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Determination of cholesterol level in different animal protein extracted from meats sold in Mbaitoli local government area, Imo state

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Abstract

The objective of this study was to detect and determine the cholesterol level in different animal proteins. Five different animal meat samples, including beef, pork, deer meat, goat meat, and rabbit meat, were used for this study. UV-spectrophotometric analysis and gas-chromatographic analysis (Gas Chromatography Mod Agilent 6890 CA, USA, equipped with an on-column automatic injector) were employed. Oils from the different meats were extracted using a Soxhlet extractor. UV-Spectrophotometric analysis of different animal meat samples revealed that beef had the highest value of cholesterol (17.73 mg/L), while pork had the least value (8.08 mg/L). Gas-chromatographic analysis of saturated fatty acids from different animal meat samples revealed that deer had the highest percentage of saturated fatty acids (85.21%), while pork had the least percentage (68.99%). The gas-chromatographic analysis of unsaturated fatty acids from different animal meat samples revealed that pork had the highest percentage of unsaturated fatty acids (31.01%), while beef had the least percentage (14.55%). From the statistical analysis of variance for saturated fatty acids in different meat samples Since the p-value of 0.11 is greater than 0.05 ($p > 0.05$), this implies that there is no significant difference in the values of saturated fatty acids in different meat samples.

Keywords: Animal; Cholesterol; Fatty acids; Saturated; Protein; Oils; Unsaturated

1. Introduction

Cholesterol is a lipid-waxy (fat-like) steroid found in the cell membrane and transported in the blood plasma of all animals (Mahan et al., 2012). It is an essential part of cells in the body and is used to make certain hormones and digest fats. A special form of cholesterol (7-dehydrocholesterol) in the skin can change into vitamin D when exposed to sunlight. There are two different types of cholesterol: Blood or serum cholesterol, which circulates in the blood and is mostly made by the body, Dietary cholesterol, which comes from foods of animal origin (Mahan et al., 2012), It is an essential component of mammalian cell membranes, where it is required to establish proper membrane permeability and fluidity (Christie, 2003).

Since cholesterol is essential for life, it is primarily synthesized within the body, with smaller contributions from diet. Approximately 1 g/day of cholesterol in the body arises by synthesis, whereas 0.3 g/day is provided by diet (Lecerf and de Lorgeril, 2011). Excessive levels of cholesterol in blood circulation are, however, strongly associated with the progression of atherosclerosis (Javiett, 1994). Cholesterol is also the principal sterol synthesized by animals, but small quantities are synthesized in other eukaryotes such as plants and fungi. Atherosclerosis is characterized by the deposition of cholesterol esters and other lipids in the connective tissues of the arterial walls. Cholesterol is an amphipathic lipid and, as such, an essential structural component of membranes.

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Over the last century, the amount and proportion of animal fat in human diets have increased in many societies. These increments have been associated with the occurrence of cardiovascular diseases (Lichtenstein, 1999; Katan, 2000). In Western societies, coronary heart disease and atherosclerosis are strongly related to the dietary intake of cholesterol and saturated fatty acids and are among the most important causes of human mortality (Sacks, 2002).

Since the relationship between plasma cholesterol concentration and atherosclerosis was demonstrated in rabbits in 1913 (Vance and van den Bosch 2000), interest in cholesterol content in foods has been driven by awareness of the association between dietary cholesterol and human disease. As a result, cholesterol has become an important component in composition studies on meat and poultry products.

Cholesterol, which is an integral lipid component, has been seen as not being good for human consumption because of its perceived negative effects on health. Public concern is more specifically related to meat products, especially red meat (Li et al., 2005). In addition, a strong relationship has been demonstrated between cellular cholesterol concentration and Alzheimer's disease (Michikawa, 2003). It is widely acknowledged that there is an urgent need to return to a balanced fatty acid diet by decreasing the intake of cholesterol and saturated fats (Evans et al., 2002). Chicken meat is low in fat and cholesterol and is usually considered healthier than other animal protein sources, especially red meats of mammalian origin. In recent years, dietary supplements such as garlic, copper, and omega-3 fatty acids have been tested in an attempt to further decrease the fat and cholesterol contents of poultry meat (Pesti and Bakalli, 1996; Konjufca et al., 1997; Matsurra, 2001; Ayerza et al., 2002; Chowdhury et al., 2002). Despite these efforts to develop novel strategies to produce meat with lower cholesterol and saturated fatty acid contents, there is still a paucity of information regarding production alternatives to achieve this important goal.

