

Comparative effect of cooling on the physico-chemicalsensory properties of ghee from cow and buffalo milk, and evaluation of the low-fat spread prepared from cow and buffalo milk ghee

Patange Dnyaneshwar Deverao

Amrita School of Agricultural Sciences, Amrita Vishwa Vidyapeetham University

Dhanake Virshasen Vinayak

Amrita School of Agricultural Sciences, Amrita Vishwa Vidyapeetham University

Deepak Chandran

Amrita School of Agricultural Sciences, Amrita Vishwa Vidyapeetham University

Radha Radha

Shoolini University of Biotechnology and Management Sciences

Manoj Kumar

ICAR - Central Institute for Research on Cotton Technology

Jose Manuel Lorenzo (imlorenzo@ceteca.net)

Centro Tecnológico de la Carne de Galicia, Parque Tecnológico de Galicia

Research Article

Keywords: Low fat spread, cow ghee, buffalo ghee, pre-cooling and exposure time.

Posted Date: May 10th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1547001/v2

License: © ① This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

Low-fat spread (LFS) is the product harmonizing with the idea of healthy nutrition. At the same time, it has a good taste and flavour, as well as very good spreadability at refrigerator temperature. The present investigation studied the effect of method of cooling on the properties of cow and buffalo milk ghee, and comparative evaluation of LFS prepared from them. Slowly pre-cooled cow ghee had intense yellow colour than rapidly pre-cooled cow ghee, whereas slowly pre-cooled buffalo ghee has creamish white colour and rapidly pre-cooled buffalo ghee had white colour. Rapidly pre-cooled cow ghee had a very smooth and pasty texture than rapidly pre-cooled buffalo ghee. The LFS of cow ghee had shown maximum sensory scores for colour and appearance, body and texture, spreadability, and overall acceptability, as compared to buffalo ghee LFS. Chemically it was observed that both the LFS differs in FFA content, while they had similar fat, protein, carbohydrate, ash and total solids content, as well as pH. Oiling off and wheying off was found higher in cow ghee LFS over buffalo ghee LFS. Colour, appearance and flavour score were found improved by the addition of butter annatto colour and diacetyl flavour respectively. Color and appearance, body and texture, spreadability, as well as overall acceptability scores were higher for cow ghee LFS when subjected to 35°C for 10, 20, and 30 minutes. It was found that after 10 minutes of exposure to 35°C, the physical qualities of both LFS were unchanged, but the sensory properties diminished as time passed.

Introduction

Spreadable food products are "plastic" in nature, allowing them to be applied on food items such as bread slices. Butter, margarine, and fat blends of milk fat and other fats (vegetable, animal, or marine origin) make up the spreadable food group. As one of the oldest methods of keeping milk fat, butter has long been used in cooking as well as for medicinal and cosmetic uses (Panchal and Bhandari 2020; Tekin-Cakmak et al. 2021). Even after the start of commercial manufacture, butter remained a pricey commodity among fat-based foods. In the diet of developed countries, butter can be utilized as a spread. However, it has poor spreadability when stored under refrigeration temperature and loses its plasticity when attending ambient temperature; which too are undesirable (Danthine et al. 2014; Duhan et al. 2018). New low-fat and low-cost fat products with better functional qualities can now be introduced by the dairy industry as a result of this development. Dairy products like low-fat spreads (LFS) fall under this category (Londhe-Patil, et al. 2019). It can be manufactured from different types of dairy and non-dairy fats, milk-derived proteins in the form of skim milk, buttermilk, whey, etc., and stabilizers, emulsifiers, plasticizers, vitamins, colorants flavors, etc. All over the world, various efforts have been made for LFS development by using butter, butter oil, cream, paneer, channa, cheese, etc. (Deshpande and Thompkinson 2000; Kulkarni 2017; Hemati 2018). Patange et al (2015) and Kumbhare et al (2021) used a general source of fat, ghee, in the production of LFS because the dairy sector needs to employ ghee in the production of LFS since it is readily available and has a longer shelf life at room temperature.

