Screening of market cow's ghee samples to detect adulteration

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Abstract

Cow ghee is a nutritious food with a lot of health benefits and popular ingredient in many vegetarian diets and indigenous medical formulations. Due to high demand, the adulteration of cow ghee with more affordable and widely available vegetable oils and animal fat is common in many industries. The detection of adulteration by instrumental techniques is expensive and time-consuming. Therefore, simple, rapid and cost-effective tests are essential for the detection of adulterants in cow ghee. The aim of this study was to compare the physicochemical parameters of pure cow ghee with the market samples using the SLS 313 and detection of adulteration using chromogenic tests and conformation through GC-MS. A pure cow ghee sample (S-01) was prepared from the curd made in the laboratory. Thirteen market samples (S-02 to S-14) were purchased from Northern, Southern, Western and Central provinces and analyzed for physicochemical parameters (moisture and volatile matter content, relative density, refractive index, acid value, iodine value, saponification value and peroxide value) based on SLS 313 standard protocols. As chromogenic tests, Modified Salkowski, and furfural tests were followed. In the Modified Salkowski test, the pure sample observed a red colour, whereas the adulterated samples showed a reddish brown to dark brown colour. The pure sample showed no colour in the furfural test, while the adulterated sample showed a light pink to crimson red. In conclusion, eleven market samples (from S-02 to S-12) were adulterated in different levels with edible oils and GC-MS analysis confirmed the adulterants and the chemical composition variation from the pure cow ghee samples.

Keywords: Adulteration, Chromogenic test, Cow ghee, Standard, Vegetable oil, GC-MS analysis

Introduction

The human food culture is inextricably linked to milk and dairy products. As it contributes good sensory and nutritional qualities as well as economy to milk and other food products, milk fat is a very desirable and expensive substance that has been consumed all over the world since antiquity[1]. Cow ghee is a valuable dairy product which is golden yellow colour[2] and produced from cow milk. It is mostly used for food industries as well as for traditional medicine[3]. Under tropical storage circumstances, it has a lengthy shelf life of approximately one year[4]. It is rich in nutrients and it's beneficial for children as well as old people.

Cow ghee generates good income in dairy industry and trades mostly tend to adulterate[5] the pure cow ghee with cheaper[6] and easily available vegetable oils such as palm oil[7,8], coconut oil[9], mee oil[10], sunflower oil[11], soybean oil[12], sesame oil[13], peanut oil[14] as well as rice bran oil[15] and animal fat[16,17] due to its higher price and the demand[4,18]. Adulteration is the process of reducing the quality or nature of a particular substance by adding an unnatural or subpar substance and removing essential components[19]. Adulteration of cow ghee is difficult

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to identify visually[4] when the adulterants have similar colour and the combination of adulterants with similar chemical composition as pure cow ghee. The detection of adulterants by instrumental techniques (GC-MS, UV spectrophotometer and Electronic sensing nose system) is expensive and time consuming[3]. Simple, rapid and cost-effective tests are essential for the detection of adulterants in cow ghee to facilitate the traders to purchase good quality cow ghee by saving the time and money.

Using the SLS 313 standards for animal and vegetable fats and oils, the objective of the study was to compare the physicochemical parameters (moisture and volatile matter content, relative density, refractive index, acid value, iodine value, saponification value, and peroxide value) between the pure and market samples and develop a colour test for straightforward adulteration detection and conformation through GC-MS. Colour tests are simple, rapid and cost-effective chromogenic tests which can be detect by the layman who is purchasing the ghee to detect the adulteration [20].

Materials and methods

Preparation and sampling of ghee

Pure ghee was prepared from the curd made in the laboratory from cow's milk. Fresh cow's milk (2 L) was purchased from a farm and boiled. After cooling, cow curd (1 table spoon) was added, stirred well and allowed to ferment for overnight. After the curd formed, distilled water (1.5 L) was added and stirred at 300 rpm using the overhead stirrer for 3 hours until the fat separated. The separated fat was collected, kept in the fridge for overnight, melted and bottled (S-01). The samples from S-02 to S-14 were purchased from Northern, Southern, Western, and Central Provinces of Sri Lanka randomly in July 2021 and from January to February 2022.

Preparation of adulterated ghee samples

A 1 mL adulterated ghee sample prepared from pure ghee was mixed with 5 %, 10 %, 15 %, 25 % and 50 % percentages of adulterants (palm oil, mee oil, sesame oil and vegetable ghee) separately and used for chromogenic tests.

Determination of acid value

The pure and market samples (2 g each) were taken in conical flasks and the acid value was determined according to SLS 313-2-6:2009 (hot ethanol method using indicator)[21]. Ethanol was taken into another flask and boiled it until bubbles formed. Phenolphthalein (2 drops) was added and neutralized using potassium hydroxide (KOH) solution (0.1M). Neutralized ethanol (25 mL) was added to sample and shaken well. Phenolphthalein (2 drops) was added and titrated with KOH solution until pink colour formed. Blank test was carried out without the ghee sample.

Determination of saponification value

The saponification value of pure and market samples of cow ghee (2 g each) was determined according to SLS 313-2-1:2014 procedure [21]. Pre-prepared ethanolic KOH (25 mL) was taken in a round bottomed flask, boiling chips added, fixed to the condenser and refluxed for 1-2 hours until clear solution formed. The solution in the flask was titrated with 0.5M hydrochloric acid (HCl) solution after added 2 drops of phenolphthalein indicator. Blank test was done using the same procedure without ghee sample.

