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Unlocking the Cholesterol Content: A Comprehensive Analysis of Cholesterol Levels in Dairy Products, Fats, and Oils Through High-Performance Liquid Chromatography

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Abstract

This study aimed to evaluate the cholesterol content in various dairy products commonly consumed by the general population, as well as fats and oils used in cooking and food preparation. The samples were collected from local markets and farmers in the Aden and Abyan Governorates. To analyze the samples, a highly sensitive high-performance liquid chromatography (HPLC) instrument was utilized. The findings of this study indicated that the highest cholesterol levels were observed in samples of animal ghee and hydrogenated vegetable ghee. Additionally, dairy products derived from full-fat animal milk displayed higher cholesterol content. Specifically, Bint ghee had the highest cholesterol percentage (495.0 mg/100g), followed by local ghee (b) (481.5 mg/100g), Qamaria ghee (453.48 mg/100g), Amazon ghee (411.83 mg/100g), Jabli ghee (368.14 mg/100g), and local ghee (a) (304 mg/100g). Butter samples had a cholesterol concentration of 245 mg/100g, while sheep cheese contained 84.3 mg/100g. Areej ghee recorded 51.9 mg/100g, and bovine cheese had 49.3 mg/100g. The cholesterol percentage in Saudi cheddar cheese reached 42.0 mg/100g. On the other hand, cream, margarine, sesame oil, and Shifa oil samples had cholesterol concentrations below the detection limits, indicating minimal or no cholesterol content.

Keywords: Cholesterol, Dairy products, Fats and oils, High-performance liquid chromatography

1. Introduction

Cholesterol, a lipid-like compound, plays a crucial role in various physiological processes within the human body [1,2]. It is present in cell membranes, the brain, nervous tissues, and the myelin sheath. Cholesterol is also found in substantial amounts in the liver, bile salts, and the skin, where exposure to sunlight converts it into vitamin D. Moreover, the adrenal gland utilizes cholesterol for synthesizing steroid hormones like cortisol, estrogen, and progesterone. The liver produces cholesterol by utilizing fats, carbohydrates, and proteins [1]. Additionally, dietary sources, such as meats, milk, and eggs, contribute to excess cholesterol in the body [3-7]. Conversely, vegetables and plant-based products do not contain cholesterol. The average American daily diet typically contains 400-500 mg of cholesterol, the highest intake worldwide, although the American Heart Association recommends intake of no more than 300 mg per day [3]. Research suggests a link between saturated fats, cholesterol, and various diseases including diabetes, breast cancer,

pancreatic cancer, colon cancer, and atherosclerosis. Atherosclerosis occurs due to the buildup of fatty protein compounds in blood vessels, leading to restricted blood flow, thereby increasing the risk of heart attacks. Factors like lack of exercise, smoking, obesity, and aging further compound the risk of heart disease. Clinically, high cholesterol levels are defined as total cholesterol levels in plasma exceeding 200 mg [8-10]. In the diet, saturated fats can trigger cholesterol production in the liver. Thus, adopting a diet low in cholesterol and saturated fats can help lower blood cholesterol levels. The American Institute for Cancer Research recommends increasing fiber and starch intake through the consumption of vegetables, fruits, and grains, along with moderate portions of low-fat, low-cholesterol foods such as fish, poultry, and lean meats. It is also advised to limit the consumption of cholesterol-rich foods, including eggs, nuts, fried potatoes, red meats, and full-fat dairy products [1]. This study aimed to estimate the cholesterol content in specific dairy products, fats, and local and imported oils. The objective was to verify the cholesterol levels in these products, which are considered significant dietary sources of cholesterol and fats, and to compare them with studies conducted in several countries, utilizing High-Performance Liquid Chromatography (HPLC) as the analytical method.

2. Materials and Research Methods

A Japanese-made high-performance liquid chromatography (HPLC) instrument, specifically the JASCO Co-2065 Plus model, was utilized in this experiment. The instrument comprised a Quaternary Gradient Pump (JASCO PU-2089 Plus) and an Intelligent UV/Vis Detector (JASCO UV-2070 Plus) capable of operating in the visible and ultraviolet ray range (UV-VIS). These instrument components were connected to a computer that housed the necessary programs for the analysis.

For the separation process, a C8 column (Mediterranea HPLC, Mediterranea C8 5µm, 15cm x 0.46cm) was employed, which contained Octyl Silyl (OS) (-Si–CH2)7-CH3) packing material. This column was accommodated in an Intelligent Column Oven (JASCO LC-Net II/ADC).

2-1 Collection of samples

Samples were obtained from various sources including the local market and farmers involved in livestock breeding in the Aden and Abyan governorates. The collection of samples was carried out in a random manner to ensure representative sampling. These samples were carefully placed in sterilized plastic bags and glass containers to maintain their integrity. Afterward, they were transported to the laboratory for further analysis.

In the laboratory, each individual sample was prepared by undergoing a mixing process, and in some cases, heating was applied to facilitate the homogenization of their components. This step was crucial in order to obtain a representative sample that was blended and homogeneous. Sufficient volume of each sample was taken into consideration to ensure accurate analysis, while also allowing for the possibility of repeating certain analyses.

Following the preparation process, the samples were transferred to clean glass containers and stored in a refrigerator to maintain their stability and prevent any degradation prior to analysis.

2-2 Types of samples under study

Assorted Dairy Product Samples: Saudi Cheddar cheese, Five Star cream .*1* packaged in the UAE, Yemeni vegetable margarine obtained from Dhamran supermarket in Mansoura-Aden, traditional cow's milk cheese, traditional sheep's milk cheese obtained from Sheikh Othman market in Aden, light yogurt from Al-Hana, Yemeni-made yogurt from Al-Hana, fresh light yogurt traditionally made from cow's milk, and cow's milk butter obtained from Al-Hisn area in Abyan .Governorate

Ghee and Oil Samples: Areej ghee made in Oman, Amazon ghee packaged in .*2* ,Malaysia, Qamaria ghee, Bint ghee, Jabali ghee, all locally made in Yemen obtained from Dhamran supermarket in Aden governorate. Locally made ghee (a) and (b) made from traditional cow's milk butter at home, sesame oil pressed at the Wali press in Sheikh Othman-Aden, cooking oil (Shifa) made in Oman, obtained .from Al-Hisn area in Abyan

2-3 Preparation of the original cholesterol solution

The primary cholesterol solution was initially prepared by dissolving 0.1 g of pure cholesterol in an adequate amount of methanol in a 100 ml volumetric flask. The solution was then further diluted with methanol to reach the marked level, resulting in an initial stock solution of 1,000 ppm [11].