It is no secret that ghee is widely available and frequently used in the Middle East and South Asian countries like India and Sudan. But ghee production on the American continent has expanded in the recent decade with the top producers, including the United States, Argentina, and Paraguay (Food and Agriculture Organization of the United Nations 2019). Ghee consumption has increased in Western countries due to globalization and the replacement of margarine with ghee as a result of industrial trans fatty acid levels in margarine in Western countries (Antony et al. 2018; Bhatia et al. 2019; Carolina and Luis Fernando 2020). Ghee is a heat clarified butter and one of the most important dairy products in Indian market. It can be prepared from cream or butter by removal of water,

protein, and other minor components (Prasanth et al. 2016; Mehta et al. 2018). Potentially used as a cooking and frying medium in Indian culture also it can serve as spread over parotha, roti, and puran poli. Out of the total milk production, 28% of milk is converted into ghee. Thus, on average, 22.68 million tons of milk gets converted into ghee annually. At least 98.8% of the triglycerides in ghee are made up of milk fat, and no more than 0.1 percent is water (Patange et al. 2018). As a complex mixture of triacylglycerol, triacylglycerol (TG), hydrocarbons, carbonyl compounds, fat-soluble vitamins (A, D, E and K), carotenoid pigments, moisture and traces of metals such as copper and iron, ghee is an excellent source of essential fatty acids and other nutrients (Kapadiya and Aparnathi 2018; Patel and Balakrishnan 2021). Cow and buffalo milk fat ghee is highly prevalent in the dairy market and both the ghee has different color, texture, and flavor due to difference in their fatty acid profile (Ahmad and Saleem 2018; Ahmad and Saleem 2020). Milk fat is a complex mixture of more than 437 fatty acids of various chain lengths and unsaturation, resulting in a wide variety of triglycerides with melting points ranging from – 40 to 40°C. There is a high probability of facile fractionation due to the milk fat's distinctive melting behaviour, which gives rise to diverse chemistry and physical properties (Kumbhare et al. 2021).

Ghee made from buffalo milk contains 8.7% more melting triglycerides than cow milk fat (16:0 and 18:0 fatty acids, respectively), which makes it unique from cow milk fat (4.9%). The triglycerides in buffalo milk fat solidify earlier than in cow milk fat because of this difference. Buffalo milk fat crystallizes at a greater temperature than cow milk fat at the same time (Bector 2002). In view of the light background of the properties of cow and buffalo milk ghee, it was utilized in the preparation of LFS and compared for its properties.

Materials And Methods

Freshly prepared cow milk ghee and buffalo milk ghee were procured from the experiential learning unit of the division of Animal husbandry and Dairy Science of the Institute whereas, spray-dried skimmed milk powder (SMP) having 3.90 % moisture, 0.60 % fat, and 0.76 ml solubility index was purchased from Kolhapur Zilla Sahakari Dudh Utpadak Sangh Ltd. Kolhapur. Carrageenan – Type II iota-carrageenan (make Hi-media) was used as a stabilizer while glycerol was used as a plasticizer and purchased from Qualigens Chemical, Mumbai. To make effective emulsion, polyoxyethylene sorbitan monooleate (Tween-80) was used as an emulsifier which was procured from S. D. Fine chem. Ltd, Mumbai. Iodized salt (Tata), annatto butter color, and citric acid were also used as other minor ingredients. The Planetary mixer (SPAR Make, Taiwan) was used for blending two phases.

