

ORIGINAL RESEARCH PAPER

COMPARATIVE STUDY ON PHYSICOCHEMICAL AND SENSORY CHARACTERISTICS OF TWO TRADITIONAL GHEE MADE FROM BUFFALO AND COW MILK

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Abstract

The physicochemical, sensory characteristics, and fatty acids composition of two traditional ghee made from native West Azarbaijan buffalo and cow milk was investigated. In order to determine the oxidative stability, free fatty acids (FFAs) content and peroxide value (PV) of ghee samples were determined during 6 months of storage at ambient temperature (25°C). The yield of buffalo ghee was significantly higher compared to cow ghee (6.01 versus 3.10%). No significant difference was observed in the saponification value, iodine value, refractive index, and slip melting point between two ghee samples ($p > 0.05$). FFAs content and PV of ghee samples increased significantly during six months of storage ($p < 0.05$). At the end of storage, the PV in buffalo ghee (0.34 meqO₂/kg) was significantly lower than that of cow ghee (0.39 meqO₂/kg) ($p < 0.05$). Fatty acid composition analysis revealed a high degree of saturation (67.93 and 72.69% in buffalo and cow ghee, respectively), with C14:0, C16:0, and C18:0 being the predominant saturated fatty acids. On the other hand, C18:1 and C18:2 were the main monounsaturated and polyunsaturated fatty acids in buffalo and cow ghee. Buffalo ghee displayed a significantly higher level of conjugated linoleic acid than that of cow ghee ($p < 0.05$). Significantly higher scores were given to buffalo ghee by the panelists for all evaluated sensory attributes ($p < 0.05$). According to the findings of this study, buffalo ghee has high nutritional potential as well as consumer acceptance, and its development would improve the livelihoods of rural herders by promoting their market share and preventing the stagnation of buffalo breeding activity.

Keywords: buffalo ghee, peroxide value, fatty acids profile, sensory analysis, physicochemical characteristics

Introduction

Ghee, or clarified butter, is a food that has traditionally been consumed in large quantities in different parts of Asia and Africa (Dhurvey *et al.*, 2012). It is known by different names in several regions, such as *roghan* in Iran, *maslee*, and *samna* in the Middle East, *meshho* in Aramea, *samuli* in Uganda, and *samin* in Sudan. It has a unique flavour profile which differentiates it from other fat-rich dairy products (Lodh *et al.*, 2018; Kumbhare *et al.*, 2021). Ghee is usually made from butter prepared from cow or buffalo milk (Fatouh *et al.*, 2003; Sujatha and Sarashetti, 2015). In recent years, the use and consumption of ghee have increased around the world, and the global consumption of ghee has increased moderately since the year 2007 (Indexbox Inc, 2019).

Iran has been ranked 6th within the group of 119 countries that FAO tracks regarding interest rates on butter and ghee production. According to the statistical report of the Food and Agriculture Organization of the United Nations, Iran's ghee production reached 204, 344 tons in 2014 (FAO, 2014). In Iran, butter and ghee are usually produced traditionally on household scales in rural areas or small workshops in the provinces of West Azarbaijan and Kermanshah by traditional milk butter method. As defined by the Iranian National Standard, ghee is a product obtained entirely from milk fat, cream, or butter by methods that almost completely remove water and non-fat solids. This product is also known as anhydrous milk fat, milk fat, and butter oil. Ghee can also be produced from fermented milk fat (INSO, 2020).

Chemically, ghee can be defined as a complex lipid made of triacylglycerol, as well as small amounts of free fatty acids (FFAs), phospholipids, hydrocarbons, carbonyl compounds, fat-soluble vitamins (A, D, E, and K), carotenoid pigments, moisture, and trace elements such as copper and iron. On average, ghee contains 99.0- 99.5% fat and less than 0.5% moisture (Kapadiya and Aparnathi, 2017), and it is usually semi-solid at room temperature (Fatouh *et al.*, 2003). Ghee has a long shelf life and can be stored at room temperature for 6-8 months (Acharya, 1997; Sserunjogi *et al.*, 1998). The obvious reason for the long shelf life is that it contains very little (0.5%) moisture and milk solids (Sserunjogi *et al.*, 1998). *Ayurveda*, a traditional (Indian) medical knowledge base, has referred to ghee as a therapeutic agent for the treatment of skin diseases and allergies (Sujatha and Sarashetti, 2015).

