

Estimation of Total Cholesterol in Selected Tropical Cow Meat Parts in Ogun State Nigeria

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ABSTRACT

Total cholesterol concentration of tropical cow meat parts was carried out using the colorimetric method and the values were calculated by extrapolating the calibration curve which represents the linear relationship between the standard concentration and the absorbance measured at 550 nm. The intestines were observed to possess the highest amount of total cholesterol, 138 mg/100g. The composition of fat in adipose tissue is highly variable as the adipose tissues of slaughtered animals contain about 50-95% of fat. The amount of total cholesterol found in the muscle was 136 mg/100g. The part with the third highest total cholesterol content was the heart, 105 mg/100 g while the lungs gave 71 mg/100 g as it is close to the heart. Excess cholesterol is eliminated from the body via the liver, which secretes cholesterol in bile or converts it to bile salts hence it contained a lower amount, 37 mg/100g. Lastly, the skin was the part with the least amount of total cholesterol 31 mg/100g. The skin and the liver with the least amount of total cholesterol can be included in a diet. The intestines having 138 mg/100ml of sample is not an ideal meat diet.

Keywords: Cholesterol, Tropical, Spectrophotometer, Absorbance, Cow.

I. INTRODUCTION

Animals have always been one of the major sources of food; both their flesh such as meat, poultry, fish and other products obtain from them such as eggs and milk products. A diet that is balanced and of a wide variety is good and plays a role in human health as an excess or deficiency in any of the components of the diet can cause an abnormality in the function of the body. Cholesterol is a soft waxy fat-soluble steroid made by animal liver and also supplied in diet through animal products such as meats, poultry, fish and dairy products. It is a natural component of fats in the blood stream. In the human body, cholesterol is an important lipid in some membranes, is needed to insulate the nerves, produce cell membranes and certain hormones. Since it is hydrophobic or water-insoluble, it is bonded or coated with a protein so that

it can be carried in the blood (Ma, 2006). Cholesterol is a substance that is essential to life as a primary component of cell membranes and as a substrate for the synthesis of steroid hormones, bile acids and vitamin D. Cholesterol is transported around in the body in various particles in the blood. High blood levels of cholesterol are linked with coronary heart disease (Meat Advisory Panel). In general, significant effects of dietary cholesterol are seen only at extreme levels of intake, for example a very high intake (> 300mg) resulting from unusually high consumption of cholesterol-rich foods. Lean red meat is low in dietary cholesterol. Excessive intakes of saturated fatty acids in the diet are the main dietary reason for high blood levels of cholesterol. Contrary to the belief of a wide majority of local population, red meat or lean meat does not contain high levels of fat or saturated fat. The total fat content of red meat has been considerably

reduced over the last few decades and the amount of fat in red meat is actually much lower than most people think. Reductions in the fat content of red meat have been achieved by breeding techniques on the farm and new butchery techniques, which trim off most of the fat. Fully trimmed lean raw beef typically contains only 5% fat, fully trimmed lean raw pork only 4% fat and fully trimmed lean raw lamb only 8% fat. In comparison, Cheddar cheese contains an average of 34% fat. Furthermore, about half of the fat found in red meat is the healthier polyunsaturated or monounsaturated types. A study has shown that with proper dietary intervention, the mean blood cholesterol level of 90% of a test population was reduced by 3 % to 23 %. Dietary habits are major factor in the development of obesity and cardiovascular heart diseases (Malekian, Khachatryan, Gebrelul and Henson 2014). Potential carcinogenic substances such as heterocyclic amines and polycyclic aromatic hydrocarbons (formed during the cooking process), high saturated fat, and cholesterol content can increase the risks for the diseases mentioned above. The addition of sodium and nitrite in processed meats also increase these risks (De Carvalho, Cesar, Fisberg, Marchioni, 2014). Red meat, which is considered to be rich in total fat and SFA, is one of the main foods restricted in these guidelines. However, the fat component of different meats is extremely variable. Different factors related to the animal source of the meat, such as breed, feedstock, processing and the particular meat cut influence both fat quantity and quality of the final product. In fact, lean red meat can be as low as or lower in SFA than white meat and have a similar effect on lipid concentrations (Mateo-Gallego, Perez-Calahorra, Cenarro, Bea, Andres, Horno, Ros and Civeira, 2012). Meat is defined as the whole of the carcass of cattle, sheep, goat, camel, buffalo, deer, hare, poultry or rabbit. Meat is the one of the most nutritive foods used for human consumption. Quantitatively and qualitatively meat and other animal food are better sources for high quality protein than plant food, for its richness in essential amino acids and organic acids

