatized-/non-derivatized-cholesterol and α-tocopherol standard peaks were presented in Supplement Fig.1. The retention time of derivatizedcholesterol and α-tocopherol standard peak were 12.15min and11.80 min, respectively whereasthe retention time of non-derivatized cholesterol and α-tocopherol peak were 11.62 min and 11.60 min, respectively (Supplement Fig.1). The retention time of non-derivatized cholesterol and α-tocopherol peaks were remarkably close to each other. As a result, cholesterol and

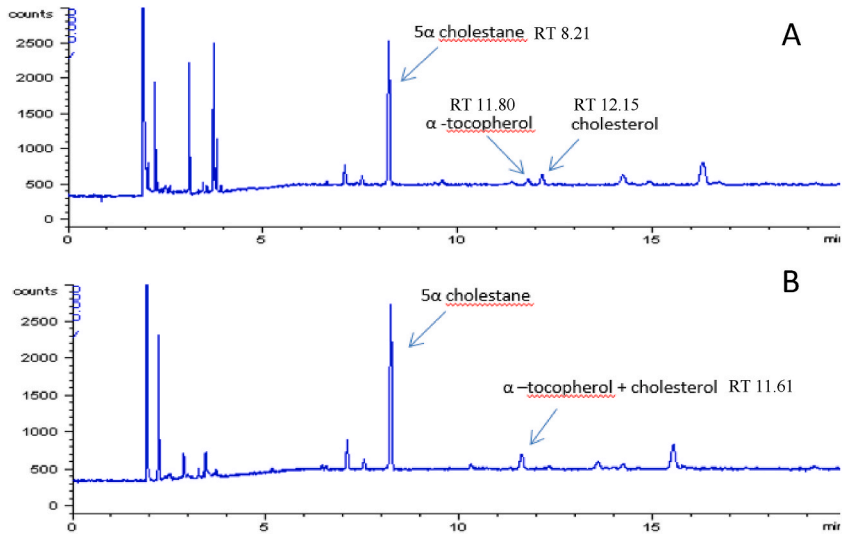

Fig. 2. GC chromatograms of cholesterol and α-tocopherol in jam bread. (A): TMS-derivatized; (B): non-derivatized sample; 5α-cholestane: internal standard.

Table 1
Limit of detection, limit of quantification, linearity,repeatability- and reproducibility-precision and accuracy of cholesterol analysis by saponification-trimethylsilyl derivatization coupled with GC-FID.

Method validation parameter		Cholesterol
LOD[1)]	(mg/100 g)	0.032
LOQ[2)]		0.078
Linearity	Linear equation	y = 10.2691x+0.0064
	R^2	0.9996
Repeatability[3)]	Mean ± SD[5)]	46.908 ± 0.70mg/100 g
	RSD[6)] (%)	1.49
	$HorRat_r$[7)]	0.26
Reproducibility[4)]	Mean ± SD (mg/100 g)	47.54 ± 0.92mg/100 g
	RSD (%)	1.93
	$HorRat_R$[8)]	0.34
Accuracy (SRM-1869)[9)]	Certified value[10)]	13.02 ± 0.47 mg/g
	Analytical value[11)]	13.30 ± 0.17 mg/g
	Recovery (%)	102.1

[1)] Limit of detection.
[2)] Limit of quantification.
[3)] Repeatability refers to the results of independent determinations carried out on a sample by analyzing five replicates of the sample on the same day.
[4)] Reproducibility refers to the results of independent determinations carried out on a sample by analyzing five replicates of the sample (once a day for 5 day).
[5)] Mean of cholesterol contents (mg/100 g) ± standard deviation.
[6)] Relative standard deviation.
[7)] Horwitz ratio for repeatability.
[8)] Horwitz ratio for reproducibility.
[9)] SRM indicates standard reference material.
[10)] Certified value provided by NIST.
[11)] The analytical value obtained by using GC-FID in this study.

α-tocopherol co-eluted together in non-derivatized sample (Fig. 2-B). However, Fenton, could not separate cholesterol from α-tocopherol even after derivatization.

A representative gas chromatogram of separated (derivatized) cholesterol and α-tocopherol and a chromatogram of co-eluted (non-derivatized) cholesterol and α-tocopherol of jam bread were presented in Fig. 2. Peak resolution of cholesterol and α-tocopherol after derivatization was 3.11 (Fig. 2). According to AOAC, for effective separation of two analytes, the resolution of two adjacent peaks must be over 1.5. In this study, the resolution of derivatized cholesterol and α-tocopherol peaks showed an excellent separation.To sum up, the non-derivatization method applied in our study could not separate α-tocopherol from cholesterol, which could overestimate the cholesterol content of the sample (Fig. 2). Derivatization plays a vital role to separate cholesterol and α-tocopherol. If samples have

Table 2
The cholesterol contents of bakery products analyzed by saponification-trimethylsilyl derivatization coupled with GC-FID.

