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Quantification of Cholesterol and comparative quality assessment of different brands of Ghee

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ABSTRACT: Background: Cholesterol is an essential component of cell membrane that is needed to maintain proper membrane permeability and fluidity; also required for synthesis of steroid hormones, vitamin D and bile acids. But cholesterol plays a major role in human heart health because high level of it increases risk of cardiovascular diseases, stroke, peripheral arterial disease along with other problems like obesity, Alzheimer's disease, gallstones etc. So, we must be concerned about the daily cholesterol intake. We consume a considerable amount of cholesterol daily through using ghee for culinary and flavouring purposes. **Aim:** In this present investigation an attempt has been made to find out total cholesterol content in some of the commercial ghees available in our state which is labelled as 'Cholesterol Free'. **Method:** The analysis was performed by using Liebermann-Burchard, Ojiako-Aukbugwo methods and the qualitative assessment was done by determining iodine and acid values of the samples. **Results:** It confirms that the ghee samples used in this procedure are not completely cholesterol free. **Conclusion:** Producers and marketers should label their products correctly with the quantity of cholesterol in the ghee brand.

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INTRODUCTIONS:

Ghee is usually used as taste enhancer in different cooking preparations. It is rich in cholesterol. While acting as a vital material in our nutrition, it is also a hazard for health. Cholesterol found in cell membrane of all cells, has been of great medical importance in recent years, because its high level in body has been associated with CHD. A person with cholesterol level of 240 mg/dl or above is more prone to risk of heart diseases than a normal person (200 mg/dl). Not the

amount of fat, but also the type of fat in diet that matters. Metabolic studies have shown that trans-fat has adverse effects on blood lipid level, increasing LDL (bad) cholesterol while decreasing HDL (good) cholesterol. Saturated fatty acid rich diets have been found to increase the level of cholesterol [1]. The objective of this work is to determine the amount of cholesterol in different Ghee samples available in the market and to check the label of the marketed product according to the amount of cholesterol.

Fig 1. Different types of ghee.

General facts about ghee and cholesterol:

Ghee (Fig 1) (comes from Sanskrit word "Ghrita") is a class of clarified butter that is originated from the Indian subcontinent; commonly used in Middle Eastern cuisine, traditional medicine and religious rituals.

Ghee is typically prepared by simmering butter, which is churned from cream, skimming any impurity from the surface, then pouring and retaining the clear liquid fat while discarding the solid residue that has settled to the bottom. Spices can be added for flavor. The texture, color and taste of ghee depend on the quality of butter and milk source used in this process and the duration of time spent boiling. Ghee mainly contains – cholesterol, fat (monounsaturated and polyunsaturated), protein, minerals (mainly potassium), vitamin A [1].

Cholesterol (Fig 2 and 3) is a sterol or modified steroid, a type of lipid molecule, is biosynthesized by all animal cells because it is an essential structural component of all animal cell membrane [2].

Importance of the work:

Cardiovascular disease (CVD) is one of the leading major causes of morbidity and mortality worldwide. It may result from the interactions between multiple genetic and environmental factors including sedentary lifestyle and dietary habits. The quality of dietary ghees and fats has been widely recognized to be inextricably linked to the pathogenesis of CVD. Ghee is one of the essential dietary components in daily food consumption. However, the benefits of ghee can be deteriorated by repeated heating that leads to lipid oxidation [3]. The practice of using repeatedly heated ghee or dalda is not uncommon as it will reduce the cause of food preparation. Thermal oxidation yields new functional groups which may be potentially hazardous to cardiovascular health. Prolonged consumption of the repeatedly heated fat has been shown to increase blood pressure and total cholesterol, cause vascular inflammation as well as vascular changes which predispose to atherosclerosis [4]. The harmful effect of heated ghee or oils is attributed to products generated from lipid oxidation during heating process. In view of the potential hazard of oxidation products [5].

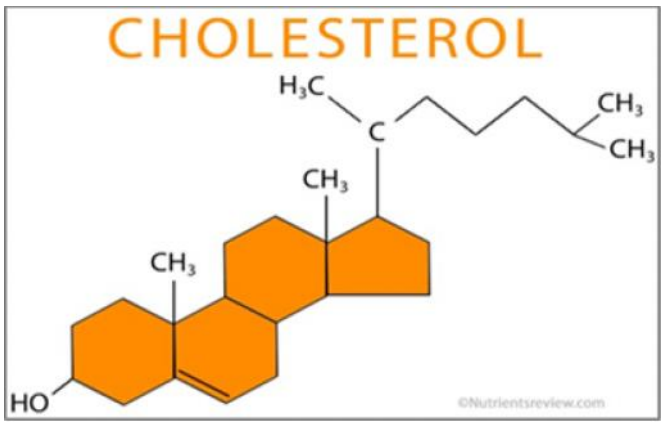

Fig 2. Molecular structure of cholesterol.