that cannot be synthesized in human are available in well balanced proportions and concentration (Siham, Daoud, Hayder, 2014). Meat can be described as the edible flesh of domestic animals (cattle, sheep, goats, pigs, wildlife and poultry). The data from FAO (2009) showed that Nigeria has a per capita meat consumption of 8.8 kg in 2009, meaning that Nigeria is among the 12 poorest nations in animal protein consumption. (Fakolade, 2015). Most consumers are often thrown into confusion with regards to the impact of visible fat and lean meat on human health. This is because it is commonly, but incorrectly believed that lean meat will have the same saturated fat content as the visible fat of meat (Duo, 2005). Some cross-sectional studies suggest that meat eaters have a significantly higher intake of total fat and saturated fat which is associated with increased plasma total cholesterol and increased LDL cholesterol compared with vegetarians. However, in these trials, hidden fats in fast foods, snack foods and other processed foods, rather than red meat, were the primary sources of saturated fat in the meat eater's diets (Meat Advisory panel). Although there are several alternative to protein that offer flavour on their own: rabbit, venison, bison and quail, chicken meat contain less cholesterol and fat content as compared to red meat, but have higher cholesterol than other poultry meat like quail (Fakolade, 2015). The determination of total cholesterol content using gas chromatography has been studied (Dinh, Thompson, Galyean and Brooks, 2012). This method combines direct saponification of 1 g meat sample and GC quantification of liberated cholesterol without derivatization. One gram of sample is saponified with potassium hydroxide (KOH) to liberate cholesterol. Unsaponified materials, including cholesterol, are extracted into toluene. The final toluene extract is diluted with a solution of 5 α -cholestane internal standard (ISTD). Total cholesterol is quantified using a GC-FID system without derivation of the toluene extract. Another method involving gas chromatography was "A simplified method for cholesterol determination in meat and meat products" has been effected (Dinh, Blanton,

Brooks, Miller and Thompson, 2008). The certified cholesterol content of this material was 75 ± 7.2 mg/100 g, which was quantitatively analysed using an isotope dilution/gas chromatography/mass chromatography (ID/GC/MS) method developed at NIST. SRM was carefully mixed, frozen in liquid nitrogen, and homogenized to a powder to use for procedure development. The three beef samples with fat content determined to be from 2.4% to 9.3% were used. Muscle samples were collected between the 10th and 13th ribs, frozen immediately using liquid nitrogen and stored in a -80°C freezer for subsequent sample preparation. Frozen samples were later trimmed of all external fat, leaving only white flecks of marbling within muscle bundles. Trimmed samples were chopped, homogenized to finely divided muscle powder, and stored at -80°C for subsequent analysis. The colorimetric determination of cholesterol was carried out by Warren, Sperry and Brand, (1943). Sample preparation process - 0.4 cc. of blood serum is pipetted into approximately 5 cc. of alcohol-acetone (1:1) in a 10 cc. volumetric flask (or 1 cc. of serum in a 25 cc. flask if replicate determinations are desired) with swirling of the solution, the solvent was brought to a boil on the steam bath, the flask is cooled, it was made up to the mark with alcohol-acetone, mixed, filtered and 5 cc. of the filtrate is pipetted into a 25 cc. Erlenmeyer flask to which 0.15 cc. of potassium hydroxide solution (10 gm. in 20 cc. of water) has previously been added. The liquid is swirled gently at intervals until the alkali has mixed completely with the alcohol-acetone, the flask is placed in an incubator at 37-40° for 40 minutes, 1 drop of phenolphthalein solution is added, it is titrated with 10 % acetic acid in absolute alcohol (about 0.6 cc. should be required), 1 drop excess is added and it is evaporated to dryness on the steam bath with the aid of a stream of air applied by means of suction through a glass tube, bent to avoid contamination from the rubber connection and clamped about 2 cm. above the surface of the solution. The colorimetric method for the determination of total cholesterol has been investigated (Cell Bio labs Inc., 2012). The assay detected total cholesterol