2-4 Preparation standard solutions from the original cholesterol solution

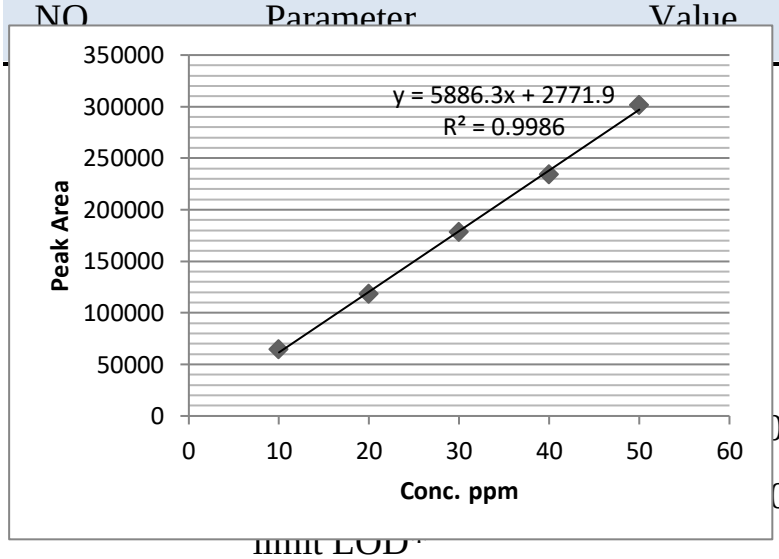
To create standard solutions with varying concentrations (10, 20, 30, 40, 50 ppm), the original cholesterol standard solution (1000 ppm) was used. These standard solutions served as reference samples, with 20 µL of each solution individually injected into the HPLC.

2-5 Calibration curve

To generate a linear calibration curve, standard solutions were prepared and injected into an HPLC/UV instrument using a mobile phase consisting of acetonitrile,

methanol, and 2-propanol in a ratio of 1:3:7. The injections were done sequentially at a flow rate of 1.6 ml/min and at room temperature. A C8 column was used, and the absorbance was measured at a wavelength of 205 nm. The concentrations of the standard solutions were plotted on the x-axis, while the corresponding response values (peak area) were plotted on the y-axis. The slope and intercept of the calibration curve were determined based on the calculated values, which can be found in Table 1 and Figure 1. The response measurements were repeated three times (n=3), and a strong linear correlation was observed with a correlation coefficient (R2) of 0.9986. The linear equation derived from the data is y = 5886.33x + 2771.9 [11].

Table 1: Linear functions of the standard curve for cholesterol.



NO	Parameter	Value
		)01
		0g
	limit LOD*	
8	The quantitative limit of detection LOQ$^{\phi}$	0.684 mg/100g

*It is the lowest detectable concentration of the analyte that can be measured with acceptable statistical accuracy.

$^{\phi}$The minimum detectable concentration for the analyte can be estimated using appropriate standard materials.

Figure 1: Cholesterol calibration curve.

Table 2: Average peak areas for different concentrations of standard solutions of cholesterol.

NO.	Concentration (ppm)	Peak area (micro volts/s) n=3	Wavelength (λ)	Flow rate (mL/min)	Retention time (min)	Mobil Phase
1	10	64680				
2	20	118486				
3	30	178375	205 nm	1.6	3.8	(Acetonitrile, Methanol, 2-Propanol) (1:3:7)
4	40	234057				
5	50	301211				

2-6 Preparation of Saponification Solution

To create the saponification solution, a precise amount of 5.6 g of potassium hydroxide (KOH) was weighed and transferred to a standard 200 ml flask. Methanol was then added to the flask until it reached the mark, resulting in an alcoholic potassium hydroxide solution with a concentration of 0.5 M, as described in references [12,13].

2-7 Preparation of the Moving Phase

The mobile phase utilized in this study consisted of a mixture of acetonitrile, methanol, and 2-propanol in a ratio of 7:3:1. To prepare the mobile phase, 350 ml of acetonitrile, 150 ml of methanol, and 50 ml of 2-propanol were measured using a calibrated standard flask and combined in a 1000 ml bottle. Prior to using the mobile

phase in the HPLC instrument, the bottle containing the mixture was subjected to 15 minutes of ultrasonic cleaning to eliminate any trapped gases [14].

2-8 Estimation of Cholesterol in the Studied Samples

1. To extract cholesterol from the dairy product samples, the direct saponification method was employed. Accurate weights of 0.2 g of butter, 0.6 g of cheeses, and 1 g of cream and yogurt were separately transferred to spiral-capped Teflon-coated test tubes. Saponification was carried out by adding 2 ml of 0.5 M methanolic potassium hydroxide solution to each tube, which was then placed in a hot water bath at 80°C. The tube contents were stirred every 5 minutes during a 15-minute saponification period. The unsaponified portion was then extracted by adding 5 ml of hexane and 2 ml of distilled water. After vigorous shaking for 10 minutes, the mixture was centrifuged at 1400 revolutions per minute for 5 minutes. This extraction process with hexane was repeated three times using 5 ml each time. The resulting hexane extracts were combined in a 50 ml round-bottomed glass flask with a cover. The total extract was evaporated to dryness at 70°C using a rotary evaporator. The remaining extract was dissolved in 2 ml of methanol, and 20 µL of the solution was directly injected into the HPLC instrument. This injection process was repeated three times, and cholesterol quantification was performed using a calibration curve of standard cholesterol solutions. These analyses were conducted at the Higher Institute for Drug Standards and Quality in Khormaksar, Aden Governorate [12,13].

2. The analysis of ghee and oil samples was performed using two different methods. In the first method, local ghee, fragrant ghee, sesame oil, and palm oil underwent direct saponification followed by cholesterol extraction from the unsaponifiable portion using hexane solvent. This extraction process was conducted following the same steps mentioned earlier for the dairy samples. Subsequently, 20 µL of the extract was taken after drying and dissolving it in 2 ml of methanol, which was then injected into an HPLC device. The injection process was repeated three times for consistency [11].

