

Quality Evaluation and Determination of Possible Adulterants in Various Marketed Butter Oil and Ghee in Bangladesh

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Abstract

Butter oil is a dairy product with high butter fat content that turns into solid when chilled and liquid when warmed. Ghee is a class of clarified butter originated in primitive India. Both butter oil and ghee are widely used for cooking various foods as well as food supplement in Bangladesh. However, low-quality butter oil and ghee may cause serious health hazard to the population who consumes regularly. In the present study, the physicochemical parameters of marketed butter oil and ghee samples were analyzed and results were compared with Bangladesh Standards and Testing Institution (BSTI) standard. Three (3) samples of butter oil (B-1 to B-3) and 24 samples (G-1 to G-24) of ghee were collected from different local market of Bangladesh. The quality determinant parameters like moisture content, acid value and free acid value, iodine value, saponification value, peroxide value and presence of sesame oil were analyzed. All the samples of butter oil and ghee were failed to meet the standard value of moisture content (butter oil, $\leq 0.5\%$ and ghee, $\leq 0.1\%$), saponification value (≥ 218 mg/gm) and sesame oil test (negative) compared to the BSTI standard. However, among all 27 samples only one sample of ghee, G-7 (35.01) met the limit of iodine value (26-35) compared to the BSTI standard. Rest of the parameters like acid value (≤ 1.9 mg/gm), free acid value and peroxide value (≤ 0.8 mEq of O₂/kg) were within the limit of BSTI standard for all the samples. However, every parameter needs to be within the BSTI standard limit to ensure the quality of butter oil and ghee. So, more concern is needed by the government authority to ensure the proper quality of marketed butter oil and ghee in Bangladesh.

Key words: Butter oil, Ghee, Quality evaluation, Adulteration, BSTI standard.

Introduction

Butter oil refers to the anhydrous milk fat or dry butter fat manufactured mainly from butter or cream by the removal of practically all the water and solids which are free from fat. Ghee, a class of clarified butter originated in primitive India, contains carbonyls, free fatty acids, lactones, and alcohols as its main flavor components¹. Both butter oil and ghee contain carbohydrates, fat, protein, vitamins and some minerals as a source of nutrition. Both butter oil and ghee are produced from fresh milk by successive fermentation, churning and heating². In India ghee is used in both religious purpose and in the diet. It was regarded pure in ancient India and was experienced to confer purity to foods cooked with it³. Butter oil and ghee possess some medicinal properties like emollient, anti-viral, anti-cancer, anti-bacterial, anti-inflammatory, antioxidant, anti-fungal and cardioprotective activities^{4,5}. Besides these therapeutic properties, Ayurveda mentions many

more medicinal properties of ghee and butter oil, such as soothes the skin, cicatrizant (healing of wounds), anti-irritant and carrier agent⁴. It may be better for people who are intolerant to butter to use ghee because ghee does not contain same amount of dairy protein as butter. Moreover, the amount of short and medium chain fatty acids is nearly doubled in ghee compared to butter⁶. Generally, butter oil is safe to use. However, if excess amount of ghee is taken, it causes indigestion and diarrhea. The risk of cardiovascular disease is increasing due to its high amount of saturated fatty acids in ghee⁷. To ensure the safety of the population using butter oil and ghee in their daily use in Bangladesh, Bangladesh Standards and Testing Institution (BSTI) tests some unique parameters to ensure the quality of market available goods. After getting approval from BSTI, company can market their product. With the increase in the number of the cases of counterfeit and adulteration, butter oil and ghee with substandard quality are entering the market. To take a measure in

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this regard, the situation of the market should be known firstly. In this purpose, different brands of butter oil and ghee were collected and their unique physicochemical parameters were tested. The values were compared with those specified in BSTI standards. Hence, the intention of the investigations was to find out the chemical characteristics and quality of butter oil and ghee of different brands collected from retail markets.

