Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 6, No. 6, p. 162-171, 2015
http://www.innspub.net

INNSPUB
RESEARCH PAPER
OPEN ACCESS

Evaluation of quality parameters in different foodstuff of Gondar, Ethiopia, with special reference to their cholesterol content

[1]Addis, K.* and [2]Pakshirajan, K.

[1]*Department of Chemistry, College of Natural and Computational Science, University of Gondar, Gondar, Ethiopia*

[2]*Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India*

Article published on June 08, 2015

Key words: Atherosclerosis, Foodstuffs, Cholesterol, Liebermann-Burchard method.

Abstract

High plasma cholesterol is a risk factor in the development of atherosclerosis and coronary heart disease, which is attributed to consumption of diets rich in cholesterol. This study examined cholesterol and other quality parameters, *viz.* acid, peroxide and saponification values, in butter, margarine and vegetable ghee products, which are commonly consumed in Gondar town, Northwest Ethiopia. The cholesterol content in these samples, followed the order butter> margarine >vegetable ghee. Among the different butter variants, Mama butter™ had the highest cholesterol values (283.67 ± 0.488 mg/100 g) and the homemade butter contained the lowest cholesterol ranging from 216-228 mg/100g compared to the industrial butter samples. Vegetable ghee contains much less cholesterol as compared to margarine and butter, thus suggesting it to be better for human consumption. Shola butter™ had the highest acid (15.167 ± 0.026 mgKOH/g) and peroxide (18.579 ± 0.006 meq/kg) values that translates to poor quality of the product for consumption. High proportion of saponification value in Shola butter™ (247.066 ± 0.799 mgKOH/g) suggests that the butter is a good raw material for soap industries.

***Corresponding Author:** Addis K. ✉ addiskokeb1@gmail.com

Introduction

Cardiovascular disease (CVD) is a major cause of disability and premature death of people throughout the world (WHO, 2007). In developing countries, there are indications that death from CVD is rising with changes in lifestyle, modernization, urbanization and sedentary occupation (Lokuruka, 2007). Within the past two decades, the prevalence of CVDs in Ethiopia has increased dramatically (Wai *et al*., 2011). There are several reasons for heart diseases but one of the most important reasons is hypercholesterolemia. The blood stream carries cholesterol in particles called lipoproteins. Cholesterol plays an important role in cell growth and development such as providing essential components of membrane and serving as a precursor of bile acids, steroid hormones and vitamin D (Man, 1998; Daksha, *et al.,* 2010; Akhtar *et al*., 2011). However, over the past few years, there has been increasing evidence indicating that high plasma cholesterol is a risk factor in the development of atherosclerosis and coronary heart disease (CHD). Thus, the cholesterol content of food has been a contentious issue for consumers when faced with the decision of consuming fat.

Weggemans *et al.* (2001) had reviewed the effects of dietary cholesterol on blood lipids and lipoproteins. Based on the results, it was found that the addition of 100 mg dietary cholesterol per day increased total cholesterol concentrations by 0.056 mmol/L, LDL cholesterol concentrations by 0.050 mmol/L and HDL cholesterol concentrations by 0.008 mmol/L. Cholesterol is synthesized in the body and, hence, it is not an essential dietary component. Higher dietary cholesterol increases blood cholesterol and, therefore, the blood cholesterol elevating effect of dietary fats increases with a high cholesterol consumption. Cholesterol intake can be reduced by limiting the consumption of high fat foods (Rao, 2010).

Many consumers have started avoiding high cholesterol containing food and turning toward low cholesterol food (Man, 1998). The results from all clinical trials show that even one percent reduction in total cholesterol can cut down the risk of developing heart attack by two percent (NIH, 1988). Hence, in the recent years the influence of diet on human health, especially the effect of dietary fat on heart disease has been repeatedly stressed. This is particularly important because several studies have indicated that the cholesterol levels in the blood and consumption of diets rich in cholesterol are closely related. This resulted in a trend to consume foods with less cholesterol content (Alogˇlu *et al.,* 2006). Diet is an important factor for controlling serum lipids and consequently the occurrence of coronary heart disease (CHD) (Grundy *et al.,* 1990). Therefore, it is necessary to identify and limit foodstuff that is rich in cholesterol (Daksha *et al.,* 2010). Besides, parameters such as acid value, peroxide value and saponification value in such foodstuff need to be analyzed to check if they are suitable for human consumption or not.