In the second method, samples of local ghee, Qamaria ghee, pent ghee, mountain ghee, and Amazon ghee were prepared by weighing 0.250 g of ghee and dissolving it in 5 ml of acetone. The solution was then transferred to a 50 ml volumetric flask and completed to volume with methanol. To ensure complete dissolution and removal of gases, the flask was subjected to ultrasonic waves for 10 minutes. A portion of the solution was then centrifuged at 1500 revolutions per minute for 10

minutes. Subsequently, 20 µL of the solution was injected into the HPLC instrument three times to obtain consistent readings [11].

2-9 Statistical analysis

The Origin 7.5 program, a powerful statistical analysis tool, was employed to perform rigorous data analysis in this study. To investigate the variations in the data, one-way ANOVA analysis was conducted, which is a robust statistical method commonly utilized in research to determine whether there are significant differences between groups. In this study, a significance level of $P < 0.05$ was adopted, implying that any observed differences between the means had to be statistically significant at this threshold to be considered meaningful.

To further ascertain the presence of statistically significant differences between the means, the least significant difference (LSD) was calculated. The LSD method is an effective post hoc test that enables researchers to compare pairs of means within the groups and determine if there are any statistically significant disparities. This approach provides a comprehensive understanding of the data by assessing the magnitude and significance of differences between multiple sets of mean values.

By employing the Origin 7.5 program for statistical analysis and conducting one-way ANOVA with a significance level of $P < 0.05$, coupled with the calculation of the LSD, this study ensures a rigorous examination of the data, allowing for precise conclusions to be drawn. The implementation of these statistical techniques contributes to the credibility and reliability of the research findings, enabling a comprehensive evaluation of the results obtained [15].

3- Results and Discussion

The chromatographic analysis results of the samples under study are presented in Figures 2-15. These results aim to differentiate between the various types of samples. In particular, the first variety corresponds to dairy products samples, which are classified based on their manufacturing origin and the type of fat added to the product. This includes products with animal fat such as local production butter, cow cheese, sheep cheese, as well as foreign production cheddar cheese. Additionally, there are products with vegetable fat, such as foreign production cream and national production margarine. Furthermore, there are national production products with animal fat, like local light yogurt, and others with vegetable fat, such as Hana light yogurt and Hana yogurt (Table 3).

Moving on to the second variety (Table 4), it pertains to ghee and oil samples. These .samples are categorized according to their country of origin and the type of ghee The classification is as follows: locally produced cow ghee with animal fat, namely local ghee A and local ghee B. Moreover, there is locally produced refined vegetable ghee, including Qamaria, Bint, and Jabali. Additionally, there is commercially ,produced refined vegetable ghee, including Arij ghee and Amazon ghee. Lastly there are vegetable oils, such as locally produced sesame oil and foreign-origin .cooking oil

To remove fats and release cholesterol from the samples, the direct saponification process was employed as an initial step. This process has proven to be successful in recovering and extracting cholesterol using appropriate solvents, thus preventing .any loss of cholesterol [16]

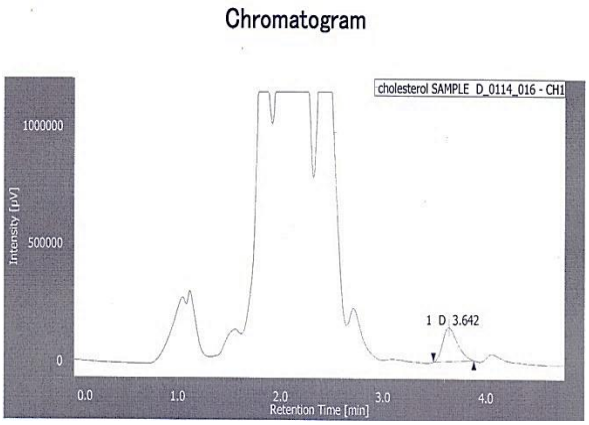


Figure 2: Chromatogram of butter sample (cow)