(cholesteryl esters plus free cholesterol) in the presence of cholesterol esterase or only free cholesterol in the absence of the esterase enzyme. Different concentrations of cholesterol samples were determined by comparison with a known cholesterol standard. Cholesteryl esters were quantified by subtracting the free cholesterol values from the total cholesterol value. Another study was carried out by Mirlohi, Madany, Hassanzadeh and Yahay, (2012) on the colorimetric method for cholesterol determination in the laboratory media. The study evaluated the modified o-phthalaldehyde colorimetric method in terms of reproducibility and sensitivity in determination of cholesterol in the *Lactobacillus* specific laboratory media. The results indicated that at the range of 0-100 mg/L cholesterol concentration, the quantity of measured cholesterol was affected by the type of cholesterol

The determination of cholesterol in milk and dairy products has been carried out using HPLC by Oh, Shin and Chang, (2001). About four different methods were used for this determination process. In one of the methods, 1.0 mL of milk was accurately weighed and transferred into a test tube fitted with a Teflon-lined screw cap. Direct saponification was achieved with 1 mL of 10% KOH in ethanol (w/v) for 30 min at 70 °C. The un-saponifiable fraction was extracted using 5 mL of diethyl ether and 2 mL of distilled water. Diethyl ether extraction was repeated 3 times and the sample was rinsed thoroughly. An aliquot of the diethyl ether extract was transferred into a 50 mL round-bottomed, glass stoppered flask and evaporated to dryness on a rotary vacuum evaporator at 50 °C, then re-dissolved in 1 mL methanol. An aliquot (20 µL) was injected directly into the HPLC.

Analysis of cholesterol has also been achieved using high performance liquid chromatography (Almeida, Perassolo, Camargo, Bragagnolo and Gross, 2006). The resulting chromatograms were processed at 210 nm. Cholesterol identification was performed by co-chromatography and by comparing sample retention

times with standard retention times. Quantification for each sample was achieved by internal standardization after saponification. The response factors were calculated daily during the sample period.

A study to evaluate the chemical composition and cholesterol level of fresh camel, beef and goat meat was successfully conducted by Siham et al, (2014). The results showed that chemical composition of camel, beef and goat meat were significantly different ($P < 0.05$). Camel and goat meat had higher moisture content compared to beef as (77.92, 75.55 and 72.12 %) respectively. Whereas beef had higher protein content as (21.07 %) compared to camel and goat meat as (19.25 and 20.32 %) respectively. Camel meat had the lowest fat content (1.17 %) compared to beef and goat meat as (2.74 and 1.66 %). However, camel meat had the highest ash content (0.78 %) followed by beef (0.47 %) and goat meat (0.43 %).

A study was carried out to evaluate the effect of age on physico-chemical, cholesterol and proximate composition of chicken and quail meat (Fakolade, 2015). One hundred poultry birds consisting of 50 chickens (Harco black) and 50 quail Japanese were randomly allocated based on a completely randomized design. Birds were kept for 20 weeks and fed with compounded feeds ad libitum and at 4, 8, 16, 20 weeks, 5 birds each were randomly selected, and the thigh and breast were evaluated for physico-chemical, cholesterol and proximate composition. Results reveal that crude protein content was significantly higher in chicken breast (21.48) at 4 weeks and at 16 weeks in quail breast (21.93), ether extract was higher in the thigh of both chicken and quail (6.33 and 5.06) at 4 weeks of age and thereafter, it decreased with increase in age of birds, respectively.

A study on the total cholesterol content and the estimation of total free fatty acid in some commercial edible oils in Ethiopia, Bahir DAR was successfully conducted by Atinafu and Bedemo, (2011). Cholesterol content was estimated using Liebermann-

Burchard reagent. Among these rapeseed oil has significantly ($p < 0.05$) maximum (257.10 ± 0.42 mg/L) cholesterol content and palm oil has significantly ($p < 0.05$) low (88.8 ± 0.85 mg/L) cholesterol content. Surprisingly, the two Niger seed oils (Niger seed k-16 and Niger seed k-7), contained nil cholesterol content. However, cholesterol content of all the BVOs was higher than that of the UVOs, and the variations were statistically significant ($p < 0.05$).