A number of studies have been conducted over recent years on the physicochemical, functional, and sensory properties of ghee prepared from different species. The physicochemical characteristics and biological importance of ghee, as well as the effect of antioxidants on stability of ghee against autoxidation during storage, were discussed by Bhavaniramy *et al.* (2018) in a review article. Singh *et al.* (2022) compared ghee made from yak, yak-cow hybrid, and cow milk and found significant differences ($p < 0.05$) in various physicochemical parameters and color profiles. Parmar *et al.* (2018) conducted a study to compare the composition of ghee made from camel, cow, and buffalo milk and concluded that the yield and recovery of the fat and chemical composition of camel ghee were significantly different from those of both cow and buffalo ghee ($p < 0.05$). Sawaya *et al.* (1984) investigated physicochemical properties of ghee and butter derived from goat and sheep milk and

found that iodine and Reichert-Meissl values of ghee and butter prepared from both species were low, while their saponification values were rather high. Moreover, a relatively high degree of saturation (63.6 - 74.1%) was observed in the fatty acid profile. Bille and Kandjou (2008) have determined the physicochemical and sensory properties of ghee produced by Herero farmers in Namibia. The high content of moisture and FFAs as well as the high peroxide value with oxidized flavor and rancid taste were found in the ghee samples. Mor *et al.* (2022) have examined the physicochemical characteristics and color parameters of cow and buffalo ghee to identify cow ghee adulterated with buffalo ghee. It was concluded that the Kirschner value and whiteness index (W) could be applied to differentiate between cow and buffalo ghee. Jing *et al.* (2019) examined the physicochemical properties and fatty acid composition of 50 ghee samples collected from seven different regions in Tibet. It was concluded that minerals and fatty acids content of ghee samples differed significantly with altitude level and region ($p < 0.05$). The main causes of differences were attributed to the quality of pastures, regional climatic conditions, husbandry method, animal diet, and feed quality.

In many Asian regions like Iran, cows and buffaloes play an important role in the livelihoods of the rural population. They are kept for the production of milk and the development of different dairy products such as butter, ghee, and cheese. Despite being a valuable dairy product in Iranian diet, there is little scientific information on the characteristics of West Azarbaijan's buffalo or cow ghee. Therefore, this study was conducted to evaluate and compare the physicochemical and sensory properties of ghee samples prepared in traditional methods from native West Azarbaijan buffalo and cow milk.

Materials and methods

Collection of milk samples

Fresh cow and buffalo milk were collected from the local herd maintained around the city of Urmia (West Azerbaijan province, Iran). The milk samples used in the preparation of ghee were analyzed for pH, acidity, fat, protein, non-fat solids, and density. Table 1 shows raw cow and buffalo milk's chemical composition.

Table 1. Chemical composition of buffalo and cow milk (Mean±S.D.).

Parameters	Buffalo milk	Cow milk
pH	6.75±0.15	6.77±0.12
Acidity (°D)	14.30±0.02	14.00±0.03
Fat (%)	5.40±0.22	3.80±0.10
Protein (%)	3.60±0.10	3.13±0.15
Non-fat solid (%)	9.23±0.30	8.48±0.44
Density (kg/m ³)	1.031±0.01	1.031±0.01

Preparation of butter

The three batches of ghee were traditionally made from cow and buffalo milk at a local dairy workshop (Urmia, West Azarbaijan Province, Iran) by the indigenous milk butter method. Cow and buffalo milk was heated separately in a stainless steel container on a medium gentle flame, at 85°C for 10-15 min; then they were cooled to 44°C and inoculated with 3% yogurt as a starter culture. The inoculated milk was kept at 44°C for 4 h for fermentation. The resulting yogurt was left at room temperature (25°C) for 2-3 days to increase lactic acid production. Then, the yogurt was poured into a butter-making machine (Parssarma, Iran), and lukewarm water, as much as the weight of the yogurt, was added. By churning yogurt in the butter-making machine, butter was prepared. Butter production was completed after 15-20 min when two phases containing butter and buttermilk were formed. The upper phase, namely butter, was manually separated from the lower liquid (dough) in the form of spherical balls.

Preparation of ghee

For ghee production, the butter balls were clarified by boiling at 100°C for few min on a flame and stirred occasionally until the bubbles disappeared and a golden liquid containing a brown solid residue appeared. In this stage, the water evaporated, and non-fat solids precipitated. Finally, the ghee was cooled to 60°C, filtered through twofold muslin cloth, packed in glass containers, and stored at room temperature (25°C) until analysis.