Sample name	Cholesterol content (mg/100 g)
Castela	114.26 ± 4.72
Croissants	80.35 ± 1.97
Mocha bread	52.08 ± 0.38
Dacquoise	50.84 ± 0.48
Soboro bread	50.53 ± 0.65
Doughnut	47.22 ± 0.35
Pizza bread	45.60 ± 0.48
Butter bread	36.21 ± 0.55
Macaron	35.38 ± 0.58
Cream puff	26.70 ± 0.43
Cheese ball	23.23 ± 0.57
Mammoth bread	23.19 ± 0.30
Chocolate conch bread	19.61 ± 0.44
Apple pie	14.39 ± 0.26
Jam bread	3.44 ± 0.15
Plain bread	3.16 ± 0.02
Chrysanthemum bread	2.76 ± 0.06

Cholesterol contents were expressed as mean ± standard deviation.

only cholesterol without α-tocopherol, the cholesterol content could be accurately measured by a simple GC without derivatization. However, derivatization should be considered for cholesterol analysis by GC for the samples containing α-tocopherol.

3.4. Method validation

3.4.1. LOD, LOQ, and linearity

Table 1 displayed the values for limit of detection (LOD), limit of quantification (LOQ), and the linearity of cholesterol analysis. An exceptionally strong linear correlation ($R^2 = 0.999$) was noted between the standard concentration and peak area across a period spanning from 0.0025 mg/mL to 0.2000 mg/mL. LOD and LOQ for cholesterol analysis were found to be 0.03 mg/100 g and 0.08 mg/100 g, respectively. It indicates that this method can detect cholesterol if cholesterol level is $\geq$ 0.03 mg/100 g and quantify cholesterol with acceptable reliability at least 0.08 mg cholesterol/100 g sample. The LOQ obtained in this study is half of the LOQs of Chen et al. [13] and Sharmin et al. [20] showing a good sensitivity in analysis.It indicates that the applied method can quantify cholesterol from very low cholesterol concentration samples.

3.4.2. Precision

Repeatability and reproducibility of cholesterol analysis were performed by a commercial infant formula (Table 1). The relative standard deviation (RSD) of repeatability and reproducibility of cholesterol analysis was 1.49 % and 1.93 % respectively. The applied method demonstrated a coefficient of variation for both repeatability and reproducibility that fell within the acceptable range ($\leq$4 % for repeatability and $\leq$8 % for reproducibility), in accordance with the AOAC guideline. The mean cholesterol content of the commercial infant formula was determined to be 47.23 ± 0.84 mg/100 g.

HorRat stands as another frequently employed performance metric for assessing the suitability of an analytical approach. HorRat values that lie in the range of 0.5–2 are regarded as entirely acceptable. Nevertheless, when the value surpasses 2, it suggests the possibility that the test sample might not beproperly homogenized before analysis or needs further optimization of the applied process or proper training.HorRat values below 0.5 indicate excellent training and experience. In this study, HorRat values of repeatability ($HorRat_r$) and reproducibility ($HorRat_R$) were 0.26 and 0.34 respectively, which indicated that the applied method is excellent in terms of HorRat ratio. Based on RSD and HorRat values of repeatability and reproducibility, the applied analytical method could be used as a precise method to analyze cholesterol.

3.4.3. Accuracy

The accuracy of the applied method was evaluated by analyzing SRM-1869 (Infant/Adult Nutritional Formula II) and presented in Table 1. TMS-derivatized GC chromatogram of cholesterol of SRM-1869 was presented in supplement Fig. 2 which shows that cholesterol and α-tocopherol clearly separated after derivatization. The analytical value obtained by using TMS-derivatization coupled with GC was within the range of the certified valuesfor SRM-1849. The recovery percentage of cholesterol from SRM-1869 was 102.1 %, showing excellent accuracy.