Determination of refractive index

Refractive index was determined using refractometer at room temperature according to SLS 313-1-5:2017 procedure [21]. Drop of distilled water was placed on refractometer prism and focused the light. Refractive index of water determined and checked with temperature in standard table. Prism of refractometer was cleaned and placed the drop of ghee sample and determined the refractive index of sample. The same way other samples also analyzed [21].

Determination of iodine value

Samples (0.634-0.793 g each) were taken in different stoppered conical flasks and iodine value was determined according to SLS 313-2-2:2019 procedure [21]. Cyclohexane and glacial acetic acid in 1:1 ratio (20 mL) was added and 25 mL of Wij's reagent pipetted into the flask. Flask was shaken well and placed in dark for 1 hour. Potassium iodide (100 g/L, 20 mL) and 150 mL of distilled water were added to conical flask. Solution was titrated with 0.1M sodium thiosulphate solution. Near to end point, few drops of saturated starch solution were added and titration was continued until blue colour just disappeared. Blank test was done without test sample.

Determination of relative density

Relative density was measured according to SLS 313-1-2:2009 using specific gravity bottle [21]. Clean and dry specific gravity bottle was weighed, filled with freshly boiled and cooled distilled water. Filled specific gravity bottle was kept in water bath until it reached 40 °C. It was maintained at 40 °C until no further alteration in volume occurs. The bottle was removed from water bath and dried outside and allowed to cool to room temperature (27 °C). Then weight of bottle was measured. The bottle was cleaned and filled with ghee sample and the above procedure followed for other samples.

Determination of moisture and volatile matter content

Moisture and volatile matter content of each sample was determined according to SLS 313-3-5:2016 procedure [21]. Empty vessel was weighed and 5 g of each test sample was added and taken initial weight. Vessels with samples were placed in an oven for 1 hour (103 ± 2 °C). Test samples were cooled to room temperature using desiccator and weighted to nearest 0.001 g. Operations of heating, cooling, weighing were repeated until constant weight.

Determination of peroxide value

Samples (2 g each) were taken into flasks and peroxide values were determined according to SLS 313-3-7:2017 standard procedure [21]. Isooctane (10 mL) and glacial acetic acid (15 mL) were added to sample. Saturated potassium iodide (KI) solution (1 mL) was added and shaken well for 1 minute. Flask was placed in dark for 5 minutes and 75 mL of distilled water added. It was titrated with 0.01M sodium thiosulphate solution. Near to end point, few drops of starch solution was added and titration was continued until solution became colourless. Blank test was done with the same procedure without ghee sample.

Modified Salkowski's test

Ghee sample (1 mL) was taken into test tubes. Petroleum ether (b.p: 40 – 60 °C, 2 mL) was added to the sample and mixed well until clear solution obtained. Concentrated sulfuric acid (5-10 drops) were added, shaken well and the colour change was observed within a minute [22]. This procedure was continued for samples prepared in the laboratory by adulterating with palm oil, mee oil, sesame oil and vegetable ghee separately with pure ghee in 5%, 10%, 15%, 25%, and 50% percentages.

Furfural test

Melted ghee (1 mL) was taken into test tubes and concentrated hydrochloric acid (2 mL) was added to each sample separately and mixed well. Ethanolic furfural solution (2%, 0.1 mL) was added to each sample and kept for 2 minutes [6]. Furfural test procedure was continued for the sample prepared in the laboratory by adulterating pure ghee with sesame oil in 5%, 10%, 15%, 25% and 50% percentages.

Gas chromatography- mass spectrometry analysis of ghee samples (GC-MS)

The ghee sample (0.1 g) was weighted, and 2 mL of iso-octane was added. The sample was well mixed, and 0.1 mL of ethanolic potassium hydroxide (2 mol/L) was added. The sample was shaken vigorously for 1 minute. It was allowed to settle for 2 minutes before adding 2 mL of saturated sodium chloride solution. After shaking the sample, the top layer (iso-octane) was separated. Anhydrous sodium sulphate (1 g) was added to the separated top layer and filtered. The supernatant was transferred to a glass vial and used for GC-MS analysis. The samples were qualitatively analyzed using a Gas Chromatograph (Thermo Scientific TRACE 1300) together with an MS (ISQ-QD, Single Quadrupole) connected with an auto-injector AI 1310 (Thermo

Scientific) and a length of 30 m, diameter of 0.25 mm, and 0.25 µm film Thermo Scientific fused silica capillary column (DB-WAX UI) with a flow of 1.0 mL/minute. The following temperature programming was used: starting temperature of 60 °C, ramping up to 225 °C at a rate of 5 °C/minute. The ion source temperature was 250 °C, and 240 °C was the injector temperature. A pre-prepared sample was injected at 5.0 mL/minute split flow and 64.20 kPa column pressure. Ion capture detector with impact energies of 70 eV was used for the MS. The constituents were identified by comparing the mass spectra to spectra from the equipment database (NIST 11).

Results

Physicochemical parameters

The hysicochemical parameters are evaluated to deterine the quality of ghee. They are moisture and volatile matter, relative density, refractive index, acid value, iodine value, saponification value and peroxide value (Table 1).