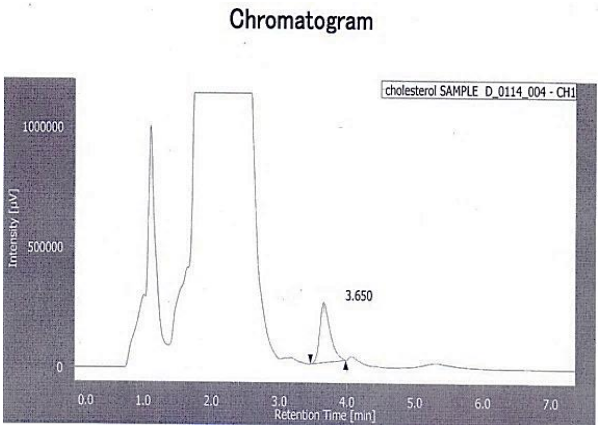


Figure 3: Chromatogram of sheep cheese sample

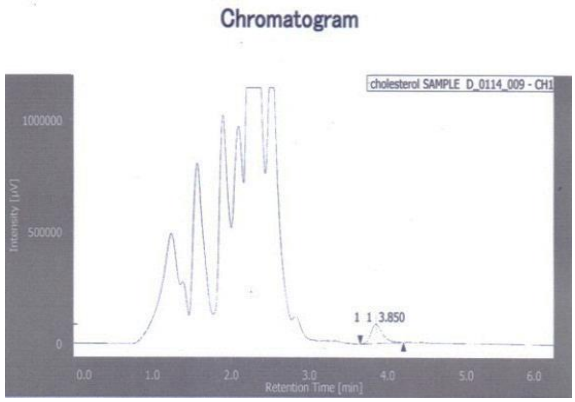


Figure 4: Chromatogram of bovine cheese sample

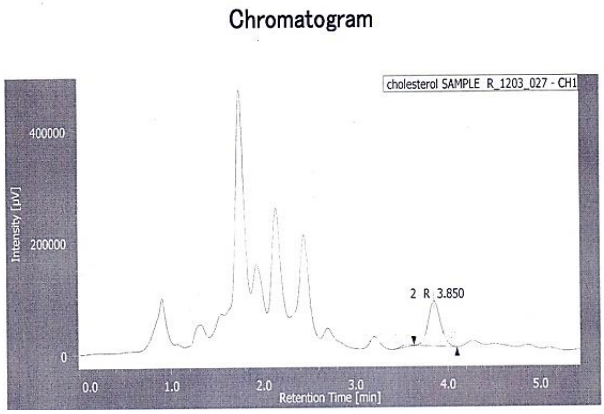


Figure 5: Chromatogram of cheddar cheese sample

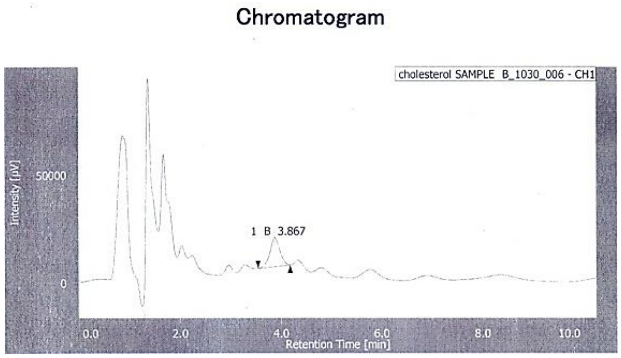


Figure 6: Chromatogram of natural light yogurt sample

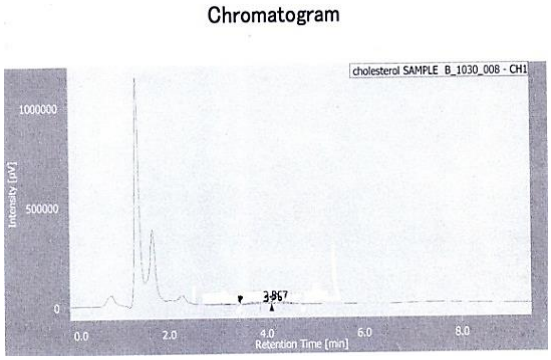


Figure 7: Chromatogram of commercial Al- Hana light yogurt sample

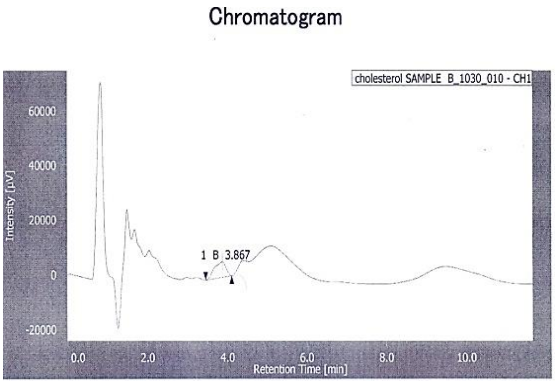


Figure 8: Chromatogram of Al-Hana yogurt sample

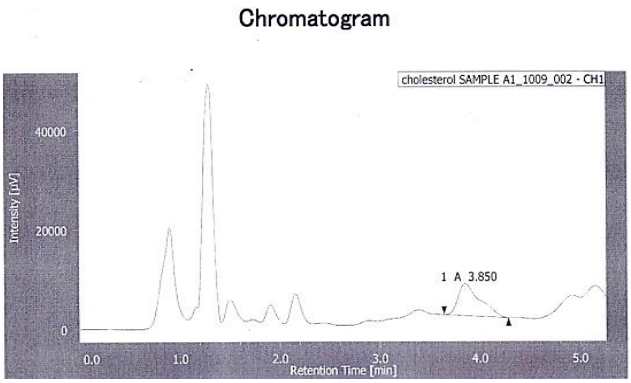


Figure 9: Chromatogram of local ghee (a) sample

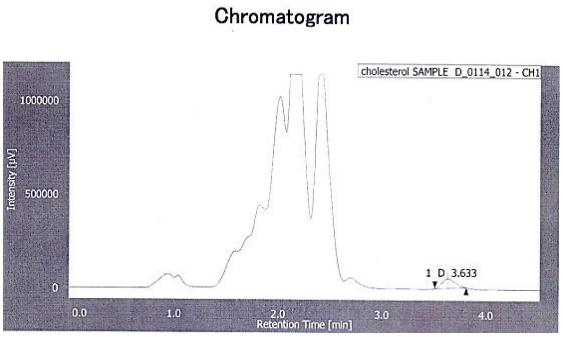


Figure 10: Chromatogram of local ghee (b) sample

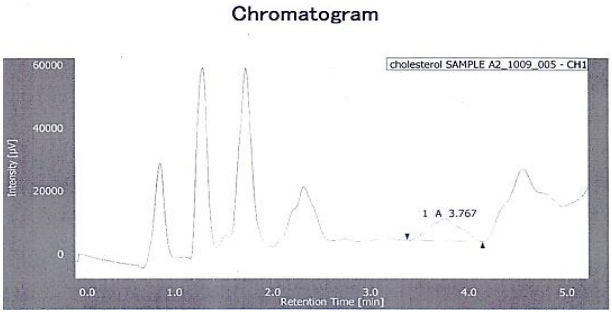


Figure 11: Chromatogram of Bint ghee sample

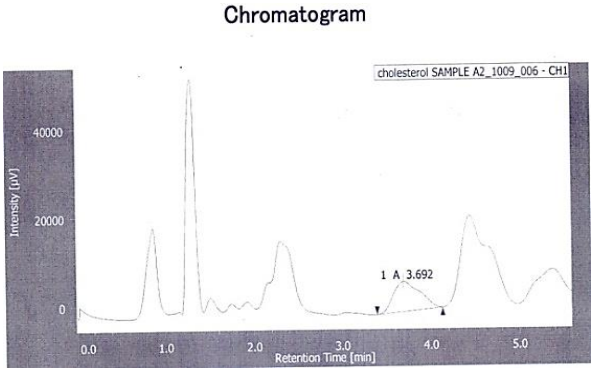


Figure 12: Chromatogram of Qamaria ghee sample

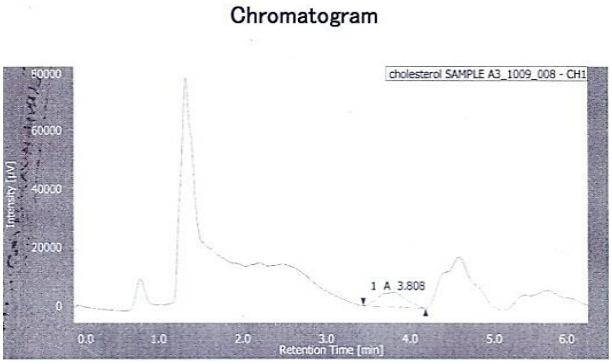


Figure 13: Chromatogram of Jabali ghee sample

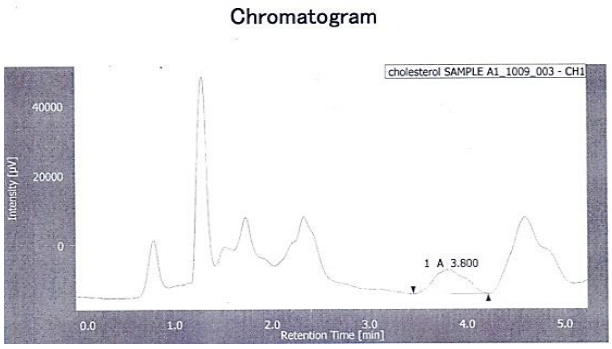


Figure 14: Chromatogram of Amazon ghee sample

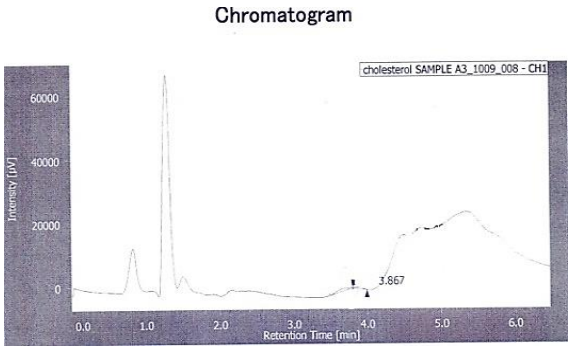


Figure 15: Chromatogram of Arij ghee sample

3-1 Dairy products samples

Based on the results presented in Table 3 and Figure 16, it is evident that there is an inverse relationship between the cholesterol content and the type of fat (animal or

vegetable) present in the dairy products under investigation. The samples belonging to the first category, which includes butter and cheeses (cow, sheep, and cheddar) with high animal fat content, as well as one domestic production sample with animal fat (local breed), exhibited the highest levels of cholesterol content (31.45 mg/100g - 245 of the product). Conversely, the commercial samples with removed animal fat and added vegetable fat (Hana light yogurt and Hana yogurt) showcased the lowest cholesterol content, aligning with the aforementioned inverse relationship. Cream and margarine samples had cholesterol levels below the detection limit of the device.

In our study, we observed that the highest concentration of cholesterol in the butter ,sample (245 mg/100g) exceeded values reported in previous studies [13,17-19] which ranged from 228.1 mg/100g to 235.6 mg/100g. However, our results were in .line with the cholesterol content recorded in [20, 21] (240 mg/100g, 244 mg/100g) Moreover, our findings were lower than values reported as 270.0 mg/100g, 286.4 mg/100g, and 307.0 mg/100g in other studies [22-24]. We also noticed that the cholesterol content in our butter sample closely matched the findings in [14], where the cholesterol content in butter was determined to be 253.5 mg/100g using the same method (HPLC). The study also indicated a cholesterol content of 84.6 mg/100g in .cheese, which aligns with our observation of sheep milk cheese (84.243 mg/100g) However, the cholesterol content in our study for