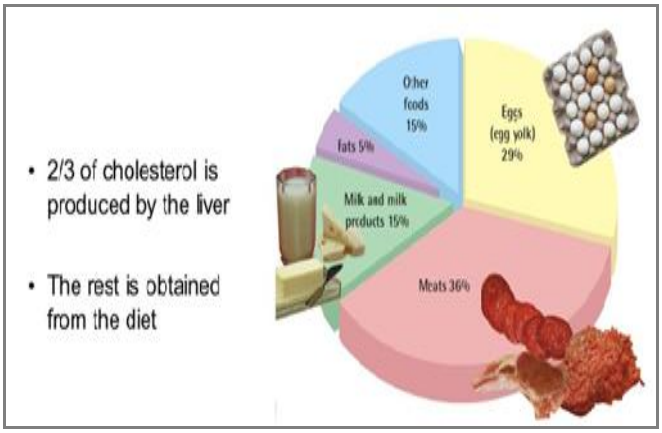

Fig 3. Sources of cholesterol.

MATERIALS AND METHODS:

The different Chloroform, Glacial acetic acid, Ferric chloride, Sulphuric acid, Acetic anhydride, WIJ's solution, Phenolphthalein indicator, Potassium hydroxide, Potassium iodide, Podium thiosulphate were

purchased from Testing Instruments Mfg. Co. Pvt. Ltd, Kolkata and all are of LR (Laboratory Reagents) Grade.

Ojiako and Akubugwo Method:

Total 0.1 ml of ghee sample was dissolved in chloroform in ratio of 1:10, it was evaporated to dryness in a water bath at 50 . Glacial acetic acid (3.0 ml) and 3.0 ml of colour reagent (a solution of ferric chloride/ glacial acetic acid/ sulphuric acid), was added to each sample and then shaken vigorously to dissolve the ghee. Blank contained 2.0 ml of chloroform, 3.0 ml of glacial acetic acid and 3.0 ml of colour reagent. After cooling for 30 min at room temperature, absorbance of blank and samples were read at 560 nm. Cholesterol content (CC in mg/ 100 ml) was estimated with the following formula [1-2];

CC = AB/ AS×CS …… (1)

Where, AB is the absorbance of ghee sample, AS is the absorbance of standard cholesterol (reference value taken) and CS is the concentration of standard cholesterol.

Liebermann – Burchard Method:

The Liebermann-Burchard reaction method is a colorimetric method in which the sample was treated with chloroform, acetic anhydride and concentrated sulphuric acid to produce a green colour which was measured spectrophotometrically at 680 nm by using the formula as mentioned in equation 1 [3-4];

Iodine value:

Chloroform (2 ml) and 5.0 ml of WIJ's solution (8.5 g iodine, 7.8 g iodine trichloride, 450 ml glacial acetic acid in 1 litre acetic acid) were added to sample from burette and mixed thoroughly. Blank contained 2 ml of chloroform and 5 ml of WIJ's solution. The test samples and the blank were left in the dark for 5 min and 3.0 ml of 7.5 % w/v potassium iodide was added to all test samples and blank. Starch indicator (0.1 ml) was added to each sample and blank and titrated to a colourless end point using 0.1N sodium thiosulphate solution. Iodine value (IV)was calculated using the formula [5];

$IV = [(TV_b - TV_g) \times 0.01269 \times 100]/W$ ….. (2)

Where, TV_b is the titer value of blank, TV_g is the titer value of ghee samples and W is the weight of ghee.

Acid value:

Each ghee sample (0.1 g) was weighed and neutralized with 50 ml of fat solvent. About 2 drops phenolphthalein indicator were added and titrated to pink end point which persists for 15 min with 0.1N potassium hydroxide solution. Acid value (AV) was calculated using the formula [6];

AV = (V×0.00561×1000)/W …….. (3)

Where, V is the volume of 0.1N KOH used and W is the weight of ghee.

RESULTS:

Ojiako and Akubugwo Method:

In this method, the cholesterol data exhibited that all the five ghee samples having cholesterol concentration more than 1 mg/ml. The lowest and highest concentration of cholesterol was found in sample no. 1 and 2, as shown in Table 1 and Fig 4.

Table 1. Cholesterol level in different ghee samples by Ojiako and Akubugwo method.

Sl. No.	Cholesterol (mg/ml)
Sample 1	1.053±0.00110
Sample 2	3.176±0.00120
Sample 3	2.263±0.00055
Sample 4	1.818±0.00115
Sample 5	2.001±0.00058

All data are presented as mean standard deviation (n = 3).