Physicochemical analysis

The yield was calculated by dividing the weight of ghee by the weight of milk and was expressed in percentage (%). Fat (Soxhlet method) and moisture of ghee samples were determined by the standard method described in AOAC Official Method (AOAC, 2010). Free fatty acids percent, peroxide value (PV), saponification value (SV), and iodine value (IV) of ghee samples were determined by the standard method described in AOCS official methods Cd 3d-63 (AOCS, 2009), Cd 8-53 (AOCS, 1997), Cd 3-25 (AOCS, 2010), and Cd 1-25 (AOCS, 1993), respectively. The refractive index was measured by an Abbe's refractometer at 25°C with automatic temperature control (RX-7000α; Atago, Co Ltd, Tokyo, Japan) according to standard methods described in AOAC official method 921.08. (AOAC, 2006) The slip melting point (SMP) was determined using the capillary tube method according to the AOCS official method Cc 3-25 (AOCS, 1996).

Fatty acids analysis

The fatty acids composition of ghee samples was determined according to the methods of AOAC-969.33 and 963.22. (AOAC, 2000). Fatty acids methyl esters (FAMES) were prepared by BF₃-Methanol method. One μL of FAMES extract was injected into a gas chromatograph (Agilent-6890) equipped with a flame ionization detector (FID) and the capillary chromatographic column HP -88 (88% - Cyanopropyl) aryl-polysiloxane, 100 m, 0.25 mm id, and 0.20 μm film thickness. The initial oven temperature was set at 120°C, held for 5 min; increased to 240°C at 4°C/min, held for 15 min. High-purity nitrogen was used as carrier gas. The injector

and detector temperatures were set at 260°C and 280°C, respectively. Identification of the fatty acid profile was undertaken by comparison with chromatograms from reference methyl esters (Sigma Aldrich, St. Louis, MO, USA).

Sensory analysis of ghee samples

Ghee samples were analyzed for sensory characteristics by a panel of 10 trained participants (5 women, 5 men) with an average age of 29-55 years old. A 9-point hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like) was used to determine the attributes of the samples (Civille and Carr, 2015). The ghee samples were placed in 5g plastic cups coded with random 3-digit numbers and presented to evaluate their color, odor, flavor, texture, and overall acceptability.

Statistical Analysis

Analysis of variance (ANOVA) was performed using the statistical program NCSS 2007 (NCSS, Statistical Software, Kaysville, UT) for the statistical analysis of physicochemical results. Student's t-test was used to determine whether there was a difference in the properties of the 2 ghee samples. The results were given as a mean \pm standard deviation. The significance of the difference was defined at the 5% level. PanelCheck software (version V1.3.2, Matforsk, Ås, Norway) was applied to monitor panelists' performance and to analyze sensory data. Multivariate comparison of sensory attributes of ghee samples was also carried out with Principal Component Analysis (PCA) using the statistical program Unscrambler® V 9.7 (CAMO Software AS, OSLO, Norway).

Results and discussion

Composition and physicochemical characteristics of ghee samples

The composition and physicochemical characteristics of ghee samples have been exhibited in Table 2. The yield of ghee is affected mainly by the fat content of raw milk, the size of fat globules (Luo *et al.*, 2018), and fat losses during ghee preparation (De, 2004). There was a relationship between milk fat content and ghee yield, the higher the milk fat, the higher yield of ghee. The yield of buffalo ghee was significantly higher than that of cow ghee because of its higher fat content ($p < 0.05$).

The moisture content of buffalo ghee was significantly lower than that of cow ghee ($p < 0.05$). According to the Iranian national standard, the maximum moisture of ghee should not exceed 0.5% (INSO, 2020). In this research, the moisture content of ghee samples was within the Iranian national standard range.

In the study of Peña-Serna and Restrepo-Betancur (2020), no significant difference was observed between buffalo and cow ghee moisture content. Parmar *et al.* (2018) also found a significant difference in the moisture content of buffalo (0.248%) and cow ghee (0.280%) ($p > 0.05$).

The fat content of buffalo and cow ghee was 99.90 and 99.80%, respectively, and no significant difference was observed between them. Similar results were reported by Peña-Serna *et al.* (2019) for the fat content of cow (98.9%) and buffalo ghee (0.99%).

In the present study, cow ghee had a non-significantly ($p>0.05$) higher iodine value (28.85 mg I₂/g) than that of buffalo ghee (28.01 mg I₂/g). This result was confirmed by the higher concentration of unsaturated fatty acids in cow ghee (32.07%) compared to buffalo ghee (27.31%) (Table 3). According to Peña-Serna and Restrepo-Betancur's (2020) report, the IV in cow ghee (50.6 mg I₂/g) was significantly higher than that of buffalo ghee (22.6 mg I₂/g). They stated that cow ghee contained more unsaturated fatty acids than buffalo ghee. Gosewade *et al.* (2017) reported that the IV of cow ghee (35.32 mg I₂/g) was insignificantly higher than that of buffalo ghee (32.36 mg I₂/g). After 8 months of storage, it decreased significantly to 33.77 and 26.85 mg I₂/g in cow and buffalo ghee samples, respectively ($p<0.05$). In the research study of Kumar *et al.* (2010), IV of cow and buffalo ghee was reported to be 35.16 and 31.89 mg I₂/g, respectively.