Acid value is a measure of the free fatty acids in oil. The acceptable levels for acid value in fats samples should be below 0.6 mg KOH/g. Normally, fatty acids are found in the triglyceride form, however, during processing the fatty acids may be hydrolyzed into free fatty acid. The higher the acid value, the higher the levels of free fatty acids suggesting high level of hydrolytic and lipolytic activities that causes deterioration in fat quality. Hence, highly acidic fats are considered a good source of raw materials for industries than for human consumption (FAO/WHO, 1982).

Peroxide value is a measure of oxidation during storage and the freshness of lipid matrix. In addition, it is a useful indicator of the early stages of rancidity occurring under mild condition and it is a measure of the primary lipid oxidation products. The accepted peroxide value is 10 meq/kg. High peroxide value is an indicator of oxidation level and the greater the peroxide value, the more oxidized the fat (AOAC, 1997).

Saponification value is an indication of the molecular weights of triglycerides in fats and oils. Higher saponification value indicates high proportion of

lower fatty acids since saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids (Muhammad *et al.*, 2011). Therefore, shorter the average chain length (C4-C12) the higher is the saponification number (Tamzid *et al.*, 2007). Despite the heightened awareness about the effect of cholesterol, there seem to have been no study undertaken to examine the cholesterol content and other quality parameters in foodstuff that are routinely included in our diet.

The present investigation was therefore, carried out for the determination of cholesterol and other quality parameters, *viz.* acid, peroxide and saponification values in industrial butter, homemade butter, vegetable ghee and margarine samples.

Materials and methods

Sample collection

Three different samples each of industrial butter, homemade butter products and two samples each of margarine and vegetable ghee products was purchased from local market in Gondar, Ethiopia. The branded butter samples included Shola butter™, Yadot butter™ and Mama Pasteurilized butter™. The homemade butter samples included butter from different locations in Gondar Town, i.e. from Ayimba, Blajig, and Fenter. Rosa margarine™ and Reima margarine™ contributed to the margarine samples tested in this study. Vegetable ghee samples were Sheno lega™ and Shadi lega™. All these products were the most commonly consumed and available in Gondar Town, Northwestern Ethiopia.

Analysis of quality parameters

The collected product samples were analyzed for different quality parameters *viz.*, cholesterol content, acid value, peroxide value and saponification value as detailed further.

Cholesterol determination

A 200 mg/dl (Human GmbH. 65205 Wiesbaden, Germany) cholesterol stock solution was used to prepare standard cholesterol solution by serial dilution with chloroform followed by treatment with Liebermann-Burchard reagent prepared by the addition of concentrated sulfuric acid (0.5 mL) and glacial acetic acid (10 mL). For cholesterol determination, Liebermann-Burchard method (Burke *et al.,* 1974) was followed, which is based on colorimetric determination of a green color complex formed. The green color formation is due to the reaction between the hydroxyl group (-OH) of cholesterol and the reagent that increases with conjugation of the unsaturation in the adjacent fused ring. In this reaction, the acetic acid in the Liebermann reagent reacts with cholesterol in samples forming a green color (Atinafu *et al.*, 2011) the absorbance of which was determined by using a UV-Visible spectrophotometer (SP65, Sanyo, UK) at 640 nm.

Flasks containing the standard cholesterol solutions mixed with the Liebermann-Burchard reagent covered with black carbon paper and kept in dark for 15 min prior to analysis. For cholesterol determination in the foodstuff, 1g sample was dissolved in 10 mL chloroform, followed by suitable dilution (up to 10 times). 1 mL each of the diluted sample solutions was mixed with 2 mL Liebermann-Burchard reagent and 7 mL chloroform followed by incubation under dark for 15 minutes. Absorbance of the resultant mixture was then determined as detailed earlier. The amount of cholesterol in the sample solutions was finally determined using a standard curve.