This work is aimed at providing adequate information about the dietary of individuals with respect to the consumption of cow meat so they can make better decisions on the meat parts that are good for them in the wake of their health status. The research will cover the determination of total cholesterol in cow body parts like heart, liver, lungs, skin, muscle and intestines.

II. EXPERIMENTAL

100 g of each of the meat samples (intestines, skin, heart, lung, liver, and the muscle) were used in this study. The samples used were collected from the local meat shops at Lusada, Igbesa, Ogun state Nigeria. The standard used in this analysis is 99% pure cholesterol (Burgoyne Burbidges & Co, India). Seven standard volumetric flasks were marked S1, S2, S3, S4, S5, S6, and S7 and were used to prepare 0.020, 0.040, 0.060, 0.080, 0.010, 0.012 and 0.014 g/100 ml of the standard solution. 0.020 g of the standard cholesterol was weighed using a weighing balance. It was then placed in a standard volumetric flask and dissolved up to 100 ml in absolute ethanol. This same procedure was carried out for the 0.040, 0.060, 0.080, 0.010, 0.012, 0.014 g standards. They are then warmed and then taken for analysis. 15 g of potassium hydroxide was weighed using a Mettler balance and then kept in a beaker. It is then diluted to 30ml with distilled water. All the six samples were macerated into very small pieces, blended, weighed (100 g of each sample) and placed in six different beakers. Each sample is placed in the soxhlet apparatus and the lipids are extracted

for about 30 minutes. 100 ml of petroleum ether is added to the flask which is placed on the heating mantel. After 30 minutes, the extracts were collected and kept in a standard volumetric flask. The extract was then filtered through a Whatman filter paper and warmed to remove most of the solvent. The samples were then dissolved in absolute ethanol up to 100 ml and saponified using 2 ml of 10 g/20ml potassium

hydroxide. The analysis was carried out using a UV-Visible Spectrophotometer at 550 nm. The absorbance values obtained were correlated by extrapolation from the calibration curve of the standard cholesterol and the results obtained were recorded accordingly.

III. RESULTS

The estimation of total cholesterol in tropical cow meat parts has been carried out and the following results were obtained.

Table 1. Concentration and absorbance of the cholesterol standards

| Standards | Concentration (mg/100ml) | Absorbance |
|-----------|--------------------------|------------|
| S1 | 20 | 0.017 |
| S2 | 40 | 0.026 |
| S3 | 60 | 0.038 |
| S4 | 80 | 0.045 |
| S5 | 10 | 0.064 |
| S6 | 120 | 0.074 |
| S7 | 140 | 0.086 |

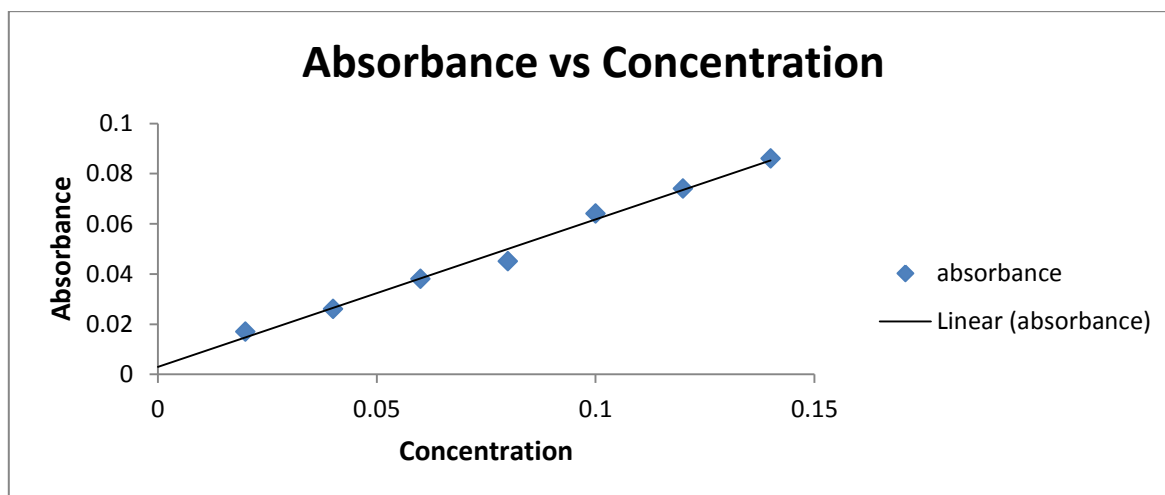


Figure 1. Calibration curve for cholesterol standard

Table 2. Absorbance of samples and concentration of cholesterol in the samples.