cow's milk cheese and cheddar cheese samples differed. The cholesterol content in the sheep milk cheese sample corresponded to the findings of a study [25] (78.8-97.9 mg/100g). In contrast, the cholesterol content in the butter sample ranged from 227.3 mg/100g to 307.0 ,mg/100g, which matched our study's measurement (245 mg/100g). Furthermore our cheese samples' cholesterol content agreed with [20] (17-114 mg/100g) and was close to [26] (52.3-76.6 mg/100g), while slightly different from [13] (61.9–143.3 mg/100g). Notably, the cholesterol content in our sheep milk cheese (84.24 .mg/100g) aligned with previous studies [13,20,26]

The observed cholesterol levels in our cow's milk cheese (49.13 mg/100g) and cheddar cheese (41.96 mg/100g) were consistent with [20], albeit slightly lower than In study 27, which aimed to determine the cholesterol content in various .[13,26] types of cheese, the values noted for blue cheese (50.38 mg/100g), natural cheese and hard cheese (58.36 mg/100g) were comparable to the findings ,(mg/100g 50.38) in cow's milk cheese and cheddar cheese. However, they were lower than the .cholesterol content in sheep's milk cheese (84.243 mg/100g) in our recent study Additionally, the cholesterol content in goat milk cheese and sheep milk cheese in

were 58.61 mg/100g and 65.06 mg/100g, respectively, which were lower than [27] our current study's results. In our study, the cholesterol content in cheddar cheese was lower than the values reported for English cheddar cheese in (mg/100g 41.96) and in both studies [17,19] (107 mg/100g and 102.41 (mg/100g 101.2) [13] .(mg/100g, respectively

Comparing the cholesterol content (mg/100g) in the samples of natural light yogurt Al-Hana injections (4.52 mg/100g), and commercial Al-Hana ,(mg/100g 6.29) yogurt (3.8 mg/100g) with [27] (8.17 mg/100g), we observe that our values are somewhat similar. The cholesterol content recorded in our study for natural light yogurt with animal fat was higher than that in the injections and Al-Hana yogurt with vegetable fat and no animal fat. This explains the lower cholesterol content in .these samples, which is consistent with the cholesterol content in skimmed dairy Furthermore, [14,20] reported higher cholesterol content in full-fat animal yogurt compared to our (mg/100g, 33.6 mg/100g, and 12.2 mg/100g, respectively 12.46) study's findings, which is expected for light yogurt and Al-Hana yogurt made from animal fat. However, study 15, which employed the HPLC method to determine the cholesterol content in yogurt, reported a cholesterol level of 12.7 mg/100g, slightly higher than our recorded value. Moreover, the cholesterol content reported for yogurt samples (7.33–7.87 mg/100g) in [28] closely aligns with our findings. The cholesterol content in the sample of natural light yogurt was 6.29 mg/100g, while ,yogurt and Al-Hana light yogurt recorded levels of 4.53 mg/100g and 3.8 mg/100g .respectively