Acid value determination

Acid value was determined according to AOAC Standard 969.17 (AOAC, 1997), and for which the fats were melted at 45°C and mixed thoroughly before weighing. Samples (1.0 g each) were weighed and dissolved in 50 mL of ethanol in a conical flask. Then, two drops of phenolphthalein indicator was added, and titrated with 0.1 N potassium hydroxide solution (KOH) to a pink color endpoint. Acid value of the sample was finally calculated using equation (1).

$$\text{Acid value} = \frac{56.1 \times V \times C}{m} \qquad (1)$$

Where, 56.1is the equivalent weight of KOH, *V* is the volume in mL of standard volumetric KOH solution used, *C* is the exact concentration in mg/L of KOH solution used and *m* is the mass in grams of the test portion.

Peroxide value determination

Peroxide value was determined according to AOAC Official Method 965.33 (AOAC, 2000). Five grams each of the samples was transferred into a 250 mL conical flask with stopper and 30 mL of glacial acetic acid-chloroform solvent mixture (3:2 v/v) was added and vortex mixed to dissolve the fat. About, 0.5 mL saturated potassium iodide solution was added and the resultant mixture was allowed to stand for 1min in dark with occasional shaking followed by addition of 30 mL distilled water. The liberated iodine was titrated with 0.1 N sodium thiosulfate solution with vigorous shaking until final disappearance of yellow color. The titration was continued by adding 0.5 mL starch solution as the indicator and by vigorous shaking to release all I_2 from $CHCl_3$ layer until blue color disappeared. The same procedure was followed for the blank. The peroxide value was finally calculated using equation (2).

$$\text{Peroxide value}(\text{meq/kg}) = \frac{\text{Titre} \times \text{N} \times 1000}{\text{w}} \quad (2)$$

Where, Titre is mL of sodium thiosulfate used (blank corrected), Titre= sample titration minus blank titration (S-B), N is normality of sodium thiosulfate solution and w is weight of test portion in gram.

Saponification value determination

Saponification value was determined according to AOAC Official Method 920.160 (AOAC, 2000). Sample (2 g) was mixed thoroughly in a 250 mL conical flask and 25 mL of alcoholic potassium hydroxide solution was added. The sample flasks connected to reflux condenser kept on a water bath maintained at 40°C for gentle boiling until a clear solution formed after 1.5 h. After cooling the contents of the flasks to room temperature, 1.0 mL phenolphthalein solution was added and the excess potassium hydroxide was titrated with 0.5N hydrochloric acid until the pink color disappeared. Saponification value was finally calculated using equation (3).

$$\text{saponification value} = \frac{56.1 \times (B - S) \times N}{w} \quad (3)$$

Where, B is the volume in mL of the standard hydrochloric acid required for the blank, S is the volume in mL of the standard hydrochloric acid required for the sample; N is the normality of the standard hydrochloric acid and w is the weight in gram of the fat sample taken for the test.

Data analysis

All the quality parameters were tested in triplicate. In all cases, both standard and blank was treated in same way as the real samples to minimize matrix interferences during analyses. The results obtained at 99% confidence level were calculated using equation (4).

$$\mu = \bar{x} \pm \frac{ts}{\sqrt{n}} \quad (4)$$

Where $\bar{x}$ is the mean of nine measurements, s is the standard deviation of the nine measurements, t is the statistical factor whose value is 3.36 at 99% confidence level at eight degrees of freedom, and n is the number of measurements (nine in this case).

Results and discussion

Cholesterol content

The cholesterol level varied among the different samples of industrial butter, homemade butter, margarine and vegetable ghee (Table 1). Among the industrial butter samples, minimum amount of cholesterol was present in Yadot butter™ (252.990 mg/100 g) and maximum was in Mama pasteurized butter™ (283.670 mg/100 g) (Table 1). In the case of homemade butter, the sample obtained from Blajig area contained the lowest amount of cholesterol (216.647 mg/100 g) and butter obtained from Fenter area contained the highest amount of cholesterol (228.51 mg/100 g).