| Samples | Samples | Conc. (mg/100ml) | Deviation from RDA (200 mg/1000ml) |
|-----------|---------|---------------------|---------------------------------------|
| Intestine | 0.085 | 138 | 118 |
| Skin | 0.021 | 31 | 11 |
| Heart | 0.065 | 105 | 85 |
| Lung | 0.045 | 71 | 51 |
| Liver | 0.025 | 37 | 17 |
| Muscle | 0.084 | 136 | 116 |

The total cholesterol content showed significant difference among the parts of the meat used. The intestines had the most total cholesterol followed by the muscle, heart, lung, liver and the skin respectively.

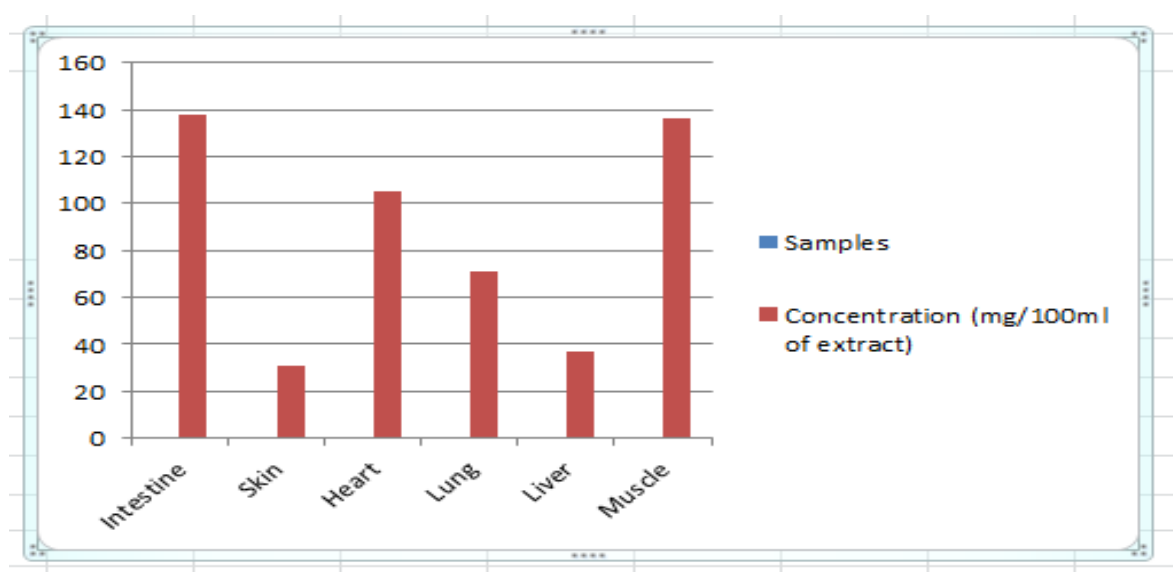


Figure .2 Bar chart showing the cholesterol content of the meat parts

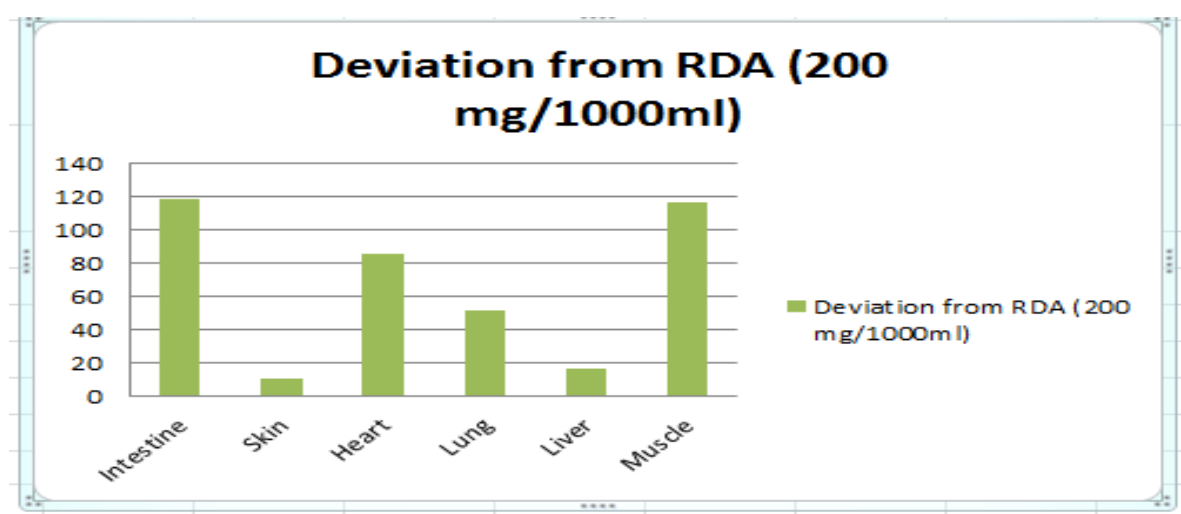


Figure 3. Bar chart showing the Deviation of cholesterol content of the meat parts from RDA

IV. DISCUSSION

Total cholesterol concentration was calculated by extrapolation using the calibration curve which represents the linear relationship between the standard concentration and the absorbance measured at 550nm. The intestines were observed to have the highest amount of total cholesterol (138mg/100g) because it adsorbs the solid onto its surface using the villi acting as a repository; hence its higher concentration of total cholesterol. The part with the second highest amount of total cholesterol is the muscle and this is because of the adipose (fat) that is usually stored in the tissue of the animal. The adipose tissues of slaughtered animals contain about 50-95% of fat. The composition of fat in adipose tissue is highly variable and depends on nutrition, breeding, age, type of animal. Significant and interesting differences have been reported in cholesterol content between muscle types. The amount of total cholesterol found in the muscle is (136mg/100g) whereas in the study conducted by Siham et al on beef muscles, the amount of total cholesterol found was 73.60 ± 6.73 mg/100gm. Another typical and indicative example is the study conducted by R. Chizzolini et al on beef muscles which reported the total cholesterol content as 60 mg/100g. The part with the third highest total