Materials and Methods

Reagents and instruments

The moisture content, acid values, free fatty acid values, iodine values, saponification values, peroxide values and presence of sesame oil were determined by standard methods using chemical reagents. Potassium hydroxide was purchased from Merck Specialities (Pvt) Ltd, Mumbai. Furfural solution was purchased from Loba Chemie Pvt. Ltd. Hydrochloric acid, acetic acid, potassium iodide, sodium thiosulfate, phenolphthalein, starch were procured from Merck KGaA, Germany. Ethanol and chloroform were procured from Sigma-Aldrich, Co., Germany. Hanus solution was prepared in the laboratory by dissolving 18.2 gm of iodine in 1 L of glacial acetic acid then 3 ml of bromine water for increasing the halogen content. Moisture analyzer was purchased from Mettler Toledo, USA. The glass apparatus used were burette, pipette, pipette filler, volumetric flask, conical flask, beaker, funnel and pycnometer. Besides these, other instruments used were reserved condenser and dryer from Pharmaceutical Technology Laboratory, University of Dhaka.

Sample collection

Total 3 samples of butter oil and 24 samples of ghee were collected from retail markets of different locations in Bangladesh. Collected butter oil samples were named as B-1, B-2, B-3 and ghee samples as G-1, G-2, G-3, G-4, G-5, G-6, G-7, G-8, G-9, G-10, G-11, G-12, G-13, G-14, G-15, G-16, G-17, G-18, G-19, G-20, G-21, G-22, G-23 and G-24. All the samples were preserved in airtight bottles. During the

study, the bottles were covered with carbon papers to prevent photo-oxidation and kept in a dried place in the laboratory at room temperature.

Determination of moisture content

Moisture content of the oil was determined by using moisture analyzer. 2 gm of oil was taken on the aluminum plate then the analyzer was set at time of 5 minutes and temperature of 110 °C. The Halogen moisture analyzer works according to the thermo-gravimetric principle, referred to as 'Loss on Drying' (LOD) principle. LOD is a method to determine the moisture content of a sample by analyzing the weight loss on heating. The moisture content was calculated by comparing the initial sample weight to the dried or final sample weight.

$$\text{Loss on Drying} = \frac{W_0 - W_1}{W_0} \times 100 \%$$

Where W_0 = initial weight, W_1 = final weight

Determination of acid value and free fatty acids

The acid value of oils was determined by using titrimetric method. About 5 gm of oils were taken in 250 ml conical flask. Then 25 ml of neutral ethyl alcohol was added to it. Phenolphthalein indicator solution (1-2 drop) was added. The mixture was titrated against with standard 0.1 N potassium hydroxide solution with constantly shaking. The end point was noted as pink color which persists for 15 seconds⁸.

$$\text{Acid value} = \frac{T \times F \times 56.1}{M}$$

Where,

T= volume of standard KOH solution in ml, F=factor for the titre, M=weight of oil sample in g

$$\text{Free fatty acids in term of oleic acid percent by mass} = \frac{2.82 \times T \times F}{M}$$

$$\text{Free fatty acids in term of lauric acid percent by mass} = \frac{2.00 \times T \times F}{M}$$

Free fatty acids in term of ricin oleic acid percent by mass $\frac{2.28 \times T \times F}{M}$

Free fatty acids in term of palmitic acid percent by mass $\frac{2.56 \times T \times F}{M}$

Determination of iodine value

About 0.5 gm of sample was taken in a 300 ml iodine flask fitted with a ground glass stopper which was dry and 15 ml of chloroform was added to dissolve the sample. Then 25 ml of iodine bromide solution was added slowly, the stopper was inserted and it was allowed to stand in a dark place for thirty minutes. 10 ml potassium iodide solution and 100 ml water were added to it. This solution was titrated with 0.1 N sodium thiosulphate VS using 1 ml of starch solution. The starch solution was added towards the end of titration as indicator. The number of ml required A. A blank under this same condition was carried out omitting the sample⁹.