The concern over the effects of dietary cholesterol on heart disease, and the obligatory nutritional labeling in the United States led to the need for an accurate and efficient cholesterol determination technique (FDA, 1993). As the source of the most validated and trusted analytical methods, the Association of Official Analytical Chemists (AOAC) adopted the first cholesterol analysis procedure for foods in 1976 (Klatt et al., 1995).

2. Material and methods

2.1. Collection of different animal meats

The different animal meat samples were obtained from selected markets in Mbaitoli Local Government Area in Imo State. The Soxhlet extraction of oils from the sample meats was carried out at New Concept Laboratory, Owerri. The animals (with their Botanical names) that their meats were used for this analysis are-: Cow (*Bos taurus*), Deer (*Dama dama*), Goat (*Capra aegagrus hircus*), Pig (*Susscrofa domestica*) and Rabbit (*Oryctolagus cuniculus*).

2.2. Preparation of samples and oil extraction

The different meat samples were washed, sliced into smaller pieces, and labeled appropriately in different sterile aluminum foils. The moist samples were then dried in a drying oven for about 4-5 hours at 70°C. After drying, the samples were closed with fat-free cotton wool. To extract the oil from different meat samples, the soxhlet extraction method was used or employed. About 50 grams of each of the meat samples were weighed and placed in the extractor, respectively. The soxhlet flask was filled with 300 ml of petroleum ether, and the soxhlet extractor was fitted to the flask and mounted on the hot plate. The reflux condenser connected with a hose to a running tap was fitted to the extractor, and the oil was extracted at a temperature of 40–60 °C for about 6–10 extraction circles for about 4–5 hours, respectively. The oil was then isolated from the solvent by evaporating and condensing the process in the Soxhlet extractor. The oil was then poured into the beaker from the soxhlet flask and placed in a drying oven at 70°C for about 2 hours and allowed to cool.

2.3. Preparation of reagents

2.3.1. Liberman Burchard Reagent

Ten (10 mg) of standard cholesterol was weighed in a small beaker and recorded; it was then dissolved in 10 ml of chloroform and shaken very well. Then 0.5 ml of sulfuric acid Liberman-Burchard reagent was dissolved in 10 ml of acetic anhydride, covered, and kept in a water bath. About one (1 gm) of the sample was weighed and dissolved in chloroform to 10 ml to further dilute to 10 times (10,000 ppm). About 3 ml of the diluted solution was then mixed with 2 ml of Liberman-Burchard reagent and 2 ml of chloroform. The tubes were covered with black carbon paper and kept

in a water bath in a dark place for 15 minutes. The Liberman-Burchard reagent reacted with sterol to produce characteristic green colour, their absorbance was determined on a spectrophotometer at 640 nm.

2.3.2. Standard cholesterol procedure

The following five test tubes of standard cholesterol solution were pipetted as 0.5, 1.0, 1.5, 2.0, and 2.5 ml whereas tube 6 was kept blank and the six tubes were labeled- S1, S2, S3, S4, S5, and S6. Then 2 ml of the Liberman-Burchard reagent was added to all six tubes and the final volume was made equal in each test tube by adding chloroform. The test tubes were covered with carbon black paper and kept in the dark for 15 minutes in a water bath. Then taken baseline on a spectrophotometer with blank (S6) at max 640 nm, the absorbance of all standards (six tubes) were determined on the spectrophotometer and the result was recorded.

2.3.3. Gas-chromatographic analysis

For the different fatty acids present in the meat samples, the gas-chromatographic analysis method was used and performed by a gas-chromatography mod. Agilent 6890 (CA, USA) is equipped with an on-column automatic injector, flame ionization detector, and HP 88 capillary column (100m x 0.25 m film thickness). Temperature detector A: 250 ^{o}C, injector temperature at 220 ^{o}C for both injectors, and integrator chart speed at 2 cm/min were used. The oven temperature was set to 180 ^{o}C, which allowed the GC to warm up. While it's warming, it was then set as follows: SIG 1 A, initial value: 180 ^{o}C, initial time: 15 minutes, rate: 0 ^{o}C/min. Final value: 181 ^{o}C; final time: 1 minute.

2.3.4. Procedure for gas-chromatographic analysis

About two grams (2g) of oil was weighed in a small beaker and the weight was recorded. The sample was dissolved in 50 ml of chloroform and transferred to a 100 ml volumetric flask and diluted to the mark. Then most of the chloroform was evaporated at room temperature. The solution was not allowed to dry up completely; this is because if the solution is allowed to dry up completely, it will be hard to re-dissolve with the esterification reagent. Then, about 1 ml of inter-esterification reagent [20 vol% benzene and 55 vol% menthanol] was added to the solution. It was sealed and heated at 100^{o}C in water bath for 30 minutes. After inter-esterification, the methyl esters were extracted with hexane and H_2O so that the final mixture of the reagent, hexane and water, is in a proportion 1:1:1 (i.e., add 1 ml each of hexane and water to the reaction mixture). The mixture was shaken vigorously by hand for 2mins. A stable emulsion was formed and it was broken by centrifugation. About half of the top hexane phase was transferred to a small test tube for injection. Caution was applied to remove only the organic layer. The injection was done directly from the reaction vial because of the risk of injecting water, as water can ruin the GC column.