Rapid and slow; pre-cooling of ghee

Initially, cow milk and buffalo milk ghee were melted at 70 °C followed by rapid and slow pre-cooling to 20 °C in a controlled condition and then kept at 5 °C by quiescent storage under refrigeration. The stored ghee was evaluated at 30 °C for sensory character viz. texture, consistency and color on a 100-point scale. The treatments were as follows:

CG1: Cow milk ghee melted at 70 °C and rapidly pre-cooled to 20 °C

CG2: Cow milk ghee melted at 70 °C and slowly pre-cooled to 20 °C

BG1: Buffalo milk ghee melted at 70 °C and rapidly pre-cooled to 20 °C

BG2: Buffalo milk ghee melted at 70 °Cand slowly pre-cooled to 20 °C

Preparation of low-fat spread using cow and buffalo milk ghee

As shown in Fig. 1., Patange (2005) devised a process for making low-fat spreads (LFS) from ghee made from cow and buffalo milk. Before blending and emulsifying, the fat and serum phases are prepared and tempered separately. The emulsifier was then added to the hot ghee. It was then heated to 70°C (in a water bath) and quickly cooled to 20°C (at a cooling rate of 12°C per minute) with continual agitation in a chilled water bath (2.5°C), and then to 5°C by quiescent storage in a refrigerator for an overnight duration. It was then held in the water bath for 6 h before usage to bring the fat phase back to a temperature of 251 °C. The aqueous phase was prepared by dissolving SMP in water with soluble components, boiling to 55 °C, filtering through a two-fold muslin cloth, pasteurizing at 72 °C for 15-20 seconds, and then cooling to 20 °C and storing overnight in the refrigerator. For final acidification and warming to blend temperature, this aqueous phase was treated with weak citric acid.

Flat beater attachment of planetary mixer was used for 30 sec at medium speed to whip the tempered fat phase into a creamy mixture. The serum phase was introduced in three equal parts. After each addition of serum phase, medium-speed blending was performed for 30 sec at a time. The 75 g of LFS was placed in plastic cups and sealed with lids before being stored in the refrigerator at 5 °C. The treatments were as follows:

CGS: LFS using cow milk ghee

BGS: LFS using buffalo milk ghee

Effect of colour and flavour on qualities of LFS

The effect of the addition of 0.1% of annatto color and 4 ppm of diacetyl flavor on LFS were evaluated for colour and flavour properties, respectively.

Effect of exposure time on sensory properties of LFS prepared from cow and buffalo milk ghee

Both LFS were removed from the refrigerator (5 ± 1 °C) and evaluated for sensory qualities at the following coded intervals. During the evaluation period, the LFS were kept in the incubator maintained at 35 ± 1 °C.

CS₁: Cow ghee LFS removed immediately from refrigeration

BS₁: Buffalo ghee LFS removed immediately from refrigeration

CS₂: Cow ghee LFS exposed for 10 min

BS2: Buffalo ghee LFS exposed for 10 min

CS3: Cow ghee LFS exposed for 20 min

BS3: Buffalo ghee LFS exposed for 20 min

CS₄: Cow ghee LFS exposed for 30 min

Analytical methods

The moisture content, sleep point (melting point), and iodine value of the ghee was determined by using FSSAI, 2015 methods whereas, fat, crude protein, carbohydrate, total ash, and TS content of LFS were determined as per AOAC (2000). The free fatty acid content of the LFS was estimated as per FSSAI (2015), whereas the pH of the spread was measured by pH meter (Lab India Instruments Pvt. Ltd., Mumbai) by inserting the electrode in the spread (sample temperature was maintained at 5±1 °C. The oiling off and wheying off in LFS was estimated as suggested by deMan and Wood (1958).