$$\text{Iodine value} = \frac{(A-B) \times F \times 1.269}{M}$$

Where

A= titre in ml for the sample, B= titre in ml for the blank, F= factor for the titre, M= mass in gm of the sample taken

Determination of saponification value

The saponification value was determined by taking about 2.0 gm of oil sample in a conical flask in which 25 ml of the ethanolic potassium hydroxide solution was added, attached with a reflux condenser and boiled for one hour. After cooling the sample, it was washed with 10 ml of ethanol neutral to phenolphthalein. After adding 1 ml of phenolphthalein, the solution was titrated with 0.5 N HCl to neutralize the excess alkali. A blank was determined at the same time without sample and with same conditions⁹.

$$\text{Saponification value} = \frac{(B-T) \times F \times 28.05}{M}$$

Where,

B= ml of HCl required by blank, T= ml of HCl required by sample, F= factor for the titre, M=weight in gm of the sample taken.

Determination of peroxide value

A Sample of 5.00 ± 0.1 gm was weighed and taken into a 250 ml glass- stopper conical flask, and 30 ml of a mixture of 3:2 volumes of glacial acetic acid and chloroform was added to it. The flask was swirled until the sample completely dissolved. Then 2 ml of saturated potassium iodide solution was added and stopped the flask and allowed to stand exactly one minute with occasional shaking. A 30 ml of water was added and titrated gradually with vigorous shaking with 0.1 N sodium thiosulfate. When the starting color of the solution was deep red orange, it was titrated slowly with mixing sodium thiosulfate until the color lightens. If the solution is initially a light amber color, starch solution was added. One ml of 1% starch solution was added as indicator. The titration was carried out until the dark blue-black color disappears from the aqueous layer (upper layer). The volume of sodium thiosulfate was accurately recorded. The same procedure was used without sample for blank¹⁰.

$$\text{Peroxide value} = \frac{(S-B) \times F \times 10}{M}$$

Where, S = titre in ml for the sample, B= titre in ml for the blank, F= factor for the titre, M= mass in gm of the sample taken.

Determination the presence of sesame oil

A 5 ml oil of melted fat was taken in 25 ml measuring cylinder with a glass stopper, and 5 ml of hydrochloric acid and 0.4 ml of furfural solution were added. Inserting the glass stopper, the mixture was shaken vigorously for 2 minutes. When the mixture was separated, development of pink or red color indicated the presence of sesame oil. The color was confirmed by adding 5 ml of water and shaking again. Persistence of the colour of acid layer indicated the presence of sesame oil, disappearance of colour denoted the absence of sesame oil¹¹.

Results

BSTI sets same physicochemical parameters with same range for moisture content, acid value, iodine

value, saponification value, peroxide value and presence of sesame oil for evaluating the quality of both edible butter oil and ghee (table 1).

Table 1: Testing parameters and their accepted values standardized by BSTI

Characteristics	BSTI Requirements
Moisture contents (for butter oil) percent by mass, max	$\leq 0.1\%$
Moisture contents (for ghee) percent by mass, max	$\leq 0.5\%$
Acid value (as KOH) mg/g, max	≤ 1.9
Iodine value	26-35
Saponification value (as KOH), mg/g	≥ 218
Peroxide value, expressed as milli equivalents of oxygen per kg, max	≤ 0.8
Sesame oil test	Negative

Moisture content

Moisture content was determined by moisture analyzer at 110 °C for 5 minutes. From the test, it was found that the moisture contents of all the samples (B-1 to B-3 and G-1 to G-24) did not meet the BSTI standard values (the value for butter oil is $\leq 0.1\%$ and for ghee $\leq 0.5\%$). Moreover, the highest moisture content was found in G-2 (1.17%) (table 2).

Acid value

From the test, it was found that all the samples meet the BSTI standard requirement. The acid values of all the samples (B-1 to B-3 and G-1 to G-24) were lower than the BSTI specified value (≤ 1.9 mg/gm, as KOH). Higher values indicate that triglycerides of oil are converted into fatty acids and glycerol which cause rancidity of the oil (table 2)¹². Free fatty acid values of different butter oil and ghee were also determined (table 3).