2.3.5. Data calculation and statistical analysis

Data obtained from the study were calculated using the Analysis of the variance test (ANOVA statistical test), tables and bar charts were also used to represent the values obtained from the study.

3. Results

Results from the UV-Spectrophotomeric analysis of different animal meat samples reveal that Beef had the highest value of cholesterol (17.73 mg/mL), while Pork had the least value (8.08 mg/mL) as shown below:

Table 1 Cholesterol level of the meat samples

Sample	Cholesterol Level/(mg/mL)
Goat (Chevon)	8.27
Rabbit (Rabbit meat)	9.04
Deer (Vernison)	13.38
Pig (Pork meat)	8.08
Cow (Beef)	17.73

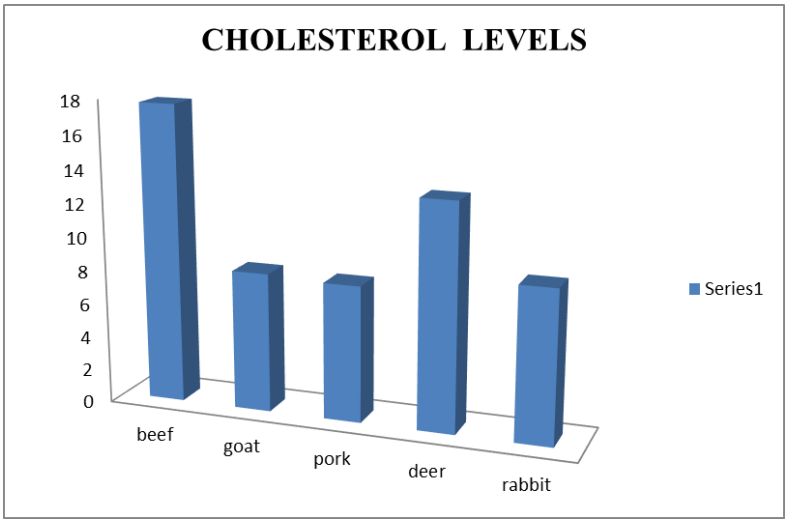

Figure 1 Cholesterol levels in different meat samples

Results from the Gas-Chromatographic analysis of saturated fatty acids from different animal meat samples reveal that Deer had the highest percentage of cholesterol (85.21%), while Pork had the least percentage (68.99%) as shown below:

Table 2 Percentage of saturated fatty acids in the meat samples

Sample	Cholesterol/(%)
Goat (Chevon)	82.77
Rabbit (Rabbit meat)	69.91
Deer (Vernison)	85.21
Pig (Pork meat)	68.99
Cow (Beef)	85.45

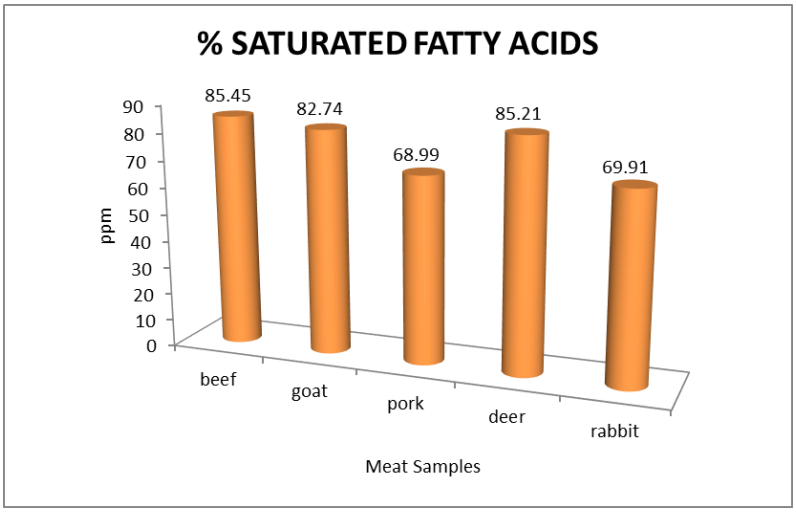

Figure 2 Percentage saturated fatty acids in the meat samples

Results from the Gas-Chromatographic analysis of unsaturated fatty acids from different animal meat samples reveal that Pork had the highest percentage of cholesterol (31.01%), while Beef had the least percentage (14.55%) as shown in Table 5 and figure 3 respectively below:

Table 3 Percentage of unsaturated fatty acids in the meat samples

Sample	Cholesterol/(%)
Goat (Chevon)	17.26
Rabbit (Rabbit meat)	30.09
Deer (Vernison)	14.79
Pig (Pork meat)	31.01
Cow (Beef)	14.55

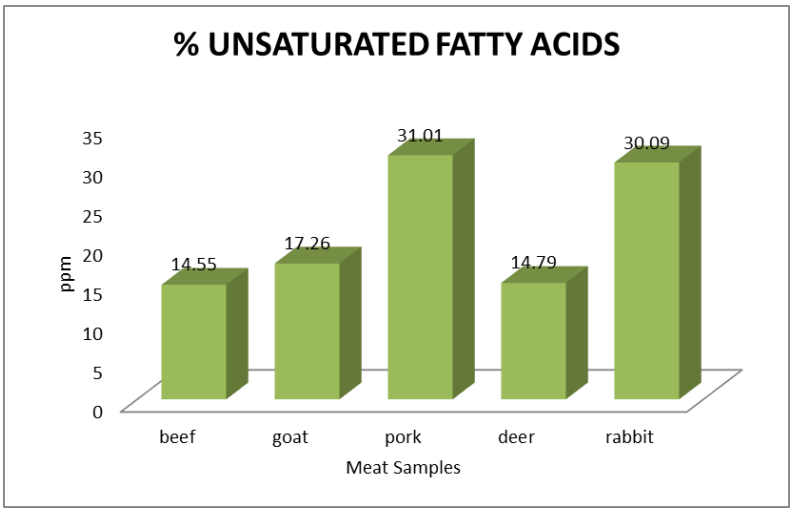

Figure 3 Percentage unsaturated fatty acids in meat samples

Table 4 Analysis of variance for saturated fatty acids in different meat samples

Anova: Single Factor						
SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Column 1	7	13.033	1.861857	1.982972		
Column 2	7	19.9357	2.847957	3.198641		
Column 3	7	6.4727	0.924671	0.926084		
Column 4	7	7.7628	1.108971	2.473841		
Column 5	7	16.1588	2.3084	2.561775		
ANOVA						
Source of Variation	***SS***	***Df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	18.22586	4	4.556464	2.044483	0.113301	2.689628
Within Groups	66.85988	30	2.228663			
Total	85.08574	34				

4. Discussion

From the observation recorded in the different animal meat samples, the cholesterol levels varied from 8.08 to 17.73 mg/mL, these values are lower than the 89 mg/mL of the USDA (2006) and the study by Lercker and Rodriguez-Estrada (2000), which varied from 100 mg/mL to 300 mg/mL in a work on beef, venison and pork.

The result of the present study revealed that Beef meat samples presented the highest proportions of cholesterol level (17.7 3mg/mL) than Deer meat (Venison) (13.38mg/mL) as revealed by Table 1 and Figure 1 respectively. This is contrary to the higher values of cholesterol, 56 mg/mL found in bologna in the study of (Novelli et al., 1998) which has values between 52.11 and 138 Mg/mL. However, in the study conducted by Baggio and Bragagnolo (2004), the quantity of cholesterol obtained in Brazilian bologna was between 39.4 and 49.8 mg/mL of product.

Nevertheless, by the FDA, the meat samples; goat (8.27mg/mL), deer (13.38 mg/mL), rabbit (9.04mg/mL), pork (8.08mg/mL) and beef (17.73/mL) investigated in this study can be considered to be "low cholesterol" meats, as they have less than 25 mg/mL of cholesterol per serving (FDA, 2014).

Dietary cholesterol has been shown to raise blood LDL cholesterol levels in some individuals. However, this effect is reduced when saturated fatty acid intake is low, and the potential negative effects of dietary cholesterol are relatively small compared to those of saturated and trans fatty acids (IOM, 2002). Moderate evidence shows a relationship between a higher intake of cholesterol and a higher risk of cardiovascular disease (Vernon and Flint, 1988). Independent of other dietary factors, evidence suggests that one egg (i.e., egg yolk) per day does not result in increased blood cholesterol levels, nor does it increase the risk of cardiovascular disease in healthy people. Consuming less than 300 mg per day of cholesterol can help maintain normal blood cholesterol levels. Consuming less than 200 mg per day can further help individuals at high risk of cardiovascular disease (Harris et al., 1992).