Modified Salkowski's test as a chromogenic test

Table 2 shows the Modified Salkowski's test results of adulterated ghee with palm oil, mee oil, sesame oil, vegetable ghee (VG)

Modified Salkowski's test results of pure ghee adulterated with palm oil (Figure 1), Modified Salkowski's test results of pure ghee adulterated with mee oil (Figure 2), Modified Salkowski's test results of pure ghee adulterated with sesame oil (Figure 3) and Figure 4 shows the Modified Salkowski's test results of pure ghee adulterated with vegetable ghee (VG).

Furfural test as a chromogenic test

Furfural test results of pure ghee adulterated with sesame oil shows in Table 3 and Figure 5.

Results of GC-MS analysis of S-01, S-10, S-13, S-14, Buffalo ghee, palm oil, sesame oil, and vegetable ghee is shown in Table 4.

Figure 6 shows the GC-MS graph of pure cow ghee (S-01).

Table 1: Physicochemical parameter values of pure ghee and market samples

Sample	Moisture & Volatile matter %	Relative Density	Refractive index at 28 ˚C	Acid value (mg KOH/g)	Iodine value % by mass	Saponification value	Peroxide value meq. (O_2)/kg of ghee
S-01	0.21±0.02	0.073±0.001	1.4581±0.0002	0.5±0.07	35±0.10	230±0.18	3±0.12
S-02	0.44±0.02	0.073±0.001	1.4559±0.0001	5.7±0.18	30±0.26	243±0.20	3±0.06
S-03	0.23±0.01	0.072±0.002	1.4551±0.0001	4.5±0.20	31±0.19	236±0.09	68±0.21
S-04	1.88±0.02	0.081±0.001	1.4581±0.0002	27.8±0.23	20±0.27	242±0.20	172±1.00
S-05	0.28±0.02	0.073±0.001	1.4581±0.0002	8.4±0.07	29±0.21	237±0.16	40±0.15
S-06	0.12±0.01	0.074±0.002	1.4612±0.0001	0.6±0.11	ND	202±0.20	ND
S-07	0.35±0.01	0.073±0.001	1.4600±0.0002	6.7±0.07	30±0.21	245±0.19	10±0.25
S-08	0.30±0.02	0.072±0.002	1.4574±0.0001	2.2±0.22	31±0.13	208±0.12	12±0.12
S-09	0.15±0.02	0.080±0.002	1.4551±0.0001	0.8±0.09	32±0.18	216±0.12	12±0.25
S-10	0.16±0.02	0.078±0.002	1.4576±0.0002	1.7±0.11	33±0.12	238±0.24	9±0.58
S-11	0.35±0.01	0.074±0.002	1.4574±0.0002	3.9±0.13	28±0.20	240±0.17	22±0.76
S-12	0.31±0.01	0.069±0.001	1.4587±0.0002	1.7±0.12	31±0.18	239±0.20	10±0.81
S-13	0.21±0.01	0.073±0.001	1.4580±0.0001	0.8±0.07	36±0.17	220±0.19	3±0.17
S-14	0.23±0.01	0.073±0.002	1.4578±0.0002	0.7±0.14	31±0.23	228±0.16	3±0.00

ND- Not determined

Table 2: Modified Salkowski's test results of adulterated ghee with palm oil, mee oil, sesame oil, vegetable ghee (VG)

1 mL of Sample		Observation			
Pure Ghee (%)	**Adulterant (%)**	**Palm oil**	**Mee oil**	**Sesame oil**	**Vegetable ghee**
100	-	Red colour	Red colour	Red colour	Red colour
95	5	Reddish brown colour	Reddish brown colour	Reddish brown colour	Reddish brown colour
90	10	Reddish brown colour	Reddish brown colour	Reddish brown colour	Reddish brown colour
85	15	Brown colour	Brown colour	Brown colour	Brown colour
75	25	Dark brown colour	Dark brown colour	Dark brown colour	Dark brown colour
50	50	Dark brown colour	Dark brown colour	Dark brown colour	Dark brown colour

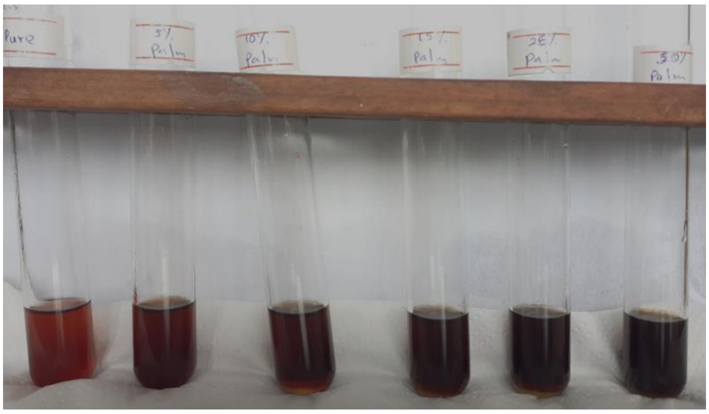

Fig. 1: Modified Salkowski's test results of pure ghee adulterated with palm oil

Fig. 2: Modified Salkowski's test results of pure ghee adulterated with mee oil

Fig. 3: Modified Salkowski's test results of pure ghee adulterated with sesame oil