Table 2: Physical and Chemical Properties of Butter oil and Ghee

Sample Code	Moisture content (%)	Acid value (mg/g)	Iodine value	Saponification value (mg/g)	Peroxide value (mEq of O ₂ /kg)	Sesame oil
B-1	0.86	0.333	44.65	134.94	0.64	+
B-2	0.86	0.333	44.43	123.47	0.64	+
B-3	0.83	0.334	44.63	133.69	0.64	+
G-1	1.11	1.344	41.40	134.26	0.41	+
G-2	1.17	1.837	38.19	117.72	0.56	+
G-3	0.83	1.214	36.24	122.70	0.39	+
G-4	0.75	1.069	45.65	132.90	0.45	+
G-5	0.96	0.667	51.65	130.28	0.32	+
G-6	0.90	1.062	50.02	134.01	0.36	+
G-7	0.85	1.115	35.01	126.65	0.48	+
G-8	0.77	1.253	48.62	137.32	0.39	+
G-9	1.01	0.719	36.81	132.24	0.22	+
G-10	0.88	1.151	50.85	135.27	0.31	+
G-11	0.79	1.320	50.11	117.99	0.33	+
G-12	0.91	1.567	48.99	126.52	0.26	+
G-13	0.93	1.550	49.01	120.70	0.36	+
G-14	0.91	0.958	39.09	133.11	0.47	+
G-15	0.77	0.883	36.75	129.56	0.28	+
G-16	1.11	0.880	45.55	134.32	0.39	+
G-17	0.88	0.763	50.97	134.26	0.40	+
G-18	0.81	1.413	51.23	135.82	0.35	+
G-19	1.10	1.515	36.11	122.11	0.39	+
G-20	1.00	0.811	51.23	123.97	0.29	+
G-21	0.95	1.114	43.85	131.21	0.36	+
G-22	0.79	1.571	36.63	134.91	0.36	+
G-23	0.77	1.117	46.44	120.75	0.41	+
G-24	0.89	0.781	42.97	131.12	0.22	+

Table 3: Free fatty acid values of samples

Sample Code	Oleic acid	Lauric acid	Ricin oleic	Palmitic acid
B – 1	0.017	0.012	0.014	0.015
B – 2	0.017	0.009	0.013	0.015
B – 3	0.015	0.011	0.005	0.019
G – 1	0.068	0.048	0.055	0.061
G – 2	0.092	0.065	0.075	0.083
G – 3	0.061	0.043	0.049	0.055
G – 4	0.054	0.038	0.043	0.049
G – 5	0.034	0.024	0.027	0.031
G – 6	0.054	0.038	0.043	0.048
G – 7	0.056	0.039	0.045	0.051
G – 8	0.063	0.047	0.051	0.057
G – 9	0.061	0.021	0.028	0.068
G – 10	0.064	0.025	0.021	0.061
G – 11	0.059	0.033	0.012	0.037
G – 12	0.052	0.039	0.015	0.041
G – 13	0.061	0.041	0.018	0.049
G – 14	0.066	0.064	0.034	0.051
G – 15	0.071	0.034	0.038	0.055
G – 16	0.058	0.038	0.045	0.049
G – 17	0.057	0.045	0.049	0.061
G – 18	0.068	0.053	0.058	0.063
G – 19	0.062	0.025	0.066	0.039
G – 20	0.061	0.035	0.051	0.045
G – 21	0.059	0.061	0.042	0.048
G – 22	0.041	0.037	0.031	0.039
G – 23	0.044	0.038	0.049	0.051
G – 24	0.033	0.046	0.051	0.057

Iodine value

Almost all samples had iodine values above the BSTI specified range of iodine value (26-35). Highest value was found in G-5 (51.65) and the lowest one was in G-7 (35.01) indicating the degradation the quality of butter oil and ghee where only G-7(35.01) had the iodine value within the BSTI standard stated limit (table 2).

