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Cholesterol Estimation in Edible Oils on the Ghanaian Market

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Abstract This study aimed to assess the levels of cholesterol present in commonly consumed edible oils available on the Ghanaian market, employing both qualitative and quantitative methodologies. The prevalence of cardiovascular diseases in Ghana underscores the significance of understanding the cholesterol content in edible oils, as excessive cholesterol intake is a known risk factor for such diseases. A total of 6 edible oil samples, including palm oil, soybean oil, sunflower oil, palm kernel oil, olive oil, and coconut oil, were collected from various retail outlets across the Ashaiman Municipality of Ghana. Qualitative analysis was conducted using standard phytochemical tests such as Salkowski's test and Lieberman Burchard's test to determine the presence of cholesterol in the oil samples. Subsequently, quantitative estimation was performed utilizing ultraviolet visible spectroscopy to precisely measure the cholesterol content in the oils. The qualitative analysis revealed the presence of cholesterol in all the sampled oils, with palm oil exhibiting the highest incidence, followed by coconut oil. Soybean and sunflower oils exhibited minimal levels of cholesterol. Quantitative analysis demonstrated significant variations in cholesterol levels among the different oil types, with palm oil recording the highest mean cholesterol content of 0.3068±0.021mg/ml, while soybean oil contained the least at 0.1946±0.011mg/ml. These findings highlight the importance of informed consumer choices regarding edible oil consumption, particularly for individuals at risk of cardiovascular diseases. Further research could explore the impact of processing methods and sourcing on the cholesterol content of edible oils, thereby providing valuable insights for both consumers and regulatory authorities in Ghana.

Keywords: *edible oils, cardiovascular diseases, cholesterol, salkowski, lieberman-burchard*

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1. Introduction

The consumption of edible oils is integral to the Ghanaian diet, serving as essential ingredients in cooking and food preparation. However, the nutritional quality of these oils can vary significantly, impacting public health, particularly concerning the prevalence of cardiovascular diseases (CVDs) [1,2]. Among the factors influencing the healthfulness of edible oils is their cholesterol content, a crucial determinant of cardiovascular risk [3,4]. Elevated cholesterol intake, particularly from dietary sources, is strongly associated with an increased risk of CVDs, including coronary artery disease, stroke, and peripheral artery disease [5].

Ghana, like many other countries, grapples with the burden of cardiovascular diseases, with a rising incidence observed in recent years. Recognizing the importance of addressing modifiable risk factors, such as dietary habits, there is a growing need to scrutinize the cholesterol content of commonly consumed edible oils available on the Ghanaian market [7]. While previous studies have examined the nutritional composition of various edible oils, comprehensive assessments specifically focusing on cholesterol content remain limited in Ghana [8].

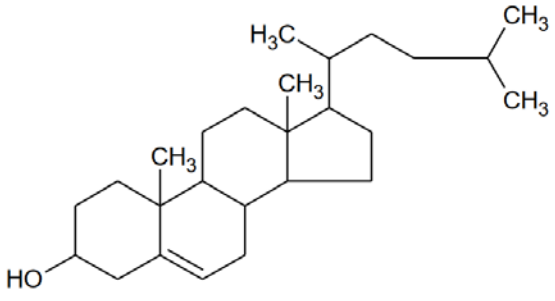

Figure 1. Structure of Cholesterol [6]

This research aims to bridge this gap by conducting a thorough qualitative and quantitative analysis of cholesterol levels in selected edible oils prevalent in the Ghanaian market. By elucidating the cholesterol profiles of these oils, this study seeks to provide valuable insights into the nutritional quality of commonly used cooking oils and their potential implications for public health in Ghana. Through a combination of qualitative and quantitative methodologies, this research endeavors to determine the presence or absence of cholesterol in selected edible oils qualitatively using Salkowski's test [9] and Lieberman-Burchard's test [7,10,11] and precisely quantify their cholesterol content using ultraviolet visible spectroscopy [7,12]. The selected oils for analysis include palm oil, soybean oil, sunflower oil, olive oil and coconut oil, which represent widely consumed varieties in the Ghanaian context.

The findings of this study hold significant implications for both consumers and regulatory bodies in Ghana. By enhancing our understanding of the cholesterol content in commonly used edible oils, this research aims to empower consumers to make informed dietary choices that promote cardiovascular health. Furthermore, insights gained from this study may inform regulatory policies aimed at improving the quality and safety of edible oils available on the Ghanaian market. In summary, this research endeavors to contribute to the existing body of knowledge concerning the nutritional composition of edible oils in Ghana, particularly regarding their cholesterol content. By shedding light on this aspect of edible oil quality, this study strives to support efforts aimed at promoting heart health and reducing the burden of cardiovascular diseases in Ghanaian communities [7].