Table 2. Composition and physicochemical characteristics of ghee samples (Mean±S.D.).

Parameter	Buffalo ghee	Cow ghee
Yield (%)	6.01 ± 0.13 ^a	3.10 ± 0.09 ^b
Moisture (%)	0.09±0.21 ^b	0.50±0.01 ^a
Fat (%)	99.90±0.14 ^a	99.80±0.12 ^a
Iodine value(mg I ₂ /g)	28.01±0.03 ^a	28.85±0.07 ^a
Saponification value (mg KOH/g)	230.57±0.87 ^a	215.38±1.01 ^a
Refractive index	1.4553±0.004 ^a	1.4561±0.006 ^a
Slip melting point (°C)	34.05±0.07 ^a	34.00±0.00 ^a

Values are means of three analyses. Different superscripts in the same row show significant differences ($p<0.05$).

The saponification value is used as a parameter to evaluate the molecular weight or chain length of fatty acids in fats and oils. A high SV indicates the presence of short and medium-chain fatty acids. In the present study, there was no significant difference between the SV of buffalo and cow ghee. The average SV of buffalo and cow ghee were 230.57 and 215.38 mg KOH/g, respectively. The SV of buffalo ghee was insignificantly higher than that of cow ghee. The variations in the SV of ghee are likely a result of a difference in fatty acid profile, as shown in Table 3. In the research work of Peña-Serna and Restrepo-Betancur (2020), the SV of buffalo ghee (233.9 mg KOH/g) and cow ghee (217 mg KOH/g) did not show a significant difference ($p>0.05$), which means that the molecular weight of fatty acids in buffalo and cow ghee was similar and consistent with long chain fatty acids above 14 carbons. In another research work, the SV for fresh buffalo and cow ghee was reported 238.1 and 234.70 mg KOH/g, respectively (Gosewade *et al.*, 2017). The average SV for milk fat ranges from 225 to 230 mg KOH/g. Significant levels of short-chain fatty acids mean the presence of significant content of butyric (C4) and caproic (C6) acids in the fat (Asif *et al.*, 2022). Variations in the saponification value of ghee samples are probably the result of differences in fatty acid profiles (Singh *et al.*, 2022).

The refractive index of a material is the ratio of the speed of light in a vacuum to its speed in the material. The RI is generally used to determine the change in saturation due to the hydrogenation of fat or oil (Katkade *et al.*, 2018). No significant difference was observed between the RI of buffalo and cow ghee samples. The RI of ghee samples in other studies was within the range of the present study (El-Hadad and Tikhomirova 2018; Gurav *et al.*, 2020).

Melting point is an important physical characteristic of fat compounds, which is useful for their identification, and it is crucial in many technological applications of fats. The MP of fatty acids increases with increasing chain length and decreases with increasing degree of unsaturation (Sulieman *et al.*, 2013). No significant difference was observed between the MP of buffalo and cow ghee. The result of the MP of buffalo and cow ghee was within the range of that reported by Bindal and Wadhwa (1993) (32.7-35.8 and 33.4-38.8°C for cow and buffalo ghee, respectively). While in the study of Sulieman *et al.* (2013), the MP of three ghee purchased in local markets in Sudan and the ghee prepared in the laboratory was reported 37, 38, and 37°C, 37°C, respectively. The MP of ghee samples in their research is higher than those of the present study.

Oxidative stability parameters (FFAs and PV) of ghee samples during storage

The acidity or FFAs content of the oil is a qualitative parameter to determine the hydrolysis of triglycerides and the levels of FFAs. As shown in Figure 1, there was a significant difference between the acidity of buffalo and cow ghee ($p < 0.05$). In both ghee samples, acidity increased significantly during storage ($p < 0.05$). According to the Iranian National Standard, the maximum acidity allowed in ghee should not exceed 0.8% (INSO, 2011). In the present study, until the 3rd month, the acidity of both buffalo and cow ghee was within the permissible limit; however, in the 6th month, it was higher than the allowed limit. As reported by Peña-Serna and Restrepo-Betancur (2020), the acidity of cow ghee (0.1%) was significantly higher than that of buffalo ghee (0.01%) ($p < 0.05$). They hypothesized that cow ghee undergoes higher oxidation and spoilage over time.