3.5. Cholesterol content of bakery products (bread, cake, and cookie)

The plant-originated foods ingredients in bakery products such as vegetable oil and flour are ampoule sources of α-tocopherol. In general, bakery products such as bread, cake, and cookies have both cholesterol and α-tocopherol derived from wheat flour, butter, milk, egg, etc. In this study, the validated derivatization-GC method was applied to analyze cholesterol from 17 kinds of bakery

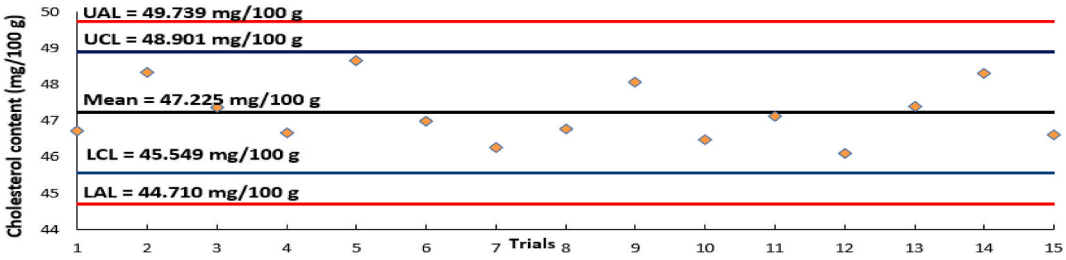

Fig. 3. A quality control chart of cholesterol analysis by saponification-derivatization coupled with gas chromatography.

products and their cholesterol contents were presented in Table 2. The applied derivatization process separated cholesterol from α-tocopherol and helped to quantify cholesterol accurately rather than overestimate. Researchers who did not apply derivatization in cholesterol analysis might have overestimatedthe cholesterol quantification if their sample contains both cholesterol and α-tocopherol. Some researchers did not apply the derivatization technique in their studies but their samples (vegetable/animal fat and oil, fish, meat, etc.) contain both α-tocopherol and cholesterol. Therefore, there is a possibility of cholesterol overestimation in their study.

High variation in cholesterol values with a range of 2.76 ± 0.06 mg/100 g (chrysanthemum bread) to 114.26 ± 4.72 mg/100 g (castella cake) can be attributed due to the different recipe and ingredient composition of the 17 bakery samples in this study. An intake of 270 g castella,375 g croissants,or 600 g mocha bread/soboro bread/dacquoise singly could provide more than 300 mg of cholesterol which is the recommended daily cholesterol level of intake.

Bread is considered one of the staple food products of many countries. Nowadays, Asian people are changing their eating habits to bakery products rather than rice. Although the concentration of cholesterol in bakery products is lower than the cholesterol-rich foods (red meat/shrimp, etc.), it can no longer be negligible if people would consume more as their daily food.Therefore, choosing an accurate procedure for cholesterol quantification in bakery products isnecessary.

3.6. Quality control of cholesterol analysis

Analytical quality control was performed to obtain the reliability of the analytical data of cholesterol levels in bakery products commonly consumed in Korea. Commercial infant formula was used as an internal quality control sample and assayed with every analysis of samples. A quality control chart of cholesterol analysis is shown in Fig. 3. The average cholesterol content of the quality control sample was 47.23 ± 0.84 mg/100 g. All the assay values of the quality control sample were within the upper and lower control lines (48.90 mg/100 g and 45.55 mg/100 g, respectively) of the quality control chart, which means that the cholesterol analysis was performed as validated under control.

4. Conclusion

The non-derivatization technique used for cholesterol analysis failed to distinguish between cholesterol and α-tocopherolin the particular gradient condition used in this study. However, the use of TMS derivatization (HMDS and TMCS) effectively separated cholesterol and α-tocopherol. Therefore, in thisparticular gradient condition the non-derivatization technique can accurately analyze cholesterol when the sample contains only cholesterol and not α-tocopherol. Nevertheless, if the sample contains both α-tocopherol/sterol and cholesterol, using the non-derivatization technique might lead to an overestimation of cholesterol levels. Consequently, when analyzing cholesterol, it is essential to opt for a derivatization technique. However further investigation is needed for different gradient condition and GC column that all the gradient conditions and column shows similar results for derivatization and non-derivatization technique. The credibility of researchers who determined cholesterol levels by similar gradient condition used in this study using the non-derivatization technique is questionable if the sample contains other fat-soluble compounds like α-tocopherol/sterols. The saponification-trimethylsilyl derivatized gas chromatographic cholesterol analysis method applied in this study, was validated based on the single laboratory method validation guideline. The method exhibited excellent accuracy and precision, following the AOAC guidelines. The limit of detection (LOD) of the analytical method was sufficient to detect cholesterol in samples as low as 0.03 mg/100 g. Consequently, reliable information regarding the cholesterol content of bakery products was obtained in this study. This information will be utilized to enhance the national food composition table, which is officially released by the Korea Ministry of Food and Drug and serves as a valuable resource for researchers, nutritionists, and policymakers.

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Data Availability Statements

All data generated or analyzed during this study are included in this article as Tables, Figures, and supplement materials.

CRediT authorship contribution statement

Md Atiqual Islam: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis. **Jiyeon Chun:** Writing – review & editing, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30596.

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