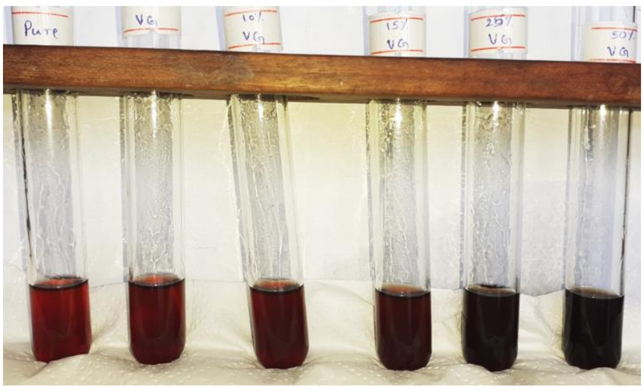

Fig. 4: Modified Salkowski's test results of pure ghee adulterated with vegetable ghee (VG)

Table 3: Furfural test results of pure ghee adulterated with sesame oil

Sample	Observation
Pure Ghee	Colourless
Pure Ghee : Sesame oil (95:5)	Light pink colour
Pure Ghee : Sesame oil (90:10)	Light crimson red colour
Pure Ghee : Sesame oil (85:15)	Light crimson red colour
Pure Ghee : Sesame oil (75:25)	Crimson red colour
Pure Ghee : Sesame oil (50:50)	Crimson red colour

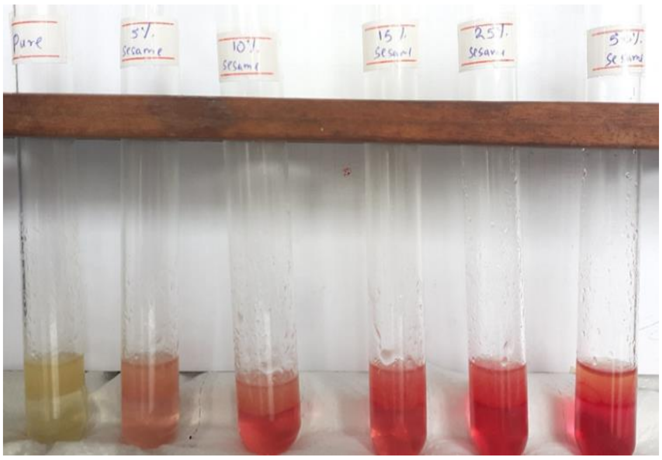

Fig. 5: Furfural test results of pure ghee sample adulterated with sesame oil

Table 4: Results of GC-MS analysis of S-01, S-10, S-13, S-14, Buffalo ghee, palm oil, sesame oil, and vegetable ghee.

Compounds	Relative abundance (%)							
	S-01	S-10	S-13	S-14	Buffalo ghee	Palm oil	Sesame oil	Vegetable ghee
Caproic acid (C6:0)	1.31	1.75	1.36	0.02	0.01	-	-	0.24
Caprylic acid (C8:0)	0.88	0.99	0.94	0.05	0.17	-	0.04	2.83
Capric acid (C10:0)	1.61	2.02	2.10	0.06	0.14	-	0.02	1.99
Undecylenic acid (C11:1n1)	0.11	0.17	0.18	-	-	-	-	-
Lauric acid (C12:0)	2.63	3.91	3.18	0.42	1.45	-	0.25	17.44
Tridecanoic acid (C13:0)	-	0.03	0.05	-	-	-	-	-
Myristic acid (C14:0)	7.66	11.36	10.23	1.26	1.51	0.01	0.11	7.89
Myristoleic acid (C14:1)	0.37	0.82	0.74	0.01	-	-	-	-
Pentadecanoic acid (C15:0)	0.52	1.07	1.13	0.06	0.03	-	-	-
Octadehydroarachidonic acid	-	-	-	-	0.02	-	-	-
Palmitic acid (C16:0)	26.95	30.70	29.08	42.69	41.77	9.85	9.55	29.48
Palmitoleic acid (C16:1)	0.91	1.41	1.44	0.18	0.13	0.13	0.11	0.06
Margaric acid (C17:0)	0.37	0.48	0.77	0.10	0.06	0.02	0.02	0.03
Stearic acid (C18:0)	17.78	10.25	14.27	4.84	4.96	5.93	5.46	3.63
Oleic acid (C18:1)	27.72	18.84	21.92	36.67	37.74	38.51	36.34	28.37
Vaccenic acid (C18:1)	3.50	3.31	0.59	-	1.03	0.01	-	0.64
*Linoleic acid (**C18:2**)*	2.82	0.81	2.07	11.06	8.63	42.83	45.37	4.74
Nonadecanoic acid (C19:0)	0.04	-	0.11	-	-	-	-	-
Linolenic acid (C18:3)	0.13	0.21	0.36	0.10	0.02	0.30	0.34	0.04
Arachidic acid (C20:0)	0.17	-	0.23	0.35	0.30	0.66	0.54	0.17
Gondoic acid (20:1)	-	0.98	0.05	-	-	-	-	-
8,11,14-Eicosatrienoic acid (C20:3)	-	-	0.04	-	-	-	-	-
Heneicosanoic acid (C21:0)	-	-	0.03	-	-	-	-	-
Arachidonic acid (C20:4)	0.05	-	0.08	-	-	-	-	-
Stearolic acid (C18:1)	-	3.09	-	-	-	-	-	-
Behenic acid (C22:0)	-	-	0.06	0.04	-	0.08	0.09	-