It is known that the duration and maintenance temperature of yoghourt used in the preparation play a significant role on pH and solubility of various fatty acids in these products. Hence, only a few days maintenance of yoghourt in the traditional method increases the contents of short and medium chain fatty acids while minimizing the content of long chain fatty acids in the final product (Parodi, 1970). Besides, innate microorganisms present in the yoghourt may convert esteric cholesterol to free cholesterol that does not participate in the resulting emulsion (Bahrami *et al.*, 2009). Thus, it can be surmised that the butter produced in traditional household method contains a low amount of cholesterol compared with that in butter produced via the industrial method.

Different researchers have compared the cholesterol level of homemade butter with industrial butter and have generally reported less cholesterol content in homemade one. For instance, Zeljka *et al.* (2000) compared the cholesterol level in butter samples from village household and industry, and showed that homemade butter was better than the industrial sample as it contained low level of cholesterol. A study conducted by Bahrami *et al.* (2009) showed that the traditional butter contains about 50% less cholesterol (155 mg/100 g) than the industrial butter (320 mg/100 g). Another study also showed that the cholesterol level of a homemade butter was lower (500 mg/100 g) than that of an industrial butter product (800-1300 mg/100 g) (Akhtar *et al.*, 2011).
The cholesterol content in milk fat is usually between 204.3 mg/100g and 382.4 mg/100g (Precht, 2001). These values are, however, different from the cholesterol content obtained in the present investigation, probably due to various factors, including milk fat content, sample variation (Oh *et al.*, 2001), extraction procedure, analytical technique used (Sweeney *et al.*, 1976; Man, 1998; Oh *et al.*, 2001), and incubation conditions (Man, 1998; Sterna, 2005).

The cholesterol concentration was 210.543 mg/100g and 206.487 mg/100g for Reima™ and Rosa™ margarine samples, respectively. These values are higher than the cholesterol content of the vegetable ghee samples. These results also showed that Shadi lega™ and Sheno lega™ vegetable ghee samples contain high amount of cholesterol i.e. 132.437 mg/100 g and 89.230 mg/100g, respectively.

The cholesterol content of margarine samples tested in this study was different from that observed by Scherr *et al.* (2009) with a reported value of zero in their study. The Japan Resources Council Agency (1982) as well reported the cholesterol level of margarine in the range 0-2 mg/100 g. The results obtained in this study are in good agreement with other reported values of cholesterol content from vegetable ghee samples in the range 100-400 mg/100 g (Sabir *et al.,* 2003) and 2700-6100 mg/100 g (Daksha *et al.*, 2010). These variations in the cholesterol level of the different margarine and vegetable ghee samples could be due to the inherent variations in their production method and raw materials used (List *et al.*, 2005). For instance, depending upon the raw material used in margarine preparation, i.e. whether vegetable or animal source, its cholesterol level can vary from zero in case of vegetable source to very high in case of animal source. However, precise determination of the effect of the different process parameters such as temperature, pH, solubility, microorganisms, raw material composition, packing material etc. is necessary to keep the cholesterol content low in such food stuff. Thus, the results obtained from this study suggest that improvement of the LDL risk profile is feasible through the replacement of butter with margarine.

Phytosterols are cholesterol-like molecules absorbed only in trace amounts, but it aids in cholesterol elimination by inhibiting the absorption of intestinal cholesterol and by recirculating endogenous biliary cholesterol (Daksha *et al.,* 2010). It has been shown that people who adopt a vegetarian diet reduce their fat intake by 26% and achieve a significant drop in

cholesterol levels in just six weeks (Maseri *et al.*, 1984). The very low level of fat consumed through a typical vegetarian diet helps in decreasing risk of heart attack (Sabir *et al.*, 2003). From the results obtained in this study, it can be well said that in order to lower the risk of CVD vegetable ghee is much preferable to butter and/or margarine owing to its low cholesterol content.

Acid value

Table 1 shows that among the industrial butter samples, highest acid value is associated with Shola butter™ (15.167 mg KOH/g) and the lowest one in Yadot butter™ (12.608 mg KOH/g). Among the homemade butter, highest acid value was obtained from Fenter (8.076 mg KOH/g) and lowest in Blajig (4.413 mg KOH/g). However, for margarine and vegetable ghee samples, maximum acid value was recorded in Reima™ margarine (8.181 mg KOH/g) and a lower value in Sheno lega™ vegetable ghee (1.705 mg KOH/g).