Lipase is one of the most important enzymes for the hydrolysis of oils and the formation of FFAs. In addition, the storage temperature and the initial content of FFAs, which may act as catalysts in the production of more free fatty acids, have a significant impact on the level of acidity (Ayton *et al.*, 2012).

The presence of FFAs in butter and ghee is undesirable because they both cause flavour degradation (fatty acids with carbon numbers less than 16), and there is a risk of the formation of long-chain unsaturated free fatty acids that may participate in oxidation reactions (Fındık and Andıç, 2017). However, it has been reported that the flavour of the oil is superior when the average FFAs are higher than 0.3% (Kirazci and Javidipour, 2008). Kirazci and Javidipour (2008), in a study of some chemical and microbial properties of 30 ghee samples produced in Eastern Anatolia, Turkey, reported that, in general, the free fatty acids of the ghee samples increased during 30 days of storage at 5°C. The increase in FFAs was attributed to the production of organic acids (*e.g.*, lactic acid) by lactic acid bacteria.

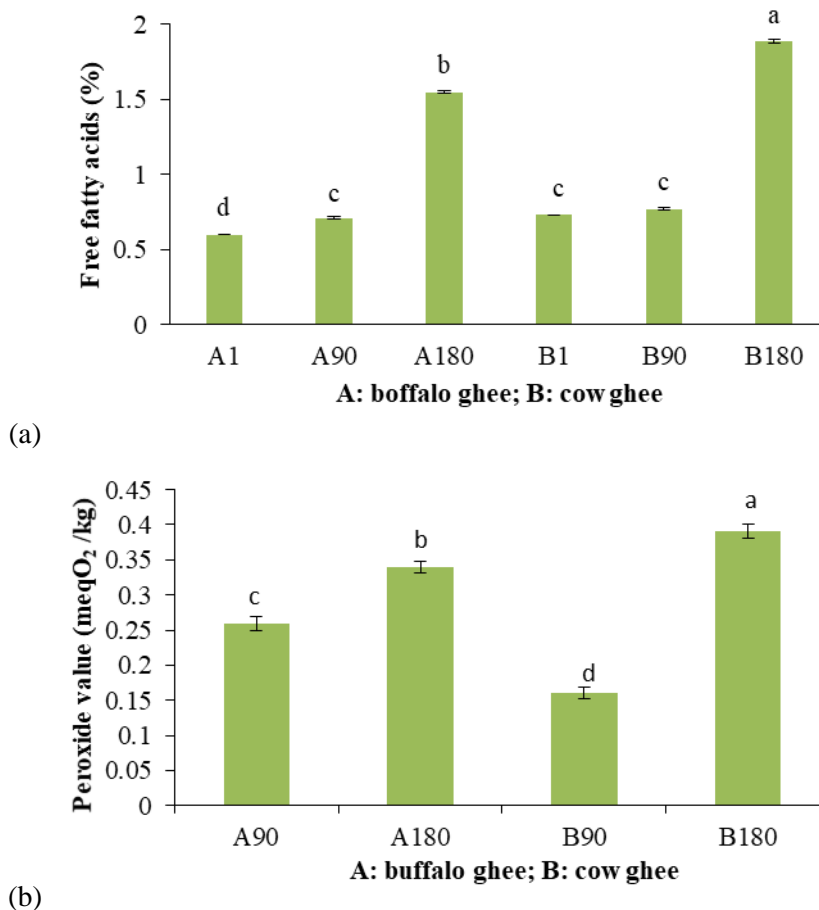


Figure 1. Free fatty acids (a) and peroxide value (Oxidative Stability) (b) of ghee samples during 6-month storage. Numbers indicate storage days. Different letters in the columns indicate statistically significant differences among samples ($p < 0.05$).

In the study of Parmar *et al.* (2018), the level of FFAs in buffalo and cow ghee prepared from the milk of local herds kept in the village near Anand was reported at 0.153 and 0.136%, respectively. In another investigation conducted by Gosewade *et al.* (2017), the FFAs content of fresh cow and buffalo ghee was found to be 0.42, and 0.21%, respectively. After 8 months of storage at 37°C, the average content of FFAs of cow and buffalo ghee increased to 0.72, and 0.61%, respectively. The higher FFAs content of buffalo ghee was attributed to the characteristics of the species. According to the results of studies carried out by different researchers, the level of acidity in ghee increased during storage due to various factors such as temperature, natural milk lipase, or microbial lipases.