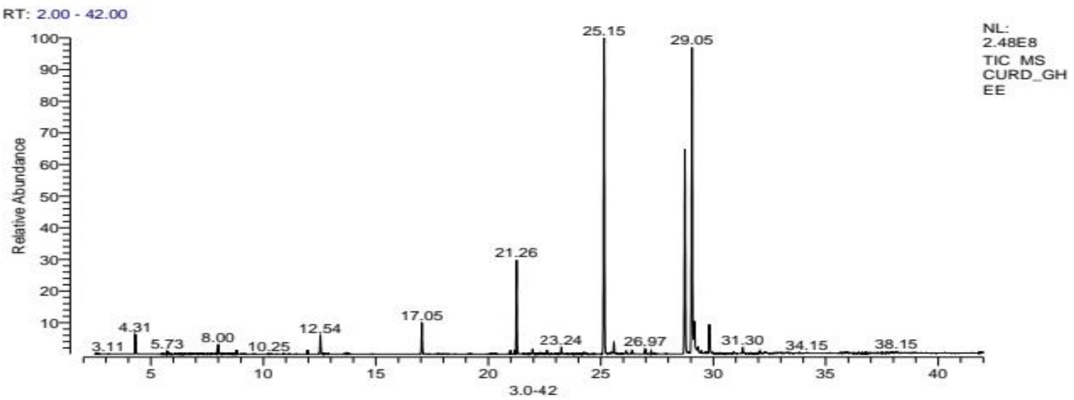

Fig. 6: GC-MS graph of pure cow ghee (S-01)

Discussion

Pure ghee

One of the most essential dairy products is ghee, which is clarified butter[17] made over heat. By taking off the water, protein, and other minor components, it can be made from cream or butter[23]. In the Middle East and South Asian nations, ghee is widely available and used often[1]. The quality of ghee is influenced by the types of milk, cream, *Dahi* (curd), and butter used in its processing, the temperature at which it is clarified, the storage environment, and the animal feed used[23,24]. The yield of cow ghee depends on the fat content of milk which was used to manufacture the ghee. The fat content of cow milk varies from 3 to 4% [25]. The yield of laboratory made ghee (S-01) was 3%. Cow's milk (2 L) gave 60 mL of pure cow ghee. In contrast to cow ghee, which is golden yellow in colour[2], buffalo milk contains no carotenoids, hence the ghee made from it is white [23].

Physicochemical parameters

The physicochemical parameters were determined to the pure ghee and market samples to see how the market sample values are deviating from the pure cow ghee due to adulteration. In specification for ghee (butter oil) standard, SLS 340:1975, maximum moisture content of ghee should be 0.25%. The moisture content of S-01, S-03, S-06, S-09, S-10, S-13 and S-14 were below 0.25% according to the standard. When the molecular weight and unsaturation of oil increase, density also increases. The high relative densities may be a sign of high molecular weight and unsaturation[26]. Sample S-04, S-09, and S-12 were highly deviating from the S-01 (Table 1).The refractive index is a crucial optical statistic for analyzing how light travels through different types of media[27]. According to SLS 313-1-5:2017, refractive index ranges from 1.3000 to 1.7000. In this study, the refractive index of S-01 was 1.4581. Samples S-06 (1.4612) and S-07 (1.4600) were highly deviating from the value for pure ghee. The free fatty acids in oil are quantified by their acid value. The level of free fatty acids [28] increase is directly proportional to the acid value, which results in lower oil quality[29]. Acid value for ghee ranged from 0.0 to 1.0[4]. The acid value of samples S-02, S-03, S-04, S-05, S-07, S-08, S-10, S-11 and S-12 were highly deviating from the value for pure ghee. The degree of unsaturation is measured by the Iodine value, which is frequently used to describe fats and oils[30]. As an oil is oxidized, a decrease in the iodine value is consistent with a decrease in double bonds[31]. Iodine value was determined to identify the unsaturation in the ghee [32]. According to SLS 313-2-2:2019, the iodine value should be between 3.0 and 200.0 for edible oil and fat. The S-01 had 35 and the S-06 didn't show any colour change during titration, which may be due to the added synthetic orange dye. The molecular weights of the triglycerides in oil are shown by the saponification value. Since the saponification value is inversely related to the average molecular weight or chain length of the fatty acids, higher saponification value denotes a higher fraction of lower fatty acids[29]. In specification for ghee (butter oil) standard, SLS 340:1975, the saponification value of pure ghee should be between 218 and 234. The results obtained for pure sample was 230 (S-01) and two market samples showed results within range, 220 (S-13) and 228 (S-14). Other market samples (S-02 to S-12) values were out of this range indicated that they were adulterated. Peroxides are one of the byproducts of the oxidation of the double bonds in unsaturated lipids. The peroxide value, which is a measure of oxidation level, is high because there is more oxidation. In this study, S-01, S-02, S-13 and S-14 showed peroxide value 3 meq/kg and S-07, S-10, and S-12 showed closer to 10 meq/kg. Due to the added synthetic dye, the sample S-06 did not show any colour change during titration. Among these samples, the physicochemical parameter values of S-04 sample were highly deviated due to the long-term storage (>1 year). Sample S-13 and S-14 showed values closer to S-01 indicated that they are pure. Samples S-02 to S-12 had deviated results when compared to S-01 because they were adulterated with adulterants.