Saponification value

Saponification value depicts the number of milligrams of potassium hydroxide required to saponify 1 gm of fat. In this test, all the samples were much less than the BSTI standard specified value ranges (≥ 218 as KOH, mg/gm) (table 2).

Peroxide value

The peroxide value is defined as the amount of peroxide oxygen per 1 kilogram of fat or oil. In general, the objective of oil production should be to manufacture oils with peroxide values as low as possible but the secondary reaction products should not be formed. All the samples showed peroxide value below the BSTI standard value (≤ 0.8 mEq of O_2 /kg) (table 2).

Sesame oil test

From sesame oil test, it was found that all the samples contained sesame oil which is considered as an adulterant in butter oil and ghee according to BSTI standard (table 2).

Discussion

In order to determine the quality of different brands of butter oil and ghee in the market, physicochemical properties such as moisture content, acid value and free acid value, iodine value, saponification value, peroxide value and presence of sesame oil are the unique parameters that are specified by BSTI to evaluate the both quality of the butter oil and ghee with same ranges value.

Moisture content

Moisture content is the ratio of the mass of water in the sample to the mass of solid in the sample which is expressed as percentage. In all the samples of butter oil and ghee, the moisture contents of all samples were higher than the normal value (butter oil at $\leq 0.5\%$ and for ghee at $\leq 0.1\%$). However, moisture contents of G-1, G-2, G-9, G-16, G-19 and G-20 were almost ten times higher than the normal value. The moisture content of butter oil and ghee is important as it determines the stability of the oil. Higher value of moisture content indicates the lower quality of that samples¹³. Moisture content of a sample of material depends on its hygroscopic

nature. Hygroscopic action is the amount of moisture a material will absorb relative to ambient temperature and humidity conditions. Temperature and humidity conditions of butter oil and ghee should be controlled.

Acid value

From the acid value analysis, it was found that all samples met BSTI standard (≤ 1.9 mg/gm).

Iodine value

From the iodine value analysis, it was found that the iodine values of all samples other than the sample G-7 (35.01) were higher than that of BSTI standard (26-35). This value indicates the amount of iodine in grams consumed by 100 gm of sample. It indicates the level of saturation of the oil. It is a good indicator of quality. The process of test was done in a safe, dry and dark place. Higher iodine values of the samples indicate the presence of more unsaturated fatty acid bonds. The greater the degree of unsaturation, the more rapid the oil tends to be oxidized¹⁴.

Saponification value

In case of saponification value analysis, all samples did not meet the BSTI standards. The saponification values found were much less than the standard value (≥ 218 mg/gm). This indicates inferior quality of the samples. Saponification value is used in investigating adulteration. It provides the information of the average chain length and hence the molecular weight of the fatty acid in the oil. Lower saponification value depicts lower medium chain fatty acids and high molecular weight of the samples^{15, 16}.

Peroxide value

In case of peroxide value analysis, all samples met the BSTI standards. The values were below the BSTI standard value (≤ 0.8 mEq of O_2 /kg). The test is valuable for assessing the extent to which spoilage has advanced during storage¹⁷.

Sesame oil test

In case of sesame oil test, all the samples were found positive for the presence of sesame oil. Sesame oil is derived from sesame seed which is an edible vegetable oil. It processes a nutty aroma and taste. According to BSTI standards, the samples should be tested negative for sesame oil. Presence of sesame oil indicates the silent adulteration.