Sensory evaluation

Sensory qualities of buffalo and cow ghee, which are commonly utilised in dairy products, were analysed using the method of sensory profile by a multidimensional approach (Patange 2005). After the ghee samples were found to be free of harmful microorganisms, sensory evaluations were carried out. Using a trained panel of five members (aged between 23 and 50 years old) in the sensory room, the evaluation was done out. Panelists were shown ghee samples in ceramic cups numbered with random two-digit codes sequentially under white fluorescent light. The panelists identified and selected the descriptors for establishing the sensory profile of ghee, and thereafter, the intensities of the descriptors were evaluated for colour (at 30±1°C) in a scale from 0 to 100 (where 0 = white and 100 = pronounced yellow), for texture (at 20±1°C) in a scale from 0 to 100 (where 0 = smooth pasty and 100 = Extremely large grain) and for consistency (at 20±1°C) in a scale from 0 to 100 (where 0 = Essentially fluid and 100 = hard solid). The acceptability of LFS was examined by the qualified panel of judges. Color and appearence, spreadability, body and texture, flavour, and overall acceptability were evaluated using a 9-point rating scale (Amerine et al. 1965). The spreadability of the product was examined by spreading LFS on pieces of bread at a consistent temperature 5±1°C.

Colour analysis

Colour values for each sample were measured five times after the colorimeter was calibrated with black and white standards. The L* (lightness; 100=white, 0=black), a* (redness, red, green), and b* (yellowness, yellow, blue) values of samples were measured with a colour flex colorimeter.

Texture analysis

The rheological properties of ghee and ghee-based low-fat spread were determined at 5°C using texture analyzer model TAT2i (stable micro system, UK) provided with texture expert exceed software. The sample was carefully filled up in a cuboidal polypropylene tub (11.0*6.4*3.5cm) so that no air pockets remained within the sample. It was allowed to set at 5 °C for an overnight period. The product was subjected to penetration to a depth 25.0 mm by a 30° PERSPEX CONE PROBE. The other test conditions included measuring force in compression mode, retest period of 5.0 mm/sec, test speed of 2.5 mm/sec and post-test speed of 10 mm/sec. It was discovered that during penetration, the force increased up to the maximum depth of penetration, and this value was referred to as "hardness" (g), and accordingly the region beneath the penetration cycled (downstroke) in the force. Work of shear, or the 'energy expended in shearing,' was represented by a distance-curve (g.s). This resulted in an

undesirable peak when the probe was removed from the sample and replaced with another one. The 'stickiness' (g), and the negative peak area was regarded as the 'work of adhesion' (g.s).

Statistical Analysis

On all experimental data sets, a one-way analysis of variance was performed using SPSS 16 for windows (SPSS Inc. in Chicago, USA). In order to determine the significance of differences between means at a P<05 level, the Duncan analysis was used.

Results And Discussion

Physico-chemical properties of cow and buffalo milk ghee

The physicochemical properties of cow and buffalo milk ghee is illustrated in Table 1. The average fat content of cow ghee and buffalo ghee was 99.5%. Ghee contains 99.5 % of fat and 0.5 % un-saponifiable matter was reported by Carolina and Luis Fernando (2020). Further iodine value of cow and buffalo milk ghee was 31.9 and 33.1 mg/gm respectively (Table 1). The lower iodine value for cow ghee might be the presence of saturated fatty acid and the absence of polyunsaturated fatty acid (Mehta 2013; Kumar et al. 2018). Free fatty acid (FFA) content in cow and buffalo milk ghee were 2.4 and 2.8 (% oleic acid) respectively, however, the presence of FFA is undesirable as it may be responsible for the rancid flavour typify by butyric acid as reported by Munro et al. (1992). It was also observed that cow milk ghee had a lower slip point (34.4 °C) than that of buffalo ghee (38.3 °C). Lakshminarayana and Rama Murthy (1985) reported an average melting point of cow ghee is 34.2 °C and melting point of buffalo ghee is 35.8 °C and it supported the present findings. Changade et al (2006) claim that the type of milk, rate of cooling, crystallization extent, entropy and enthalpy changes have an effect on ghee's melting point. Ahmad and Saleem (2018) discovered that heating desi ghee modifies its molecular makeup, and they concluded that temperatures between 140°C and 170°C are safe for cooking or frying.

Effect of rapid and slow pre-cooling on qualities of cow and buffalo milk ghee.