Chromogenic tests

Chromogenic tests show colour change when the reagents are added to the test samples. Modified Salkowski's test gives positive results for cholesterol and plant steroids (phytosterols). The natural pure ghee contains cholesterol but no phytosterols, but the vegetable oil contains phytosterols[12]. Pure ghee gave red colour due to the presence of cholesterol[33]. Phytosterols show a brown ring in Salkowski's test[34]. When pure ghee was adulterated with vegetable oil, the presence of phytosterols gave it a reddish brown colour, which turned dark brown when the amount of phytosterols increased. Sample S-01, S-13 and S-14 gave red colour only but other samples gave reddish brown to dark brown colour. The colour different from red colour indicated that the samples S-02 to S-12 were adulterated with vegetable oils. Sample S-08, S-09 and S-10 showed reddish brown colour indicated that they were adulterated with 5-10 % of vegetable oils.

Furfural test was mainly done for the ghee adulterated with sesame oil (*Vanaspathi* ghee). Sesame oil contains sesamolin which breaks into sesamol and oxonium ion when concentrated hydrochloric acid added[6]. This sesamol reacted with furfural and produced crimson red colour[35,36]. If pure ghee adulterated with sesame oil, it gives crimson red colour. Furfural test was done for 1 mL of pure ghee sample adulterated with sesame oil in different percentages. The colour intensity increased when the sesame oil percentage increased. Sample S-03 and S-05 gave light pink colour and S-07 had light crimson red colour. Therefore, those samples also adulterated with sesame oil. The rest of the samples (S-01, S-02, S-04, S-06, S-08, S-09, S-10, S-11, S-12, S-13, and S-14) were not showed crimson colour, indicating that those samples were not adulterated with sesame oil.

Compounds detected in GC-MS analysis of ghee samples

The analytical technique known as gas chromatography-mass spectrometry (GC-MS) combines the advantages of gas chromatography and mass spectrometry to identify various compounds in a test sample[37]. Pure cow ghee sample (S-01) contains caproic, caprylic, capric, Undecylenic, lauric, myristic, myristoleic, pentadecanoic, palmitic, palmitoleic, margaric, stearic, oleic, vaccenic, linoleic, nonadecanoic, linolenic, arachidic, and arachidonic acid as fatty acids. Oleic acid is the fatty acid with the highest proportion. The other two greatest percentages are palmitic and stearic acid respectively. According to the S-01, market samples S-13 and S-14 gave closer results to it, which indicates those were pure.

In market sample (S-10), palmitic acid shows highest percentage within these fatty acids. The oleic acid percentage of the market sample (S-10) is lower than that of pure cow ghee (S-01) when compared to pure ghee. Gondoic acid and stearolic acid are present in the S-10 sample. Gondoic acid was detected in S-10 in considerable percentages (0.98%), but only in small amounts (0.05%) in S-13, suggesting that the latter sample may have been contaminated with plant oil. It is commonly found in plant oils such as those from the Fabaceae family [38,39]. Stearolic acid was found in high concentrations (3.09%) in market sample (S-10) and is commonly found in plants. That indicate the S-10 market sample was adulterated with plant oil [40].

The fatty acid composition of buffalo ghee was shown to be closer to that of pure cow ghee, but in different percentages. Undecylenic, Tridecanoic, myristoleic, nonadecylic, and arachidonic acid were not present in buffalo ghee as they were in pure ghee (S-01). Buffalo ghee contains high amounts of palmitic (41.77%) and oleic acid (37.74%).

Palm oil contains myristic, palmitic, palmitoleic, margaric, stearic, oleic, vaccenic, linoleic, linolenic, arachidic, and behenic acid only according to results. Linoleic acid showed the highest percentage (42.83%) among those fatty acids. Comparing sesame oil to pure cow ghee, linoleic acid was more prevalent in sesame oil. The following acids can be found in vegetable ghee: caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, margaric acid, stearic acid, oleic acid, vaccenic acid, linoleic acid, linolenic acid, and arachidic acid. Compared to pure cow ghee, lauric and linoleic acids were present in significant concentrations.

Conclusion

According to the results obtained in the physicochemical parameter tests for the market samples deviating from the pure ghee samples indicated that they appear to have adulterated with different degree of adulteration and chromogenic tests confirmed it. The GC-MS analysis is helpful to identify the adulterants and the variation of chemical composition among adulterated samples. The chromogenic test developed in this research with different percentage of several adulterants will be helpful to do the test on the spot when the customer purchase the ghee sample.

Acknowledgement

The Treasury Grant (TG 21/201) of Industrial Technology Institute.

References

1. Serunjogi, M. L. Abrahamsen, R. K., Narvhus, J. (1998). A Review Paper: Current Knowledge of Ghee and Related Products. *Int. Dairy J.* 8: 677–688.
2. Dhurvey, Y. R., Kawtikwar, P. S., Sakarkar, D. M. (2012). Evaluation of Physicochemical Properties of Cow Ghee before and after Hydrogenation. *Int. J. ChemTech Res*. 4 (1): 185–189.
3. Jirankalgikar, N. M., De, S. (2014). Detection of Tallow Adulteration in Cow Ghee by Derivative Spectrophotometry. *J. Nat. Sci. Biol. Med.* 5 (2): 317–319. https://doi.org/10.4103/0976-9668.136174.
4. Ahmed, S., Hamid, M. A., Rahman, M. M. (2020). Assessment of Ghee Adulterated with Oils and Fats in Bangladesh. *J. Adv. Vet. Anim. Res.* 7 (4): 678–684. https://doi.org/10.5455/javar.2020.g467.
5. Gandhi, K., Upadhyay, N., Aghav, A. D., Sharma, V., Lal, D. (2014). Detection of Adulteration of Ghee (Clarified Milk Fat) with Palmolein and Sheep Body Fat Using Reichert-Meissl (RM) Value Coupled with Solvent Fractionation Technique. *Indian J. Dairy Sci*, 67 (5): 387–393.
6. Shukla, A. K., Dixit, A. K., Singh, R. P. (2005). Detection of Adulteration in Edible Oils. *J. Oleo Sci.* 54 (6): 317–324. https://doi.org/10.5650/jos.54.317.
7. Tarmizi, A. H. A., Lin, S. W., Kuntom, A. (2008). Palm-Based Standard Reference Materials for Iodine Value and Slip Melting Point. *Anal. Chem. Methods Appl.* 3: 127–133. https://doi.org/10.1201/b12872-15.