The variation in cholesterol content between previous studies and our study may be attributed to various factors, including differences in milk fat content, sample variations, extraction methods, and the cholesterol determination or estimation .methods employed

Table 3: Cholesterol concentration in selected dairy product samples in mg/100g

Origin of samples	Type of fat added to the product	Sample Name	**Peak area mean**	Average concentration (mg/100g)	Standard Deviation (SD)	Relative Standard Deviation (RSD)
Local samples made in a traditional manner.	product animal fat	Butter	1444494	245a	3.357	1.37
		Sheep cheese	1490516	Eighty-four point two four billion	1.226	1.455
		Bovine cheese	870409	49.12c	1.189	2.42
		Cheddar cheese	743768	42nd	1.497	3.57
				LSD= 3.078.		
External samples	Animal fat	Local light yogurt	187921	6.3e,g	0.046	0.734
		Hana light yogurt	136062	4.5f,e,g	0.091	2.03
		Hana yogurt	115044	3.81g	0.140	3.66
				LSD = 0.159		
	Vegetable fat	Cream (Five Star)	N.D			
		Margarine (Momtaz)	N.D			

3-2 Samples of ghee and oil

The analysis of clarified butter and oil samples, as presented in Table 4 and Figure 17, yielded interesting findings. The sample of clarified butter labeled "Bint" displayed the highest cholesterol content (495.04 mg/100g) after undergoing dissolution in methanol without direct saponification and subsequent extraction with

hexane. Conversely, the vegetable ghee "Areej" exhibited the lowest cholesterol level (51.96 mg/100g).

Regarding sesame oil and palm oil samples, which underwent the same treatment as Areej and local clarified butter (ghee b) (saponification followed by extraction of the unsaponified portion with hexane), their cholesterol content fell below the detection limit of the device (N.D). This observation aligns with existing scientific literature [29], which identifies cholesterol as an animal sterol.

The remaining samples, including local clarified butters (ghee a) made from cow's milk fat, Qamiria fats, Bint, Jabali (locally made), and Amazon (imported), are primarily composed of vegetable oil, mostly palm oil. These products are commonly referred to as vegetable ghee or hydrogenated ghee. The labels attached to them state that they are "cholesterol-free".

To analyze these five samples, a distinct approach was employed, involving the dissolution of 0.250g of each sample in an appropriate amount of acetone. The volume was then completed with methanol up to the mark in a 50 ml volumetric flask, as described in [11]. Subsequent analysis using HPLC-UV revealed the following cholesterol content in these samples: 304 mg/100g, 453.5 mg/100g, 495.04 mg/100g, 368.1 mg/100g, and 411.8 mg/100g respectively.

Comparing the cholesterol content in local clarified butter (a) (304 mg/100g) and local clarified butter (b) (481.5 mg/100g), we note that sample (a) has a higher cholesterol content despite both being local clarified butter. This disparity can be attributed to the different treatment methods employed. Saponification, a process that releases cholesterol from fatty acids bound to it in esters, as well as from cholesterol bound to lipoproteins, significantly contributes to the overall cholesterol content. The subsequent extraction with hexane, an effective solvent for cholesterol extraction, further aids in capturing the released cholesterol. On the other hand, local clarified butter sample (a) only contains free cholesterol since it did not undergo saponification. Consequently, the cholesterol content is limited to free cholesterol, resulting in a lower level compared to sample (b).

This interpretation is corroborated by the striking similarity in cholesterol content between basil ghee (306.5 mg/100g), Pharma ghee (304.1 mg/100g), and Almarai ghee (307.6 mg/100g) as reported in [11]. Moreover, the cholesterol content in sample (b) (304 mg/100g) treated in the same manner is lower than that in sample (a), which underwent saponification and hexane extraction, indicating the effectiveness of these processes in enhancing cholesterol extraction.

The cholesterol content in local clarified butter (a) falls within the range reported in [30] (303 mg/100g to 328 mg/100g), slightly surpasses that in [31] (301 mg/100g), and significantly exceeds the value reported in [32] (256 mg/100g).

Interestingly, the cholesterol content in samples of hydrogenated vegetable ghee (Qamaria, Bint, Jabali, and Amazon) exceeds that in local clarified butter (ghee a), despite undergoing the same treatment. This disparity contradicts the "cholesterol-free" claim on the commercial packaging labels and the assertions made in promotional TV advertisements for these products, as they are supposed to be cholesterol-free due to their plant origin. Furthermore, the cholesterol content in these studied hydrogenated vegetable ghee samples surpasses the highest observed levels in Mazola ghee (302.8 mg/100g) and Halwani ghee (226.6 mg/100g) [11].

The variation in cholesterol content observed in previous studies compared to our current study may be attributed to sample discrepancies or lack of standardization, ineffective extraction methods employed, differences in fat content in milk, lack of specificity in the estimation methods utilized, and seasonal variations. Additionally, the density of the fat emulsion, and consequently the cholesterol content, may differ, with high-density fat emulsion showing greater cholesterol amounts compared to low-density fat emulsion.

Table 4: Concentration of cholesterol in samples of ghee and oil in mg/100g

Sample origin	Type of ghee	Sample Name	Peak area mean	Cholesterol concentration (mg/100g)	Standard deviation (SD)	Relative standard deviation (RSD)
Local	Cow ghee	Local ghee a	92630	304	11.464	3.77
		Local ghee b	569543	481.5	21.505	4.46
Local	Vegetable ghee	Qamaria ghee	136243	453.50	11.11	2.45
		Bint ghee	148475	495.0	15.74	3.17
	Commercially hydrogenated	Jabali ghee	111223	368.14	4.277	1.159

Imported	Vegetable ghee Commercially hydrogenated	Arij ghee	63957	51.96	2.365	4.55
		Amazon ghee	123984	411.83	1.632	0.395
Local	Vegetable oil	Sesame oil	N.D			
Imported	Vegetable oil	Cooking oil (Shifa)	N.D			
LSD = 14.843						