This acid value was higher than the recommended and reported value of less than 0.8 mg KOH/g (FAO/WHO, 2006) which indicates a poor quality of the butter sample. Another study reported the acid value of butter samples from family plants and small dairies in the range 1.05-25.05 mg KOH/g and 1.39-6.91 mg KOH/g fat, respectively,(Celik *et al.*, 2000) which is closer to the value obtained in this work (Table 1). The higher acid value for the butter samples may be due to processing, storage conditions, light and temperature exposure and activity of lipase enzyme (Bendixen, 1940; Koczon *et al.*, 2008).

The acid value of margarines and vegetable ghee samples can be in the range 0.5771-0.995 mg KOH/g and 0.4577-0.9154 mg KOH/g (Naz *et al.*, 2012), respectively. In another study conducted by Gizaw (2007) the acid value of margarine and vegetable ghee samples was in the range 0.227-0.394 mg KOH/g and 0.334-0.438 mg KOH/g, respectively. Compared to these literature reports, the acid value obtained in this study for the margarine samples was 2.775 mg KOH/g for Rosa™ and 8.181 mg KOH/g for Reima™, whereas for the vegetable ghee samples it was 1.705 mg KOH/g for Sheno lega™ and 1.989 mg KOH/g for Shadi lega™. These values are higher than the literature reported results and the recommended value. A high acid value may indicate a higher tendency to become rancid (Karim, 1997). The difference in the acid values for margarine and vegetable ghee samples could be attributed to the presence of moisture, elevated temperature (above ambient room temperature), packing material used and most importantly due to the enzyme lipases secreted by contaminating microorganisms present in the fat (Naz *et al.*, 2012).

The lowest acid value of Shadi lega™ and Sheno lega™ suggest that these are suitable for consumption purpose. On the contrary, the high acid value of Shola butter™, Mama butter™, and Yadot butter™ indicate high free fatty acids content with a tendency to become rancid, suggesting the requirement of further refining for a better edibility. Else, they may be better utilized for industrial purposes.

Peroxide value

Peroxide value of a substance gives an estimate of its oxidation level, which is used to obtain the amount of the primary product of hydroperoxides. The result obtained by Celik *et al.* (2000) in the samples of butter from family plants and small dairies showed peroxide value in the range 1.35-5.86 meq/kg and 1.99-4.23 meq/kg fat, respectively. In this study, all the samples except the commercial butter samples yielded peroxide value within the recommended limit of <10 meq/kg (Joint FAO/WHO, 2006) (Table 1).

The peroxide value for industrial butter ranged from 13.383 to 18.579 meq/kg and for the home made butter it was in the range 2.018-2.779 meq/kg (Table 1). The higher peroxide value for the industrial butter samples may be due to the effect of moisture, light, heat and transition metals during its processing and packing (Akinoso *et al.*, 2010). A lower peroxide value for homemade butter than the industrial butter may

be attributed to long-term storage of industrial butter under non-refrigerated condition compared to the homemade one, which was collected afresh and analyzed immediately after a brief storage period in a refrigerator. However, determination of peroxide value of fresh butter samples collected based on their manufacturing date will be necessary to ascertain this aspect.

Table 1. Cholesterol content, acid value, peroxide value and saponification value in different samples of industrial butter, homemade butter, margarine and vegetable ghee.