8. Che Man, Y. B., Haryati, T., Ghazali, H. M., Asbi, B. A. (1999). Composition and Thermal Profile of Crude Palm Oil and Its Products. *Jaocs.*76 (2): 237–242.
9. Deen, A., Visvanathan, R., Wickramarachchi, D., Marikkar, N., Nammi, S., Jayawardana, B. C., Liyanage, R. (2021). Chemical Composition and Health Benefits of Coconut Oil: An Overview. *J. Sci. Food Agric.* 101 (6): 2182–2193. https://doi.org/ 10.1002/jsfa.10870.
10. Suryawanshi, Y. C., Mokat, D. N. (2019). Chemical Composition of Essential Oil of *Madhuca longifolia* Var. Latifolia (Roxb.) A. Chev. Flowers. J. Essent. Oil-Bearing Plants 22 (4): 1034–1039.https://doi.org/10.1080/ 0972060X.2019.1667879.
11. Pal, U. S., Patra, R. K., Sahoo, N. R., Bakhara, C. K., Panda, M. K. (2015). Effect of Refining on Quality and Composition of Sunflower Oil. *J. Food Sci. Technol*. 52 (7): 4613–4618. https://doi.org/10.1007/s13197-014-1461-0.
12. Ozawa, Y., Sato, H., Nakatani, A., Mori, O., Hara, Y., Nakada, Y., Akiyama, Y., Morinaga, Y. (2001). Chemical Composition of Soybean Oil Extracted from Hypocotyle-Enriched Soybean Raw Material and Its Cholesterol Lowering Effects in Rats. *Journal of Oleo Science.* P.217–223. https://doi.org/10.5650/jos.50.217.
13. Rounizi, S. K., Mohajeri, F. A., Broujeni, H. M., Pourramezani, F., Jambarsang, S., Kiani, H., Sadrabad, E. K. (2021). The Chemical Composition and Heavy Metal Content of Sesame Oil Produced by Different Methods: A Risk Assessment Study. *Food Sci. Nutr.* 9 (6): 2886–2893. https://doi.org/10.1002/ fsn3.2245.
14. Aluyor, E. O., Aluyor, P., Ozigagu, C. E. (2009). Effect of Refining on the Quality and Composition of Groundnut Oil. *African J. Food Sci.* 3 (8): 201–205.
15. Oluremi, O. I., Solomon, A. O., Saheed, A. A. (2013). Fatty Acids, Metal Composition and Physico-Chemical Parameters of Igbemo Ekiti Rice Bran Oil. *J. Environ. Chem. Ecotoxicol.* 5 (3): 39–46. https://doi.org/ 10.5897/JECE2012.0005.
16. Smith, S. B., Gill, C. A., Lunt, D. K.; Brooks, M. A. (2009). Regulation of Fat and Fatty Acid Composition in Beef Cattle. *Asian-Australasian J. Anim. Sci.* 2009, 22 (9): 1225–1233. https://doi.org/10.5713/ ajas.2009.r.10.
17. Nagalla, S. Y., Sahoo, J. P., Samal, K. C., Sahoo, S. (2020). Detection of Adulteration in Ghee - A Spoonful of Yellow Magic. *Biot. Res. Today*, 2 (9): 912–914.
18. Upadhyay, N. A. K., Padghan, P. V., Gandhi, K., Lal, D., Sharma, V. (2015). Detection of Vegetable Oil and Animal Depot Fat Adulteration in Anhydrous Milk Fat (Ghee) Using Fatty Acid Composition. MOJ Food Process. *Technol.* 1 (3): 46–54. https:// doi.org/ 10.15406/mojfpt.2015.01.00013.
19. Ayari, F., Mirzaee-Ghaleh, E., Rabbani, H., Heidarbeigi, K. (2018). Detection of the Adulteration in Pure Cow Ghee by Electronic Nose Method (Case Study: Sunflower Oil and Cow Body Fat). *Int. J. Food Prop.* 21 (1): 1670–1679. https://doi.org/10.1080/10942912.2018.1505 755.
20. Rmani, A., Hazra, T., Parmar, MP., Sindhav, R.G., Ramani, V. A. (2019). Simple Rapid Technique for Detection of Palm Oil in Ghee. *Indian J. Dairy Sci.* 72 (04): 441–444. https://doi.org/10.33785/ijds.2019.v72i04.01 5.
21. Sri Lanka, S. (2017). Methods for analysis of animal and vegetable fats and oils. In part 1 – determination of physical characteristics Section 5: Determination of refractive index; 2017.