Peroxide value is a good indicator of fat oxidation, and it measures the early stages of fat oxidation. The PV of both buffalo and cow ghee samples increased significantly during 6 months of storage at ambient temperature ($p < 0.05$) (Figure 1).

The PV of both fresh ghees was zero; however, after 6 months of storage, it was significantly lower in buffalo ghee (0.34 meqO₂/kg) than that of cow ghee (0.39 meqO₂/kg) ($p < 0.05$). A similar result was reported by Gosewade *et al.* (2017). In an investigation of oxidation in buffalo and cow ghee in the presence or absence of antioxidants during 8 months of storage at 37°C, they observed a gradual increase of PV over time in all samples. The increase of PV in ghee samples containing added antioxidant (BHA) was lower than that of control samples (without antioxidant).

According to the report of Peña-Serna and Restrepo-Betancur (2020), fresh buffalo and cow ghee samples did not show any oxidation. The result of a research study carried out by Kirazci and Javidipour (2008) also indicated an increase in peroxide value in ghee samples during 30 days of storage at 5°C. They concluded that 5°C was not cold enough to stop the oxidation reactions. However, a shelf life of 6–8 months, even at ambient temperature, has been reported for the oil, and a longer shelf life, up to 2 years, has been reported for the oil produced in Ethiopia by the Burano tribe.

Fatty acids composition of buffalo and cow ghee

The fatty acids composition of buffalo and cow ghee samples are presented in Table 3. Saturated fatty acids were the predominant fatty acids in both buffalo and cow ghee (72.69 and 67.93%, respectively). Palmitic acid (C16:0), followed by myristic acid (C14:0) and stearic acid (C18:0), were dominant saturated fatty acids. Palmitic (C16:0) and stearic (C18:0) acid content of buffalo ghee were significantly higher than those of cow ghee; on the contrary, myristic acid (C14:0) in cow ghee was significantly higher than that of buffalo ghee ($p < 0.05$).

According to Peña-Serna and Restrepo-Betancur (2020), palmitic (C16:0) (24–28%), stearic (C18:0) (9–14%), and myristic (C14:0) (8–10%) acids were the three primary saturated fatty acids in buffalo and cow ghee. A similar report was also presented by Antony *et al.* (2018). In the study of Peña-Serna and Restrepo-Betancur (2020), the average content of palmitic (C16:0) and stearic (C18:0) acids in buffalo ghee were 28.84% and 14.04%, respectively which was significantly higher than those found in cow ghee (24.03% and 9.36%, respectively).

In our study, the palmitoleic acid (C16:1) content of cow ghee (2.35%) was significantly higher than that of buffalo ghee (2.05%) ($p < 0.05$). In Peña-Serna and Restrepo-Betancur's (2020) study, no significant difference was observed between the palmitoleic acid (C16:1) content of buffalo (1.13%) and cow ghee (1.18%).

Oleic (C18:1) and linoleic (C18:2) acids were the main monounsaturated and polyunsaturated fatty acids in buffalo and cow ghee samples. A similar result was reported by other researchers (Peña-Serna, *et al.*, 2019; Peña-Serna and Restrepo-Betancur, 2020). Oleic acid (C18:1) content in cow ghee (24.27%) was significantly higher than that of buffalo ghee (20.42%) ($p < 0.05$). However, no significant difference was observed between the linoleic acid (C18:2) content of buffalo (2.03%) and cow (1.95%) ghee. In Peña-Serna and Restrepo-Betancur's (2020) research study, oleic (C18:1) and linoleic (C18:2) acids content of cow ghee (20.04%

and 1.64%) were significantly higher than those of buffalo ghee (18.64% and 0.92%) ($p < 0.05$).

The linolenic acid (C18:3) content of buffalo ghee was significantly lower than that of cow ghee ($p < 0.05$). Similar results were reported by other researchers (Peña-Serna *et al.*, 2019; Peña-Serna and Restrepo-Betancur, 2020). In our study, conjugated linoleic acid in buffalo ghee was significantly higher than found in cow ghee ($p < 0.05$).

Differences in the diet or microbial ecosystem of the animals may have influenced the observed differences in fatty acid profiles. Diet (forage quality, pasture quality, etc.) has a great influence on the milk fatty acids profile of ruminants (Khiaosa-ard *et al.*, 2015). The specific adaptation of livestock to the harsh environment, such as specific metabolic efficiency, local climates, husbandry practices (Guo *et al.*, 2012), or differences in rumen microbial composition (Chen *et al.*, 2015), may also be factors that interfere with lipid metabolism. There may also be a systemic effect due to different times of milking (morning, evening, or both) and the presence or absence of calves (Marquardt *et al.*, 2016).