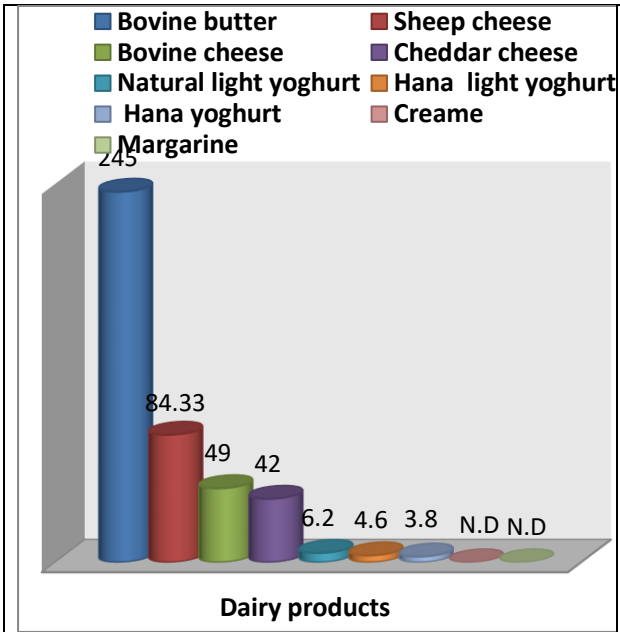


Figure 16: Cholesterol content graphically in samples of dairy products (mg/100g)

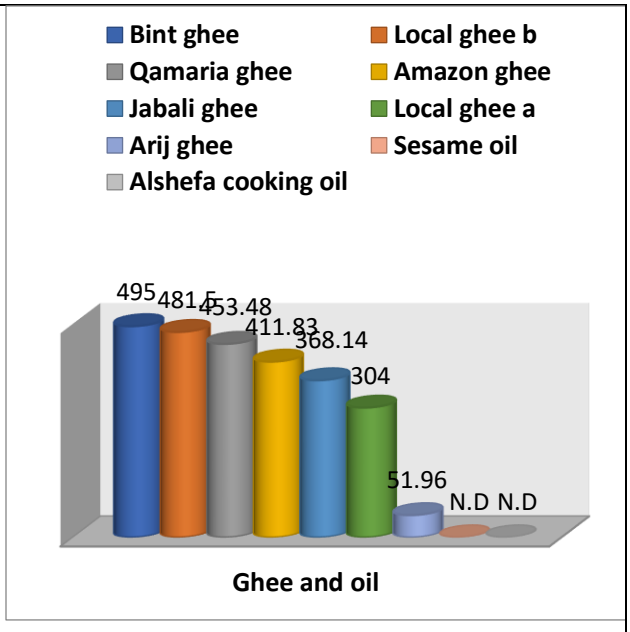


Figure 17: Cholesterol content graphically in ghee samples (mg/100g)

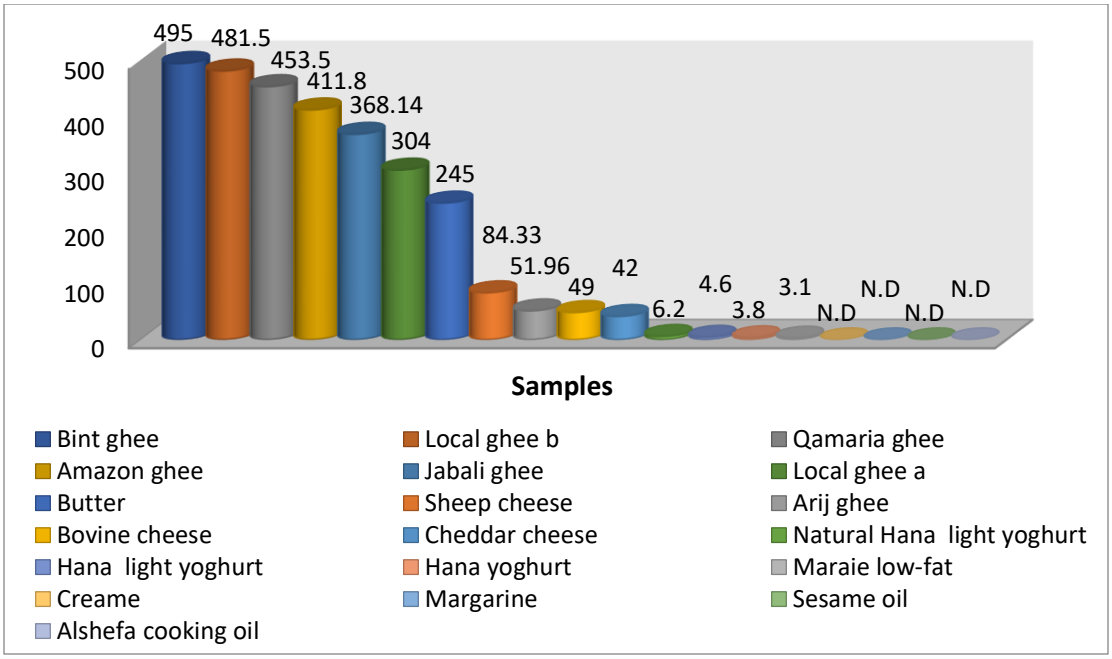


Figure 18: Comparison of cholesterol content in samples of dairy products and ghee (mg/100g)

3-3 Comparison of results of samples of dairy derivatives, fats, and oils

In the comparison of cholesterol content in samples of dairy products, ghee, and oil (as presented in Table 5), it is observed that ghee samples exhibit the highest average cholesterol content at 285.10 mg/100g. On the other hand, dairy product samples show the lowest cholesterol content at 48.32 mg/100g. Previous studies attribute this difference to the animal fat content (suet) present in most animal ghee samples and dairy products, which is higher compared to pure vegetable ghee and dairy products. It is worth noting that there is a direct relationship between the percentage of animal fat and the percentage of cholesterol. In response to the association between consumption of full-fat dairy products and cardiovascular diseases and arteriosclerosis, various food industries have adopted measures recommended by the World Health Organization (WHO). These measures include using dairy products made from skimmed animal milk, low-fat animal milk, and sometimes incorporating vegetable fat into the products.

These adjustments aim to reduce the cholesterol content in the final product, aligning with the WHO's recommendations and addressing potential health concerns associated with the consumption of high-fat dairy products.