The color of rapidly pre-cooled cow ghee was light yellow (score 67.32) whereas, the slowly pre-cooled cow ghee was yellow color (score 73.92). It indicates that when cow ghee pre-cooled slowly had shifted towards pronounced yellow color compare to pre-cool rapidly (Table 2). The observed trend in colour of ghee was also reflected in terms of L*, a* and b* colour values (Table 3). All these values were significantly affected due to variations in treatments. The color of fat always appears deeper in eyes when melted than when in solid form as reported by Frankel et al (1958). Similarly, buffalo ghee when pre-cooled rapidly had a white appearance (score 22.44) as compared to slowly pre-cooled buffalo milk ghee which had a creamish white appearance (score 30.36). It was observed that buffalo ghee pre-cooled rapidly had appearance more white than pre-cooled slowly. Carotene pigment, which is found in cow ghee, may be responsible for its vivid yellow colour. Beta-carotene is abundant in cow ghee, while it is lacking from buffalo ghee due to the buffalo's metabolism of beta-carotene into vitamin A (Ahmad and Saleem 2018; Ahmad and Saleem 2020). Buffalo ghee lacks in carotene resulted in whitish with slightly greenish tinge due to the presence of bilirubin and biliverdin which gives it a greenish tint (Achaya 1997; Kumar et al. 2018).

The texture score of cow ghee and buffalo ghee were significantly (p<0.05) affected by the method of precooling (Table 2). The texture of cow ghee that had been rapidly pre-cooled (score 5.28) was smoother and

pastier than buffalo ghee (score 11.88). Further, the texture characteristic of both slow pre-cooled ghee samples also shows variation. The buffalo ghee had shown large grains compared to cow ghee, which observed fine to medium grain. The present finding is well supported by the reports of Ahmed et al (2008) who reported that the fat globule of buffalo milk ghee is course and bigger than cow milk ghee. Elzein et al (2006) also reported much average globule size of buffalo milk fat (8.7µm) as compare to 3.8 µm for cow milk. A Higher percentage of large fat globules has been observed only in buffalo milk. Pre-cooled buffalo ghee was found to have a solid hard consistency score of 94.16%, which is higher than the pre-cooled cow ghee (score 85.12). It indicated that slowly pre-cooled cow ghee has less solidity (score 67.77) than slowly pre-cooled buffalo ghee (score 71.45). The smaller number of large crystals forming under slowly pre-cooled conditions would have a smaller surface area for the liquid fat to adhere, thus resulting in a higher ratio of liquid fat to the surface area at the crystal interfaces. Similar findings were reported by Bhaskar et al (2003). It is also evident from Table 3 that instrumentally measured hardness and work of shear was significantly lower when slow initial cooling was followed. Also, with the increasing degree of initial rapid cooling, the firmness increased which was in accordance with the sensorily perceived crystal size and its impact on the crystal network in the bulk of cooled product. The crystal size and the relative quantity of the liquid phase within the interstitial spaces also had an impact on the adhesiveness in terms of adhesive force on work of adhesion, both of which were lower in slowly cooled ghee. Further, within the rapidly cooled ghee a higher degree of initial cooling showed significantly greater stickiness. Thus, stickiness was directly related to the crystal surface area and the proportion of liquid fat at the interface.

Effect of type of ghee on sensory attributes of LFS

Ideally, LFS should possess glistening light-yellow color and be easy to spread on the bread slice i.e., neither too hard nor too soft. It should be smooth, non-sticky, and free from visible moisture, and have a butter-like pleasant flavor. The effect of the type of ghee on color and appearance (score) of LFS is depicted in Fig. 2. A significant (p<0.05) difference was observed in the score of color and appearance of cow ghee LFS and buffalo ghee LFS. The score of cow ghee LFS (7.70) was significantly (P<0.05) higher score than buffalo ghee LFS (7.13). It was recorded that cow ghee-based LFS had more scores due to light yellow to pale yellow colour and appearance. Naturally, butter spread has an occurrence of pale yellow colour was desired (Patange 2005). Further, cow milk ghee contains a higher amount of carotene pigment which was a lack in the buffalo milk ghee (Achaya 1997; Kumbhare et al. 2021). Colour units of the cow ghee (15.90) were about five times higher than those from buffalo ghee (3.56) as reported by Changade et al (2006).