Samples	Cholesterol (mg/100 g)	Acid value (mg KOH/g)	Peroxide value (meq/kg)	Saponification value (mg KOH/g)
Mama butter™	283.670 ± 0.488	14.792± 0.106	15.353± 0.050	222.037 ± 0.168
Yadot butter™	252.990 ± 0.243	12.608± 0.015	13.383± 0.005	231.187 ± 0.212
Shola butter™	262.133 ± 0.288	15.167± 0.026	18.579± 0.006	247.066 ± 0.796
Fenter (home made butter)	228.510 ± 0.298	8.076 ± 0.030	2.779 ± 0.005	226.940 ± 0.287
Ayimba (home made butter)	223.020 ± 0.242	5.240 ± 0.024	2.023 ± 0.009	212.650 ± 0.578
Blajig (home made butter)	216.647 ± 0.613	4.413 ± 0.083	2.018 ± 0.016	215.163 ± 0.953
Reima margarine™	210.543 ± 0.123	8.181 ± 0.114	13.579± 0.003	197.342 ± 0.773
Rosa margarine™	206.487 ± 0.691	2.775 ± 0.003	2.184 ± 0.005	190.980 ± 0.123
Shadi lega ghee™	132.437 ± 0.208	1.989 ± 0.005	1.983 ± 0.003	190.057 ± 0.787
Sheno lega ghee™	89.230 ± 0.459	1.705 ± 0.002	1.203 ± 0.007	187.703 ± 0.589

™ stands for the branded samples.

The results of peroxide value for margarine and vegetable ghee are in the normal range specified for these foodstuffs except that the peroxide value of Reima™ margarine (13.579 meq/kg) is higher than the recommended level. FAO/WHO (2006) joint committee standard for such products indicates that peroxide value should not exceed 10meq/kg for vegetable ghee and margarine products. Naz *et al.* (2012) reported the peroxide value for samples of vegetable ghee in the range 0.93-3.76 meq/kg and for margarine in the range 1.43-4.96 meq/kg. In a study, Gizaw (2007) reported the peroxide value for vegetable ghee samples in the range 11.87-18.95 meq/kg and for margarine samples within 8.81-19.98 meq/kg. It is well known that factors such as temperature, light, moisture, metals and oxygen affect the rate of oxidation of fats and are, therefore, the major cause of fat deterioration (Akinoso *et al.*, 2010). In general, the peroxide value of fats increases with their storage time. Fats exposed to both atmospheric oxygen and light showed a much larger increase in peroxide value during storage (Othman *et al.*, 2010). A high peroxide value for Reima™ margarine may be attributed to one or more of the above reasons.

Saponification value

Among the six samples of butter investigated for their saponification value, Shola™ butter had the highest saponification value (247.066 mg KOH/g) and Ayimba homemade butter contained the least saponification value(212.650 mg KOH/g) (Table 1). Celik *et al.* (2000) determined the saponification value of butter products in the range 186-244 mg KOH/g. Sengul *et al.* (1998) also reported a similar saponification value of butter samples in the range 124.70-272 mg KOH/g. The variation of the saponification value in the butter samples is probably due to the differences in both their preparation method and their composition. The highest value obtained for Shola™ butter in this study showed that it contains high amounts of short chain fatty acids (< C_{12}) suggesting its suitability as a good raw material for soap industries than for human consumption.

The saponification value for margarine and vegetable ghee samples tested in this study is high for Reima™ margarine (197.342 mg KOH/g) and low for Rosa™ margarine (190.980 mg KOH/g). In the case of vegetable ghee, a high value was obtained with Shadi™ lega (190.057 mg KOH/g) and a low value was obtained with Sheno™ lega ghee (187.620 mg KOH/g). Gizaw (2007) reported the saponification value for margarine samples in the range 190.07-192.09 mg KOH/g and for vegetable ghee samples; it was 192.41-194.84 mg KOH/g. A similar study showed that saponification value for margarine samples was in the range 189.05-192.30 mg KOH/g and for vegetable ghee samples; it was 184.40-190.84 mg KOH/g (Naz *et al.*, 2012). FAO/WHO joint committee standard for margarine and vegetable ghee products suggests saponification value to be in the range 185-200 mg KOH/g (FAO/WHO, 2006). The results obtained from this investigation were in agreement with the standard saponification value that translates to its use as a good raw material for soap production and suitable for consumption. The slight variation of this parameter value from the literature reported studies might be due to differences in the production method and the type of raw material used for its production.