Quality		Type of Fat	Sample Name	Cholesterol Concentration (ppm)	Cholesterol Concentration (mg/100g)
Dairy Products		Whole Animal Full-Fat	Cow butter	2450	245.0
			Sheep cheese	843.3	84.33
			Bovine cheese	490	49.0
			Cheddar cheese	420	42.0
			LSD	5.825	3.078
		Whole Animal Full-Fat	Natural yogurt	62.9	6.29
		Defatted with Added Vegetable Fat	Hana light yogurt	46	4.6
			Hanayogurt	38	3.8
			LSD	0.789	0.159
		Defatted with Added Vegetable Fat	Cream	N.D	N.D
			Margarine	N.D	N.D
		Whole Animal Full-Fat	Local ghee (a)	3040	304
			Local ghee (b)	4815.0	481.50
			LSD	5.356	31.634
Ghee and Oil		National Industry Processed Vegetable Ghee	Bint ghee	4950	495.0
			Qamaria ghee	4535.0	453.50
			Jabali ghee	4118.3	411.83
			LSD	0.420	18.072
		Foreign Industry Processed Vegetable Ghee	Amazon ghee	3681.4	368.14
			Arij ghee	519.6	51.96
			LSD	0.591	3.531
		Local Oil	Sesame oil	N.D	N.D
		Foreign Oil	Shifa Cooking Oil	N.D	N.D

Table 6 and Figure 18 present the cholesterol content in all the studied samples, including dairy products and ghee. The samples of clarified butter exhibited the highest concentration of cholesterol, with values of 495.0 mg/100g, followed by local ghee (b) at 481.5 mg/100g, Qamaria ghee at 453.48 mg/100g, Amazon ghee at 411.83 mg/100g, Jabli ghee at 368.14 mg/100g, and local ghee (a) at 304 mg/100g.

Among the dairy products made from whole animal full-fat milk, the sample of butter displayed a cholesterol content of 245 mg/100g, while sheep milk cheese had a content of 84.3 mg/100g. Arij ghee had a cholesterol content of 51.96 mg/100g, while cow milk cheese had 49.3 mg/100g. Saudi cheddar cheese followed with a value of 42.0 mg/100g.

Samples of cream, margarine, cooking oil (Shifa), and sesame oil were below the detection limits for cholesterol content. Additionally, the samples of raw natural light yogurt contained 6.2 mg/100g, while the commercial Hana light yogurt had 4.6 mg/100g, and the commercial Hana yogurt had 3.8 mg/100g of cholesterol.

Table 6: Comparison of cholesterol content in samples of dairy products and ghee in ppm and mg/100g units

3-4 Comparison between studied local and imported vegetable ghee samples

Upon comparing local and imported samples, it was observed that local vegetable ghee exhibited higher levels of cholesterol in comparison to imported vegetable ghee. The cholesterol content in local vegetable ghee ranged from 368.14 to 495.0 mg/100g, whereas the cholesterol content in imported vegetable ghee samples varied from 51.96 mg/100g to 411.83 mg/100g. These findings suggest that locally produced vegetable ghee might have been adulterated with animal fat, as indicated by the results presented in Figure 19 and Table 6. Surprisingly, both local and imported vegetable ghee samples contained cholesterol, despite the product label claiming to be cholesterol-free and derived from hydrogenated palm oil. This discrepancy can be attributed to the lack of regulatory oversight and monitoring by relevant authorities, both for local and imported food products.

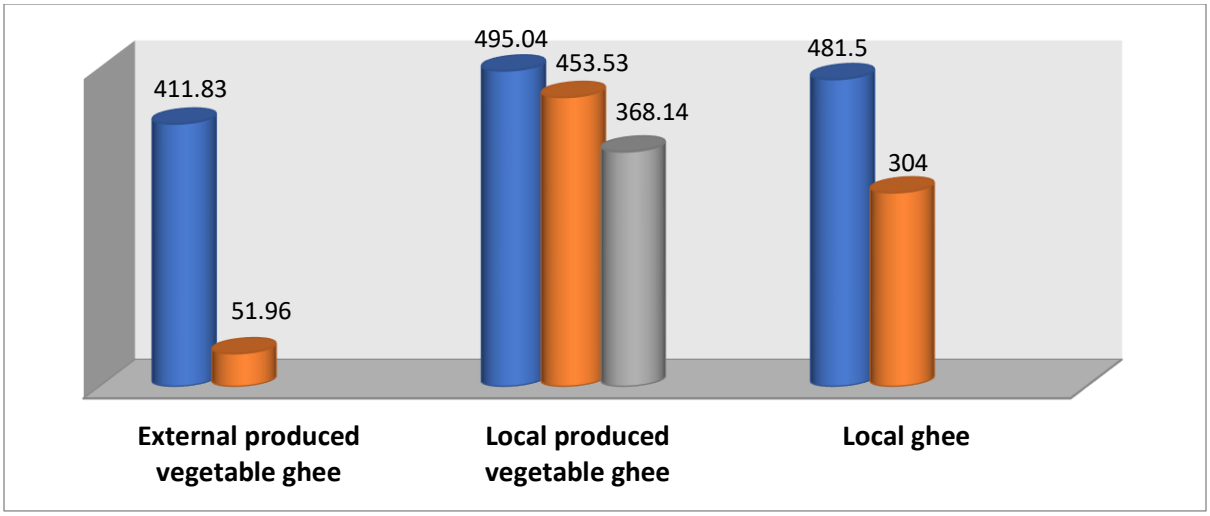


Figure 19: Cholesterol content in samples of vegetable ghee, animal ghee, local and external oils in mg/100g graphically.

4- Conclusion

In conclusion, the findings of this study highlight significant disparities in the cholesterol content among the analyzed samples. There was a noticeable elevation in cholesterol concentration in samples derived from animal sources in comparison to those obtained from plant sources. Moreover, the results displayed variations in cholesterol content when compared to similar studies conducted in different countries, utilizing standardized analytical techniques such as high-performance liquid chromatography. These dissimilarities may be attributed to various factors, including divergent origins of dairy and fat derivatives in both animal and plant forms, discrepancies in cattle breeds or plant species used for the extraction of ghee and oil, disparities in dietary practices, processing and extraction methods, and the influence of physicochemical and environmental factors. Overall, these findings emphasize the complex nature of cholesterol content in food products and the need for further investigation to comprehend the underlying mechanisms influencing these variations.

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