22. Sharma, V., Agarwal, A., Chaudhary, U., Singh, M. (2013). Phytochemical Investigation of Various Extracts of Leaves and Stems of Achyranthes Aspera Linn. *Int. J. Pharm. Pharm. Sci.* 5 (SUPPL.1), 317–320.
23. Ganguli, N. C., Jain, M. K. (1973). Ghee: Its Chemistry, Processing and Technology. *J. Dairy Sci.* 56 (1), 19–25. https://doi.org/10.3168/jds.S0022-0302(73)85109-4.
24. Hazra, T., Parmar, P. (2014). Natural Antioxidant Use in Ghee-A Mini Review. *J. Food Res. Technol*. 2 (3): 101–105.
25. FAO, F. and A. O. of the U. N. Milk Composition Database. Gateway to dairy production and products.
26. Otunola, G. A., Adebayo, G. B., Olufemi, O. G. (2009). Evaluation of Some Physicochemical Parameters of Selected Brands of Vegetable Oils Sold in Ilorin Metropolis. *Int. J. Phys. Sci.* 4 (5): 327–329.
27. Sarkar, A., Pandey, J. P., Singh, A., Tiwari, L., Kumar, A. (2012). A Novel Method of Using Refractive Index as a Tool for Finding the Adulteration of Oils. *Res. J. Recent Sci.* 1 (7): 77–79.
28. Zhang, W., Li, N., Feng, Y., Su, S., Li, T., Liang, B. (2015). A Unique Quantitative Method of Acid Value of Edible Oils and Studying the Impact of Heating on Edible Oils by UV-Vis Spectrometry. *Food Chem.* 85, 326–332. https://doi.org/10.1016/j.foodchem.2015.04.005.
29. Atinafu, D. G., Bedemo, B. (2011). Estimation of Total Free Fatty Acid and Cholesterol Content in Some Commercial Edible Oils in Ethiopia, Bahir DAR. J. Cereal. Oil seeds, 2 (6): 71–76. https://doi.org/10.5897/jco11.025.
30. Yu, X., van de Voort, F. R., Sedman, J. (2007). Determination of Peroxide Value of Edible Oils by FTIR Spectroscopy with the Use of the Spectral Reconstitution Technique. Talanta, 74 (2): 241–246. https://doi.org/10.1016/j.talanta.2007.06.004.
31. Alireza, S., Tan, C. P., Hamed, M., Che Man, Y. B. (2010). Effect of Frying Process on Fatty Acid Composition and Iodine Value of Selected Vegetable Oils and Their Blends. *Int. Food Res. J.* 17 (2): 295–302.
32. Diop, A., Sarr, S. O., Ndao, S., Cissé, M., Baldé, M., Ndiaye, B., Diop, Y. M. (2014). Effect of Deep-Fat Frying on Chemical Properties of Edible Vegetable Oils Used by Senegalese Households. *African J. Food, Agric. Nutr. Dev.* 14 (6): 2218–2238.
33. Paul G. Weston, M. D. (1912). A Colorimetric Test for Cholesterol. No. 1, 47–54.
34. Sanjeeva Kumar, A., Raveendra Reddy, J., Rama Mohan Gupta, V. (2014). Preliminary Phytochemical and Standardization Parameters of *Ipomoea Quamoclit* Linn Whole Plant - An Ethnomedicinally Important Plant. *Int. J. Pharm. Pharm. Sci.*, 6 (7), 162–165.
35. Shukla, A. K., Singh, R. P. (2005). Detection of Sesame Oil in Other Oils and Fats. *J. Oleo Sci.* 54 (10): 543–544. https://doi.org/10.5650/jos.54.543.
36. Pardeshi, S. (2019). Assessing the Genuinty of Sesame Oil Available in Markets of India Using Bellier Turbidity Temperature Test (BTTT*). Int. Res. J. Eng. Technol*. 6 (8): 1110–1113. https://doi.org/10.22214/ijraset.2019.9092.
37. Sim, S. F., Lee, T. Z. E., Mohd Irwan Lu, N. A. L., Samling, B. (2014). Synchronized Analysis of FTIR Spectra and GCMS Chromatograms for Evaluation of the Thermally Degraded Vegetable Oils. *J. Anal. Methods Chem.* https://doi.org/10.1155/2014/271970.

38. Shin, E. C., Hwang, C. E., Lee, B. W., Kim, H. T., Ko, J. N. M., Baek, I. Y., Lee, Y. B., Choi, J. S., Cho, E. J., Seo, W. T., Cho, K. M. (2012). Chemometric Approach to Fatty Acid Profiles in Soybean Cultivars by Principal Component Analysis (PCA). *Prev. Nutr. Food Sci.* 17 (3): 184–191. https://doi.org/10.3746/pnf.2012.17.3.184.
39. Shin, E. C., Craft, B. D., Pegg, R. B., Phillips, R. D., Eitenmiller, R. R. (2010). Chemometric Approach to Fatty Acid Profiles in Runner-Type Peanut Cultivars by Principal Component Analysis (PCA). *Food Chem.* 119 (3): 1262–1270. https://doi.org/10.1016/j.foodchem.2009.07.058.
40. Kenar, J. A., Moser, B. R., List, G. R. (2017). Naturally Occurring Fatty Acids; Elsevier Inc., Vol. 2600. https://doi.org/10.1016/b978-0-12-809521-8.00002-7.