2. Materials and Methods

2.1. Sample Collection

Samples of 6 different edible oils including palm oil, soybean oil, sunflower oil, palm kernel oil, olive oil, and coconut oil, were obtained from various retail outlets in the Ashaiman municipality of Ghana. Two of the sampled oils (palm oil and palm kernel oil) were locally manufactured whereas the others were imported and most of them were labelled as having no cholesterol.

2.2. Chemicals and Reagents

All chemicals and reagent were of analytical grade. Solvents used includes acetic anhydride, concentrated sulphuric acid, chloroform, and were obtained from Dae-Jung Chemicals and Metal Company Limited, South Korea. Pure standard cholesterol (200mg/dl) solution and distilled water was obtained from the Central University school clinic and Chemistry laboratory respectively.

2.3. Equipment and Instrumentation

Amber glass vial, Black carbon paper, Bucket, droppers, Beakers, Burette, Graduated pipette (5ml, 10ml), Measuring cylinders, stirring rod, watch glass, Funnel, Volumetric flask (100ml, 250ml) and the Ultraviolet Visible Spectrophotometer were used in this research.

2.4. Preparation of Standard Cholesterol Solutions

A stock solution of 0.1mg/ml was prepared by pipetting a volume of 5ml pure cholesterol (200mg/dl) stock solution. It was slowly added into a 100ml volumetric flask. The solution was topped up to the volume with chloroform. The flask was then stoppered, shaken, labelled appropriately and refrigerated for later use. Various concentrations (0.08mg/ml, 0.06mg/ml, 0.04mg/ml, and 0.02mg/ml) of standard cholesterol solutions were prepared by pipetting calculated volumes of the stock solution and diluting to the required volumes with chloroform.

2.5. Preparation of Lieberman-Burchard's Reagent

50ml of acetic anhydride was measured into an amber glass vial and kept in an ice bath. After 30 minutes, 5ml of concentrated sulfuric acid was pipetted and added carefully to the acetic anhydride in the vial. It was then covered tightly, mixed by inversion and kept in the refrigerator for use [7,13].

2.6. Qualitative Analysis (Identification of Cholesterol)

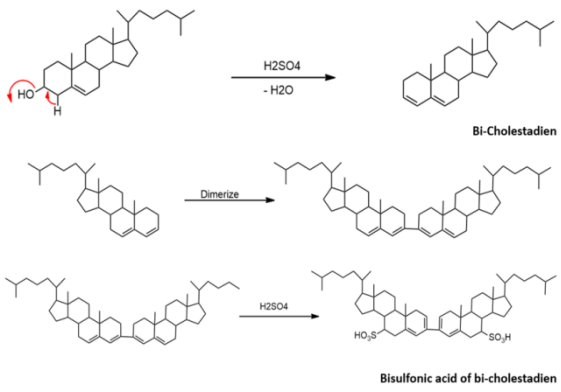

Figure 2. Mechanism of Salkowski's Test [14]

For qualitative analysis to detect the presence of cholesterol, Salkowski's and Lieberman-Burchard's test are the test carried out to detect the presence of cholesterol. This is a relatively simple and readily available analytical method that employs concentrated sulfuric acid, acetic anhydride and chloroform [7]. From a historical point of view, modern cholesterol determinations had their beginnings in the late nineteenth century when Salkowski described a color reaction for this analyte, which was isolated from gallstones about a century earlier. Salkowski described the first color reaction, by adding sulfuric acid to a chloroformic solution of cholesterol to obtain a brick red color. Concentrated sulfuric acid is highly hygroscopic and it removes two molecules of water from two molecules of cholesterol. It then causes a connection at position 3, forming a bi-cholestadien. Simultaneously the sulfuric acid sulfonates the molecules of the bi-

cholestadien at positions 7, 7' aromatic rings, and as a final product, red colored bi-sulfonic acid of bi-cholestadien is formed.

Lieberman-Burchard's test is a colorimetric test used to detect cholesterol by producing deep green color [15]. This color begins as a purplish, pink color and progresses through to a light green then very dark green color. The color is due to the hydroxyl group (-OH) of the cholesterol reacting with the reagents and increasing the conjugation of the unsaturation in the adjacent fused ring.