From the Fig. 2., it is observed that the LFS prepared from cow ghee had a significantly (p<0.05) higher score for body and texture and spreadability than prepared from buffalo ghee. Figure 2 shows that the LFS created from cow ghee had a considerably (p 0.05) higher grade for body and texture and spreadability than prepared from buffalo ghee. This was attributable to a higher concentration of long-chain saturated fatty acids (16:0 and 18:0) in the coconut oil than in the fat from cow's milk. Buffalo milk ghee has a higher percentage of triglycerides (8.7%) than cow milk fat (4.9%). Triglycerides crystallize significantly earlier in buffalo milk fat than in cow milk fat because of this difference. As a result, the amount of crystallized fat in buffalo milk fat was significantly larger than that in cow milk fat, as reported by Bector (2002). The lower score of buffalo LFS was due to the hard body and texture of buffalo ghee. It was observed that at 5 °C temperature LFS prepared from buffalo ghee is harder than the LFS prepared from cow ghee. It was due to the cow milk ghee contains short-chain fatty acid (4:0 to 12:0) and unsaturated fatty acids which contributes softness to the fat whereas long-chain fatty acid

contribute to its hardness and long-chain acids were more in buffalo milk ghee. Due to this reason buffalo milk fat was harder than cow milk fat, as reported by Bector (2002). As the buffalo ghee was harder than the cow milk ghee, therefore, the LFS prepared from buffalo ghee was harder than the LFS prepared from cow milk ghee.

The flavor score of LFS prepared from buffalo milk ghee was significantly (P<0.05) higher (score 7.18) than from the cow milk ghee (score 6.70). Cow ghee LFS has a more pleasant flavor than buffalo ghee (Wadhwa and Jain 1984). In the present investigation, it was not reflected in LFS because the expectation of the flavor from LFS was essential to have a slight butter-like flavor rather than ghee flavor. Here it was also recorded that as in case of spread made from cow ghee had more pronounced ghee flavor resulted in decreased in flavor score and vice versa for buffalo ghee spread. The overall acceptability score of LFS prepared from cow ghee (score 7.90) was more than LFS prepared from buffalo milk ghee (score 7.23). It was due to the cow ghee LFS scored higher in the color and appearance score, body and texture score and spreadability score therefore overall acceptability score was also higher in the case of cow ghee LFS. Patange et al (2015) observed an overall acceptability score of LFS 7.90 which was higher than present findings. It was might be due to not addition of annatto butter color and diacetyl flavor in experimental LFS. According to Carolina and Luis Fernando (2020), the sensory profile of buffalo and cow ghee is predominantly characterized by a lactic aroma, followed by cooked and fatty overtones. As for the flavour, it was described as fatty, lactic, sweet, and fried, with greasy notes in the texture.

Effect of type of ghee on CIELAB color values, texture and physical properties

The CIELAB values of the sample exhibited significant (P<0.05) for both the product (Table 4). Being a natural while colour of buffalo colour milk fat, the L* values of BGS was higher than GGS. Similarly, all the textural properties were significantly varied for both the spread. The oiling off is the percentage (weight %) of oil a butter sample releases under specified conditions (Frede and Buchheim 1994; Kulkarni 2017) and it was significantly (P<0.0) affected by the type of ghee used for LFS (Table 4). The LFS prepared from the cow ghee has more oiling off (4.4%) than that of LFS prepared from the buffalo milk ghee (3.70%). It might be because cow ghee has a higher liquid fraction (83%) than buffalo ghee (62%) as reported by Armugham and Narayan (1979). Earlier research reports in the literature indicated oiling off of LFS were ranged from 3.1 to 42.03% (Bullock 1966, Prajapati et al. 1991, Reddy et al. 2000 and Bhaskar et al. 2003). Wheying off (%) of the CGS was higher than BGS (6.94%). It was also observed that CGS was least firm than the BGS. A similar observation was found by Dalaly et al. (1968) who reported that the body firmness was inversely related to the tendency of wheying off. Due to these reasons, cow ghee LFS had more wheying off ability.