Conclusion

In conclusion, as none of the samples analyzed were found to be free from cholesterol, there is a risk of developing cardiovascular diseases in the consumers of these products. This study showed that in the traditional method of butter production, several health beneficial changes are incorporated which improves the buttery quality in terms of its cholesterol content. Further, vegetable ghee contains much less cholesterol as compared to margarine and butter, thus suggesting it to be better for human consumption. Unlike the saponification values, some of the samples show high peroxide and acid values in comparison with the maximum permissible level that translates to poor quality of the products.

Acknowledgement

The author Addis Kokeb gratified to Dr. Mehretie Kokeb and Sister Medenekia Melese, University of Gondar, for their financial support to carry out this research work. The authors have declared no conflict of interest.

References

Akhtar MS, Jabbin D, Irfan HM, Abbas N. 2011. Cholesterol, omega-3 and oleic acid contents of various oils, butter and vegetable ghee used as dietary fats. Journal of Technical Research **02(01),** 14-19.

Akinoso R, Aboaba SA, Olayanju TMA. 2010. Effects of moisture content and heat treatment on peroxide value and oxidative stability of unrefined sesame oil. African Journal of Food and Agricultural Nutrition Development **10(10),** 4268-4285.

Alogˇlu H, Öner Z. 2006. Assimilation of cholesterol in broth, cream, and butter by probiotic bacteria. Journal of European Lipid and Science Technology **108**, 709–713.

AOAC. 2000. Official method of Analysis 17th ed. Gaithersburg, Maryland. AOCS International press.

AOAC. 1997. Official methods of Analysis 16th ed. Gaithersburg, Maryland: Association of Analytical chemists International.

Atamer M. 1993. Guidebook of butter technology. Agriculture Faculty Press. Ankara University, Turkey 1-52.

Atinafu DG, Bedemo B. 2011. Estimation of total free fatty acid and cholesterol content in some commercial edible oils in Ethiopia, Bahir Dar. Journal of Cereal and Oil seeds **2**, 71-76.

Bahrami G, Vanak ZP. 2009. Improvement of fatty acids composition and reduction of cholesterol contents in traditionally prepared butter and oil in west of Iran. Journal of ARYA Atherosclerosis **4**, 152-156.

Bendixen HA. 1940. Acid values and acid ratios as related to the keeping quality of salted butter. Journal of Dairy Science **23**, 275-284.

Burke RW, Diamondstone BI, Velapoldi RA, Menis O. 1974. Mechanisms of Liebermann-Burchard & Zak color reactions for cholesterol. Journal of Clinical Chemistry **20,** 794-801.

Celik S, Bakirci I. 2000. A study on the physical and chemical properties of cookery-type butter. Pakistan Journal of Biological Science **3**, 596-598.

Daksha A, Jaywant P, Bhagyashree C, Subodh P. 2010. Estimation of sterols content in edible oil and ghee samples. International Journal of Pharmaceutical Sciences Review and Research **5,** 135-136.

FAO/WHO. 1982. Codex Alimentarius Commission: Recommended internal standards for edible fats and oils 1st ed. Rome, Italy.

Gizaw E. 2007. Evaluation of hydrogenation process, trans-fatty acid level and some qualities of selected hydrogenated food products in the markets of Addis Ababa. Addis Ababa. MSc thesis, Addis Ababa University Ethiopia, 35-60.

Grundy SM, Denke MA. 1990. Dietary influences on serum lipids and lipoproteins. Journal of Lipid Research **31**, 1149-1172.

Joint FAO/WHO. 2006. Food standards program: Codex Committee on nutrition and foods for special dietary uses. Geneva, Switzerland.

Karim MR. 1997. Purification and characterization of proteins from mulberry silkworm *Bombyx mori* L. and biochemical analysis of two varieties of pupae bivoltein and multivoltein. MSc thesis, Rajshahi University, Bangladesh.

Koczon P, Gruczynska E, Kowalski B. 2008. Changes in the acid value of butter during storage at different temperatures as assessed by standard methods or FT-IR spectroscopy. American Journal of Food Technology **3**, 154-163.

List GR, Wang T, Shukla KS. 2005. Storage, Handling, and Transport of Oils and Fats. In Fereidoon, S. (ed). Bailey's industrial oil and fat products, volume 5, 6th ed., John Wiley & Sons 191-192.