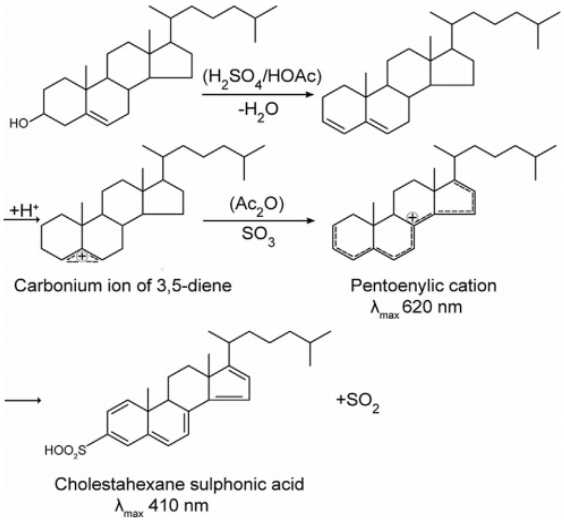

Figure 3. Mechanism of Lieberman-Burchard's Test [11,16]

2.6.1. Salkowski's Test

2ml of the oil samples were measured with graduated pipette into different test tubes containing 2ml of chloroform. It was then shaken for the oil to dissolve. 2ml of concentrated sulfuric acid was then added to the resulting solution in the test tubes [17]. A brick-red coloration was obtained indicating the presence of cholesterol.

2.6.2. Lieberman-Burchard's Test

2ml of the oil samples were measured with graduated measuring cylinder into different test tubes containing 2ml of chloroform. It was shaken for the oil to dissolve. 2 – 4 of freshly prepared Lieberman-Burchard's reagent was added to the resulting solution in the test tubes. A deep-green coloration was obtained indicating the presence of cholesterol [15].

2.7. Quantitative Analysis (Estimation of Cholesterol)

2.7.1. Assay of Standard Cholesterol Solutions

5ml of the pure cholesterol solutions were measured using a graduated pipette into five different test tubes labelled 0.1mg/ml, 0.08mg/ml, 0.06mg/ml, 0.04mg/ml and 0.02mg/ml. 2–4 drops of freshly prepared Lieberman-Burchard's reagent were added to each test tube and a deep-green coloration was produced. The test tubes were then covered with a black carbon paper, placed in an ice bucket and kept in the dark for 15 minutes. After 15 minutes they were removed from the ice bucket and their absorbance was obtained using the ultraviolet visible spectrophotometer and their absorbances were recorded.

2.7.2. Assay of Cholesterol from Edible Oil Samples

2ml of the oil samples were measured using a graduated measuring cylinder into six different test tubes. 2 – 4 drops of freshly prepared Lieberman-Burchard's reagent were added to each test tube and a green coloration was produced. The test tubes where then covered with a black carbon paper, placed in an ice bucket and kept in the dark for 15 minutes. After 15 minutes, they were removed from the ice bucket and their absorbances were obtained using the ultraviolet visible spectrometer and their absorbances were recorded.

2.8. Statistical Analysis

Statistical analyses were carried out in GraphPad Prism 8 software using one-way analysis of variance. Significance was set at $P < 0.05$. Data are presented as the mean ± standard error of the mean (SEM).

3. Results and Discussion

3.1. Salkowski's Test

The qualitative test results for Salkowski's test are given in Table 1. From the results, all the six (6) oil samples were found to contain cholesterol. All the oil samples tested positive for Salkowski's test by producing a brick- red coloration during the experiment.

Table 1. Results of Salkowski's Test

Oil Sample	Results
Olive Oil	positive
Palm Kernel Oil	positive
Sunflower Oil	positive
Coconut Oil	positive
Palm Oil	positive
Soyabean Oil	positive

3.2. Lieberman-Burchard's Test

The qualitative test results for Lieberman-Burchard's test is given in Table 2. From the results, all the oil samples were found to contain cholesterol. All the six oil samples tested positive for the Lieberman-Burchard's test. A green coloration was produced for all the six oil samples.

Table 2. Lieberman-Burchard's Test

Oil Sample	Results
Olive Oil	Positive
Palm Kernel Oil	Positive
Sunflower Oil	Positive
Coconut Oil	Positive
Palm Oil	Positive
Soyabean Oil	Positive

3.3. Assay of Standard Cholesterol Solutions

The absorbances of the standard cholesterol solutions were obtained and recorded as shown in Table 3.

Table 3. Absorbance of Standard Cholesterol Solutions

Conc. (mg/ml)	Absorbance(nm)
0.1	0.312±0.001
0.08	0.282±0.002
0.06	0.230±0.001
0.04	0.184±0.000
0.02	0.141±0.003

Table 4. Absorbance of Oil Samples

Oil Sample	Absorbance(nm)
Olive Oil	0.06±0.0212
Palm Kernel Oil	0.092±0.0331
Sunflower Oil	0.052±0.0231
Coconut Oil	0.093±0.0392
Palm Oil	0.095±0.0401
Soyabean Oil	0.044±0.0191