Table 3. Fatty acids composition of buffalo and cow ghee (Mean±S.D.).

Fatty acid (%)	Buffalo ghee	Cow ghee
Butyric acid methyl ester (C4:0)	1.70±0.07 ^a	1.51±0.04 ^b
Caproic acid methyl ester (C6:0)	0.41±0.02 ^b	0.67±0.08 ^a
Caprylic acid methyl ester (C8:0)	0.05±0.02 ^a	0.03±0.02 ^a
Capric acid methyl ester (C10:0)	1.03±0.03 ^b	2.09±0.05 ^a
Lauric acid methyl ester (C12:0)	2.10±0.02 ^b	3.01±0.04 ^a
Myristic acid methyl ester (C14:0)	11.84±0.47 ^b	12.25±0.03 ^a
Methyl myristoleate (C14:1)	0.75±0.02 ^b	1.35±0.03 ^a
Pentadecanoic acid methyl ester (C15:0)	1.70±0.03 ^a	1.66±0.02 ^a
Palmitic acid methyl ester (C16:0)	37.19±0.02 ^a	32.55±0.45 ^b
Palmitoleic acid methyl ester (C16:1)	2.05±0.42 ^b	2.35±0.02 ^a
Heptadecanoic acid methyl ester (17:0)	1.54±0.02 ^a	1.09±0.01 ^b
Stearic acid methyl ester (C18:0)	14.10±0.02 ^a	12.77±0.32 ^b
Oleic acid methyl ester (C18:1)	20.42±0.03 ^b	24.27±0.18 ^a
Linolelaidic acid methyl ester (C18:2)	2.03±0.95 ^a	1.95±0.44 ^a
Linolenic acid methyl ester (C18:3n3)	0.66±0.01 ^b	1.17±0.12 ^a
Arachidic acid methyl ester (C20:0)	1.03±0.02 ^a	0.30±0.02 ^b
Conjugated linoleic acid (CLA)	1.40±0.07 ^a	0.98±0.01 ^b
Saturated fatty acid	72.69	67.93
Monounsaturated fatty acid	23.22	27.97
Polyunsaturated fatty acid	4.09	4.10

Values are means of three analyses. Different superscripts in the same row show significant differences ($p < 0.05$).

Sensory evaluation

The sensory quality of dairy products is influenced by factors such as the chemical and microbial quality of the milk. The difference in the taste of dairy products can

be caused by the difference in the composition of fatty acids according to the feed consumed by the animal. In particular, unsaturated fatty acids appear to be broken down by microbial enzymes in the rumen to produce compounds that are responsible for the aroma of dairy products. Furthermore, processing and storage conditions significantly affect the sensory properties of butter and ghee (Van Ruth *et al.*, 2008). The development of ghee flavour is greatly influenced by compounds formed in the heat-induced breakdown of milk components (fat, proteins, amino acids, and lactose) during filtration stage. Aldehydes, ketones, free fatty acids, carboxylic acids, lactones, and alcohols are major classes of compounds formed during ghee production (Wadhwa and Jain, 1990; Newton *et al.*, 2012). Lactones have a coconut-like aroma, which is primarily associated with the distinctive flavour of ghee. The main types of lactones found in ghee are δ -lactones and γ -lactones, of which, δ -decalactone, δ -dodecalactone, and δ -tetradecalactone are the most important compounds contributing the flavour of ghee (Duhan *et al.*, 2020).

The results of the sensory evaluation showed a significant difference between the two ghee samples in terms of color, odor, taste, texture, and overall acceptability. Buffalo ghee was scored significantly higher for all evaluated attributes ($p < 0.05$). The buffalo ghee had a pale-yellow color, while the cow ghee had a deep yellow color. Both ghee samples were free of unpleasant, sour, fodder, cowy, and chemicals smell as well as free of rancidity, sweetness, bitterness, cooked, and metallic taste. The buffalo ghee had a more pleasant taste and a finer and more uniform texture than those detected for the cow ghee. The sensory scores of both ghee samples were within the acceptable range and, after three months of storage, did not show a significant decrease compared to the first day (fresh ghee).

In a recently published work, a highly significant difference was observed between the sensory characteristics of cow and buffalo ghee. According to their report, the characteristics of ghee odor were mainly defined as lactic, followed by cooked and fatty, along with sweet and butyric odor. In the taste profile, mainly fat and then lactic, cooked, and sweetness were evident. Cow and buffalo ghee samples showed a little sandy texture, but there were more particles in cow ghee. The overall acceptability of buffalo ghee was higher due to its better taste (Peña-Serna and Restrepo-Betancur, 2020).