Lokuruka MNI. 2007. Role of fatty acids of milk and dairy products in cardiovascular diseases. African Journal of food and Agricultural Nutrition Development **7**, 3-10.

Man X. 1998. An affinity chromatography procedure to remove cholesterol from butter oil, lard and tallow. Guelph, Canada: University of Guelph, MSc thesis.

Maseri JR, Rouse IL, Lynch WJ. 1984. Vegetarian diet lipids and cardiovascular risk New Delhi. Journal of Agricultural Research New Delhi **14**, 400-404.

Muhammad N, Bamishaiye E, Bamishaiye O, Usman L, Salawu M, Nafiu M, Oloyede O. 2011. Physicochemical Properties and Fatty Acid Composition of Cyperus esculentus (Tiger Nut) Tuber Oil. Bioresearch Bulletin **5**, 51-54.

Naz R, Anjum FM, Rasool G, Nisar MA, Batool R, Saeed F. 2012. Total trans fat content in comercially available hydrogenated vegetable oils. Pakistan Journal of Nutrition **11**,145-149.

NIH Report. 1988. Modified from Pakistan NIH report of the National Cholesterol Education program Expert panel on Detection, Evaluation, and treatment of High blood Cholesterol in adults. Journal of Architecture Internal Medicine **148**, 36-69.

Oh HI, Shin TS, Chang EJ. 2001. Determination of cholesterol in milk and dairy products by high-performance liquid chromatography. Asian-Australasian Journal of Animal Science **14**, 1465-1469.

Othman OC, Ngassapa FN. 2010. Physicochemical characteristics of some imported edible vegetable oils and fat marketed in Dar es Salaam. Tanzanian Journal of Natural and Applied Science **1**, 138-147.

Parodi PW. 1970. Fatty acid composition of Australian butter and milk fat. Australian Journal of Diary Technology **25,** 200-201.

Precht D. 2001. Cholesterol content in European bovine milk fats. Journal of Food/Nahrung **45**, 2-8.

Rao DR. 2010. Dietary guidelines for Indians: National Institute of Nutrition 2nd ed. Hyderabad, India, 48.

Resources Council, Science and Technology Agency, the Government of Japan. 1982. Standard Tables of Food Composition in Japan, 4th ed. Tokyo: Ministry of Finance Printing Bureau, 13.

Sabir SM, Hayat I, Gardezi SDA. 2003. Estimation of sterols in edible fats and oils. Pakistan Journal of Nutrition **2**,178-181.

Scherr C, Ribeiro JP. 2009. Fat content of dairy products, eggs, margarines and oils: Implications for atherosclerosis. Brazil: Gama Filho University, Phd dissertation.

Sengul M, Cakmakci S, Unsal M. 1998. The determination of physical and chemical properties of Trabzon butter. National Productivity Centre, Ankara, Turkey 230-243.

Sterna V. 2005. Cholesterol dynamics regularities in Latvia cow's milk in connection with breeds and keeping conditions. PhD thesis, Latvia University of Agriculture, Jelgava, 45-50.

Sweeney JP, Weihrauch JL. 1976. Summary of available data for cholesterol in foods and methods for its determination. Critical Review in Food Science and Nutrition **8**, 131-159.

Tamzid HM, Alam MT, Islam MA. 2007. Physicochemical and nutritional studies of *Terminalia belerica* roxb. Seed oil and seed kernel. Journal of Biosciences **15**, 117-126.

Wai WS, Dhami RS, Gelaye B, Girma B, Lemma S, Berhane Y, Bekele T, Khali A, Williams MA. 2011. Comparison of measures of adiposity in identifying cardiovascular disease risk among Ethiopian adults. Journal of Obesity **10**, 1-9.

Weggemans RM, Zock PL, Katan MB. 2001. Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol in humans: a meta-analysis. American Journal of Clinical Nutrition **73(5),** 885-891.

WHO. 2007. Prevention of cardiovascular disease. Pocket Guidelines for assessment and management of cardiovascular risk, Geneva.

Zeljka BN, Davorin B, Mirza A. 2000. The quantity of cholesterol in butter. University of Zagreb, Croatia.