Presently, food adulteration is a great concern in Bangladesh. As the demand of butter oil and ghee is very high, there are many brands available in our local market. So, there is also a great chance to adulterate by its immoral manufacturers and low-quality butter oil and ghee may be available in the market. To determine the quality and possible adulterants this study was conducted. In this study, different samples of butter oil and ghee from different Bangladeshi market were analyzed to determine their quality and possible adulterants. In Bangladesh, marketed brands of butter oil and ghee need to meet the requirements of BSTI standards as

it is the government authority to give approval to market any product in Bangladesh. So, the test results of the samples were compared with the BSTI standard of butter oil and ghee. Result showed that most of the samples did not meet BSTI standards. Moreover, sesame oil should not be present in butter oil and ghee but all the samples showed the presence of sesame oil. So, the study showed that the quality of butter oil and ghee in Bangladesh is not up to the marks. Therefore, more concern is needed by the government authority to ensure the proper quality of marketed butter oil and ghee in Bangladesh.

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References

1. Rama Murthy MK. Factors Affecting the Composition, Flavour and Textural Properties of Ghee. *Indian Dairyman* 1980: 32:765-68.
2. Ndb.nal.usda.gov.USDA Food Composition Databases 2019.
3. Acharya KT. Ghee, vanaspati, and special fats in India. In: Gunstone FD, Padley FB, editors. *Lipid Technologies and Applications*. New York: Marcel Dekker Inc 1997: 369-90.
4. Sharma H, Zhang X and Dwivedi C. The effect of ghee (clarified butter) on serum lipid levels and microsomal lipid peroxidation. *Ayu* 2010;31(2):134-40.
5. Rani R and Kansal V. Effects of cow ghee (clarified butter oil) & soybean oil on carcinogen-metabolizing enzymes in rats. *Indian J Med Res* 2012;136(3):460 - 65.
6. Montgomery M, Osborne B, Brown S, Small L, Mitchell T, Cooney G and Turner N. Contrasting metabolic effects of medium- versus long-chain fatty acids in skeletal muscle. *Journal of Lipid Research* 2013;54(12):3322-33.
7. Dwivedi C, Crosser AE, Mistry VV and Sharma HM. Effects of dietary ghee (clarified butter) on serum lipids in rats. *J Appl Nutr* 2002;52:65-8.
8. cmd.gov.hk 2019: http://www.cmd.gov.hk/hkcmms/vol6/eng/E_Appendices_EXIV.pdf

9. Odoom W and Edusei VO. Evaluation of Saponification value, Iodine value and Insoluble impurities in Coconut Oils from Jomoro District in the Western Region of Ghana. *Asian J Agric Food Sci* 2015;03(05):494-99.
10. BtShahidan F. Determination of Peroxide Value In Deep-Frying Cooking Oil Collected At Night Market In Kepala Batas. *Eprintsusummy* 2015.
11. Shukla A and Singh R. Detection of Sesame Oil in Other Oils and Fats. *J Oleo Sci* 2005;54(10):543-44.
12. Bhattacharya R and Hilditch T. The fatty acids and component glycerides of Indian ghee. *The Analyst* 1931;56(660):161.
13. Quehl A and Lampert LM. *Modern Dairy Products - Composition. Food Value. Processing Chemistry. Bacteriology. Testing. Imitation Dairy Products. I. Auflage, 446 Seiten, 84 Abb., 64 Tab. New York: Chemical Publishing Company Inc* 1975: 21(10): 967-967.
14. Chebet J, Kinjanjui T and Cheplogoin PK. Impact of frying on iodine value of vegetable oils before and after deep frying in different types of food in Kenya. *J Sci Innov Res* 2016: 5(5): 193-96.
15. Özcan MM and Chalchat JC. Chemical composition of carrot seeds (*Daucus carota* L.) cultivated in Turkey: characterization of the seed oil and essential oil. *Grasas y aceites* 2007;58(4):359-65.
16. Seneviratne K and Dissanayake DMS. Effect of method of extraction on the quality of coconut oil. *J Sci Kelaniya Univ Sri Lanka* 2011;2:63-72.
17. Decker EA, Elias RJ and McClements DJ. *Oxidation in foods and beverages and antioxidant applications: management in different industry sectors. Elsevier* 2010;2:1-528.