The value of cholesterol obtained in this study is similar to that obtained in Table 1 and Figure 1 respectively for goat (8.27mg/mL) by (Badiani, 2002) and (7.54 mg) by (Hutchion, 1987). Although the cholesterol content of beef meat (35mg) as reported by (Piironen et al., 2002) is higher than that of beef (17.73 mg/mL) as revealed in this study. This supports the notion that the type of dietary fatty acid, rather than the level of dietary cholesterol, is the most potent regulator of serum cholesterol levels (Schaefer, 2002). It is known that dietary cholesterol has an inverse effect on endogenous cholesterol synthesis (Jones, 1997). The lower the LDL cholesterol number of an individual, the better it is for the person's health. Lower than 100 mg per dl (2.6 mmol/L) is ideal. The cholesterol values between 100 and 125 mg per dL (2.6 and 3.3 mmol/L) are close to ideal while between 130 and 159 mg per dL (3.4 and 4.1 mmol/L) are borderline elevated. The values of 160 and 189 mg per dL (4.1 and 4. 9mmol/L) are elevated and when more than 190 mg per dL (4.9 mmol/L), it is very high. When one is at a high probability of having a cardiac arrest, LDL cholesterol below 70 mg per dL(1.8 mmol/L) is highly advisable.The inverse effect of body weight on LDL cholesterol uptake was detected largely in the liver (receptor-mediated process). The uptake pattern and extent seem to vary in the adrenal gland and ovary (receptor-mediated) regardless of animal size; however, it is similar in other tissues such as the intestines (receptor-independent), adipose tissues, and muscles. Muscle tissues synthesize most of their cholesterol demand (Dietschy et al., 1993).

The findings from this study on saturated fatty acids as represented in Table 2 and Figure 2 respectively reveal that pork had the least percentage of (68.99%), rabbit (69.91%), goat (82.77%), deer (85.21%), while beef meat had the highest percentage of saturated fatty acids (85.45%). This agrees with the findings of Sacks (2002), which reported (66 32% in pork, 82.14% in beef, and 65.64% in rabbit). He further stated that in Western societies, coronary heart disease and atherosclerosis are strongly related to the dietary intake of cholesterol and saturated fatty acids and are among the most important causes of human mortality.However, table 3 and Figure 3 respectively show that pork meat had the highest percentage (31.01%) of unsaturated fatty acids.

From the statistical analysis of variance for saturated fatty acids in different meat samples. Since the p-value 0.11 is greater than 0.05 ($p>0.05$), this implies that there is no significant difference in the values of saturated fatty acids in different meat samples. There was no significant difference in the cholesterol obtained in the different meat analyses for deer, goat, beef, pork, and rabbit when Anova statistical test was applied. Data from recent studies show that the consumption of saturated fatty acids, when part of a low-fat diet, does not adversely affect the human hormone particularly low-density lipoprotein concentration (Harman et al., 2008; Spence et al., 2010). Cholesterol is essential for the production of bile acids; without bile acids, fats cannot be digested. Bile acids are crucial for the assimilation of fat fat-solubleamines, such as vitamins A, D, E, and, K.Since p-value 0.11 is greater than 0.05 ($p>0.05$), this implies that there is no significant difference in the values of saturated fatty acids in different meat samples. Current evidence indicates that dietary cholesterol has a modest effect on plasma cholesterol (1.9-mg change in LDL and 0.4-mg change

in HDL per 100 mg/d of dietary cholesterol) in the general population; more importantly, dietary cholesterol does not appear to influence the ratio of LDL to HDL cholesterol (the most important predictor of CHD) in the general population (Katz et al., 2005).

5. Conclusion

The cholesterol content of meat and poultry has been important in making nutritional decisions. Selection of protein sources always includes the consideration of fat and cholesterol which is the inseparable components of meat and poultry; because meat and poultry are the most important protein sources with abundance and affordability. In this study, the cholesterol level of beef, goat, rabbit, pork, and deer meats was determined. It was revealed that beef had the highest value of cholesterol (17.73mg/mL), while Pork had the least value (8.08mg/mL). all the samples meet up with the serving quantity according to FDA rules, which state that cholesterol quantities less than 2 mg per serving may be labeled as zero (CFR, 2005). Therefore, normal levels of HDL cholesterol are between 40 and 49 mg per dL (1 and 1.3 mmol/L) for men and between 50 and 59 mg per dL (1.3 and 1.5 mmol/L) for women. It is recommended that people should watch their daily intake of cholesterol and regular cholesterol checkup is also encouraged.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

This research work does not contain any study performed on animals/humans subjects by any of the authors.

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