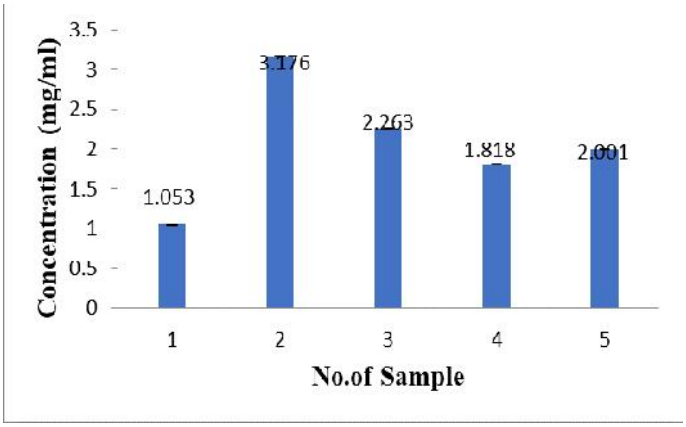

Fig 4. Cholesterol content in different ghee samples by Ojiako and Akubugwo method.

Liebermann – Burchard Method:

In this method, it was shown that all the five ghee samples possessed cholesterol concentration more than 1 mg/ml. The lowest and highest concentration of cholesterol was found in sample no. 1 and 2 (Table 2 and Fig 5).

Iodine and Acid values:

The experimental data revealed that the lowest iodine and acid values was exhibited by sample no. 5 and 4 respectively. The highest iodine and acid values was exhibited by sample 1(Table 3).

Table 2. Cholesterol level in different ghee by Liebermann – Burchard method.

Sl. No.	Cholesterol content (mg/ml)
Sample 1	1.06±0.012
Sample 2	4.99±0.01
Sample 3	3.72±0.006
Sample 4	2.83±0.0012
Sample 5	3.20±0.0057

All data are presented as mean standard deviation (n = 3).

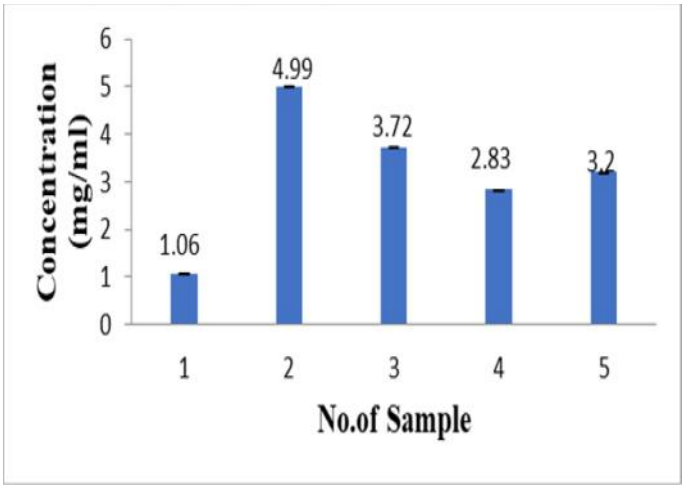

Fig 5. Cholesterol content in different ghee samples by Liebermann – Burchard method.

Table 3. Iodine and acid values of different ghee samples.

Sl. No.	Iodine Value	Acid Value
Sample 1	270.93±0.057	28.05±0.2
Sample 2	187.81±0.06	10.83±0.06
Sample 3	121.57±0.11	5.04±0.058
Sample 4	145.42±0.057	1.6±0.12
Sample 5	68.39±0.2	5.61±0.06

All data are presented as mean standard deviation (n = 3).

DISCUSSIONS:

We have used two different methods in our quest to find out if there is any cholesterol present in the various brands of ghee available in market. Most of them claim to be cholesterol free. Due to increasing awareness on the health implications of high cholesterol in our diets, most people nowadays prefer to purchase cholesterol free ghee.

This study shows that cholesterol is present in the ghee samples, although in small proportion (up to 5 percent of the total sterol). Our result may substantiate this claim as all the samples analysed by the two methods led to the detection of cholesterol in varying proportion. This contradicts the label claim by the brands of ghee. Cholesterol is known as "oily killer" because it is proven that high level of it in blood causes atherosclerotic lesions which are major cause of coronary heart disease. So, we must be concerned about the amount of cholesterol that we take via out diet and should be more careful in choosing different brands of ghee. Popular branded ghee may be an option for local ghee products or loose ghee because it has less percentage of cholesterol. Overall, we should try to consume ghee as minimum as possible.

The qualitative analysis of the ghee samples was done by determining iodine and acid value separately. Iodine value is the measure of degree of unsaturation of the edible fats or ghee. It is the amount of iodine in gm that is taken up by 100gm of fats. Acid value is the number of mg of KOH required to neutralize the free fatty acid present in 1gm of oil or ghee. They indicate stability of ghee or oils and susceptibility to oxidation. High iodine values indicate more unsaturation which is good health and high acid value indicates more prone to oxidation as well as degradation.

CONCLUSION:

Our research confirms that the ghee samples used in this procedure are not completely cholesterol free. It is pertinent that producers and marketers should label their products correctly with the quantity of cholesterol in the ghee brand no matter how minute the quantity therein.

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