cholesterol content is the heart (105 mg/100g) and this is as a result of the cholesterol carried in the blood to and from the heart. They form fatty deposits around the heart. The next is the lung with (71mg/100g) as it is close to the heart. Excess cholesterol is eliminated from the body via the liver, which secretes cholesterol in bile or converts it to bile salts hence it contains a lower amount (37 mg/100g). Lastly, the skin is the part with the least amount of total cholesterol (31 mg/100g). These results are expected because the intestines, muscle and heart have greater quantity of fat than the other parts of the cow used for the analysis. The chemical composition, including the total cholesterol content can be

influenced by different factors such as animal species, breed, age, muscle fibre type and muscle fat content etc.

V. CONCLUSION

A review of some studies provides substantial evidence that lean red meat, trimmed of visible fat, does not raise total blood cholesterol or LDL (bad) cholesterol levels. When consumed as part of a diet low in saturated fat, lean, trimmed beef does not increase cardiovascular risk factors (plasma cholesterol levels or thrombotic risk factors). The skin and the liver with the least amount of total cholesterol can be included in a diet. The intestines having 138mg/100ml of sample is not an ideal meat diet. Chemically, beef has high fat and cholesterol content. Reductions in the cholesterol content of red meat have been achieved by breeding techniques on the farm and new butchery techniques, which trim off most of the fat. Based on the results from the study, the recommended way to prepare beef so that it can be healthier and free of fat and cholesterol is by grilling so that the fat can melt and drip off and what is left is relatively lower in fat and cholesterol; hence it will provide more health benefits. Current evidence strongly supports a new approach that emphasizes the value of naturally nutrient-rich foods, such as lean unprocessed red meats, as part of a healthy balanced diet.

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