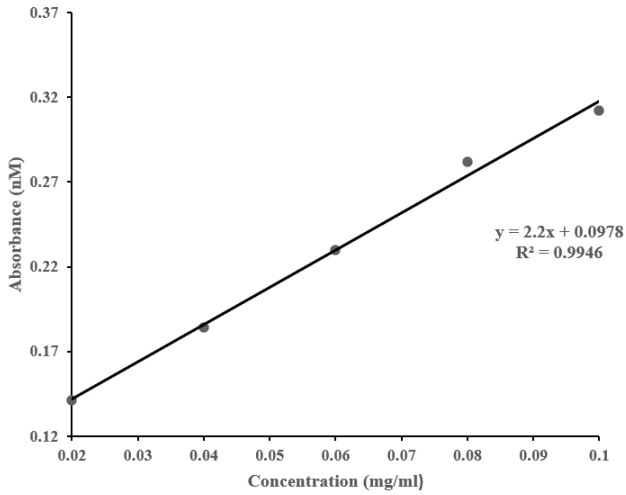

Figure 4. Calibration curve using standard cholesterol

Dietary cholesterol can modestly influence blood cholesterol levels, particularly LDL cholesterol, which is associated with cardiovascular disease (CVD) risk [18]. However, the impact varies among individuals due to factors like genetics and overall dietary patterns. While early research suggested a strong link between dietary cholesterol intake and blood cholesterol levels, more recent studies have shown that saturated and trans fats in the diet may have a more significant impact [19]. As a result, current dietary guidelines focus less on strict limits for dietary cholesterol intake and more on promoting a balanced diet rich in fruits, vegetables, whole grains, and lean protein sources to reduce the risk of CVDs.

The cholesterol content in cooking oils can have a minimal direct impact on human health because cholesterol is found exclusively in animal products, not plant-based oils. However, the type of fat in cooking oils can significantly influence health outcomes. Saturated Fats: cooking oils high in saturated fats, such as coconut oil and palm oil, can raise LDL cholesterol levels [20], which is associated with an increased risk of cardiovascular diseases (CVDs) when consumed in excess. Therefore, it's recommended to use these oils sparingly and opt for healthier alternatives. Trans Fats: partially hydrogenated oils, which contain trans fats, were once commonly used in processed foods and cooking oils. However, extensive research has shown that trans fats significantly increase LDL cholesterol levels and the risk of CVDs [21]. Many countries have implemented regulations to limit or ban the use of trans fats in food products. Monounsaturated and Polyunsaturated Fats: oils high in monounsaturated and polyunsaturated fats, such as olive oil, canola oil, and sunflower oil, have been associated with beneficial effects on heart health [22,23,24]. These oils can help lower LDL cholesterol levels when used as replacements for saturated and trans fats in the diet. Omega-3 Fatty Acids: some cooking oils, like flaxseed oil and walnut oil, are rich in omega-3 fatty acids, which have been linked to various health benefits, including reducing inflammation and improving cardiovascular health [25].

Overall, the cholesterol content in cooking oils is a primary concern, the type of fat they contain can significantly impact human health, particularly cardiovascular health. Choosing cooking oils high in unsaturated fats and low in saturated and trans fats can contribute to a heart-healthy diet and help reduce the risk of CVDs [26].

The cholesterol content of each of the oils was determined by substituting their absorbances into the formula, y = 2.2x+0.0978 obtained from the calibration curve of standard cholesterol solutions (Table 5). The qualitative analysis revealed the presence of cholesterol in all the sampled oils, with palm oil exhibiting the highest incidence, followed by coconut oil. Soybean and sunflower oils exhibited minimal levels of cholesterol. Quantitative analysis demonstrated significant variations in cholesterol levels among the different oil types, with palm oil recording the highest mean cholesterol content of 0.3068±0.021mg/ml, while soybean oil contained the least at 0.1946±0.011mg/ml. These findings highlight the importance of informed consumer choices regarding edible oil consumption, particularly for individuals at risk of cardiovascular diseases. The correlation coefficient was found to be greater than 0.99 which manifests a linear relationship between concentration and the peak area. Cholesterol content in the edible oils was found to be in the range of 0.2 – 0.3 mg/ml.

Table 5. Estimated Cholesterol Content of Oil Samples

Oil Sample	Estimated Cholesterol Content(mg/ml)
Olive Oil	0.2298±0.012
Palm Kernel Oil	0.3002±0131
Sunflower Oil	0.2122±0.031
Coconut Oil	0.3024±0.100
Palm Oil	0.3068±0.021
Soyabean Oil	0.1946±0.011

4. Conclusion

The presence of cholesterol was detected and quantified in all the 6 oil samples. Although the amount of cholesterol found in them was minute and not in excess, we call the attention of all manufacturers to indicate the amount of cholesterol present in the oil, no matter how small the quantities may be. According to the American Heart Association (2015), the recommended daily intake of dietary cholesterol is 300mg/day for average healthy

person and 200mg/day if you are at high risk of heart diseases or diabetes. Findings from this study support previous work that cholesterol is present in vegetable oils, although in small proportion as described by [10,12,15,27].

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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