Figure 2 illustrates the principal component analysis (PCA) plot that describes the sensory attributes and some physicochemical properties of buffalo ghee (A) and cow ghee (B) during 90 days of storage at ambient temperature. In this plot, each component is described as the maximum possible variance, and each new component is based on the previous component. The higher the percentage of variance described, the more reliable the information obtained (Shaviklo, 2018). Accordingly, multivariate analysis indicated that 98.5% of the distribution or dispersion of data between the two ghee samples was classified in the first two principal components (Figure 2). The buffalo ghee (A) and the cow ghee (B) are situated on different sides of the PCA plot. It can be observed that buffalo ghee samples are located in the upper left part of the plot, and their predominant sensory characteristics are color, odor, taste, texture, and overall acceptability. While the cow ghee samples are placed in

the upper right part of the plot and have no predominant sensory characteristics. The oxidation indices, including FFA and PV, are located in that area and indicate that cow ghee contained higher levels of FFA and PV at the end of the study. The PCA also reveals that the two samples had the same values of fat, iodine value, slip melting point, saponification value, and refractive index.

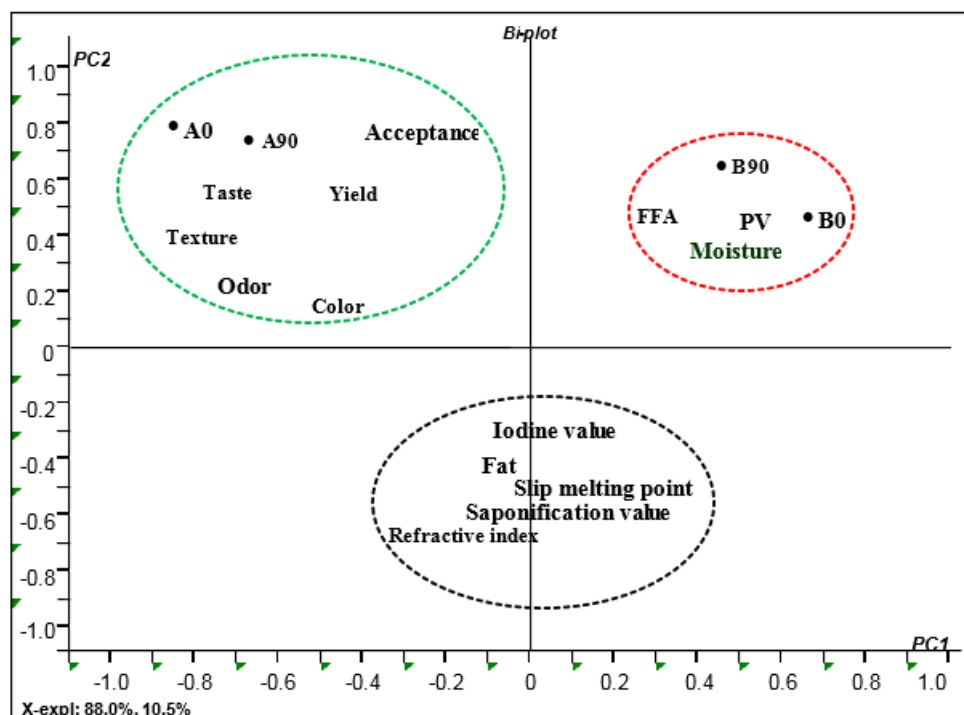


Figure 2. Principal component analysis (PCA) describing sensory changes of buffalo ghee (A) and cow ghee (B) during 90 days of storage at 25°C as evaluated by an expert panel. Numbers show the storage days.

Conclusion

In some physicochemical parameters, significant differences between the two ghee samples made traditionally from buffalo and cow milk were observed. Higher total solids was observed in buffalo milk and the ghee obtained from buffalo milk fat. The ghee prepared from buffalo milk revealed a significantly higher yield than that found for cow milk. Both ghee samples contained higher levels of saturated fatty acids, followed by monounsaturated and polyunsaturated fatty acids. Buffalo ghee displayed a higher level of conjugated linoleic acid (CLA cis-9, trans-11). The overall acceptability score of buffalo ghee was higher than that of cow milk ghee. Based on these results, it can be concluded that the development of buffalo dairy products would improve the livelihoods of rural buffalo ranchers living in rural areas, promotes their market share, and prevents the stagnation of this livestock breeding

activity. It would be recommended to investigate the application of synthetic or natural antioxidants to improve oxidative stability and shelf life enhancement of ghee during storage.

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