The chemical analysis of CGS and BGS samples revealed that there was a non-significant difference in all the parameters under study. The fat content was ranged from 40.25 ± 0.10 % to 40.30 ± 0.05 %., while crude protein content was from 6.42 ± 0.01 % to 6.48 ± 0.04 % for CGS and BGS, respectively. The total solids, pH, and FFA for CGS were 59.28%, 5.7, and 0.278 % oleic acid respectively, whereas BGS had 59.31%, 5.7 and 0.352 % oleic acid, respectively.

Effect of addition of annatto on color and appearance score and diacetyl on flavour of LFS

The effect of addition of annatto on color and appearance of LFS, and effect of diacetyl on flavour of LFS is illustrated in Table 5 and Table 6 respectively. It was observed that the LFS prepared with the addition of the annatto color had obtained an extra score for color and appearance than LFS prepared without addition of annatto color in both the cow and buffalo ghee spread. Due to the addition of the annatto cow and buffalo

ghee, LFS imparted an attractive yellow color to the both the cow and buffalo ghee spread. Therefore, the LFS prepared with the addition of the annatto butter color (0.1%) has obtained more scores than of LFS prepared without addition of the annatto color. The LFS prepared with the addition of diacetyl flavor had obtained significantly (P<0.05) additional flavour score than the LFS prepared without addition of diacetyl flavor in both the LFS. From the above statement, it was concluded that diacetyl flavor incorporated in to spread had more acceptability.

Effect of exposure time on sensory attributes of LFS prepared from cow milk ghee and buffalo milk ghee at $35\pm1\%$ C

From Table 7, it is seen that the scores for color and appearance ranged from 6.90 to 7.70. The maximum score was recorded for CS_1 followed by CS_2 (score = 7.45). The color and appearance score was significantly (P<0.05) decline for both the spread with increasing exposal time. When the time for exposing the LFS was more than 20 min, the appearance of the product was observed to be dull similarly the color of the product was not accepted by the panel of judges. Considering the exposure time of cow ghee LFS, it was observed that the score for body and texture of CS₁ and CS₂ were at par with each other. Body and texture scores were significantly (p< 0.05) decreased from CS₁ to CS₄. It might be due to cow ghee contains a more short-chain of saturated fatty acids as compared to buffalo ghee. Short chain saturated fatty acids were responsible for smoothness in the product as reported by Bector (2002). In LFS prepared from buffalo ghee, the body and texture score was 7.30 at BS₁ which was decreased to 6.25 at BS₄. Body and texture score in buffalo ghee spread was less at BS₁ and BS₂ treatment as compared to cow ghee spread at CS₁ (7.90) and CS₂ (7.79). It was also observed that body and texture score in cow milk ghee spread from the CS₃ treatment rapidly decreased as compared to buffalo milk ghee spread. It might be due to cow ghee had less melting point and harder structure than the buffalo milk ghee as reported by Lakshminarayana and Rama Murthy (1985). The spreadability scores of LFS prepared from CGS at treatment CS₁, CS₂, CS₃, and CS₄ were 7.98, 8.10, 7.92, and 6.15, respectively whereas the spreadability score for BGS at BS₁, BS₂, BS₃ and BS₄ were 7.55, 7.65, 7.50 and 7.25 respectively. It was observed that the spreadability score was increased from CS₁ to CS₂ and BS₁ to BS₂ however, the increase in score was statistically at par with each other. As the time of exposure increased up to 10 min the CGS showed appreciable results for spreadability. After the CS₂ treatment as the exposure time increased, the LFS was shown a slight sticky appearance and resulted in significant decrease in spreadability score. As the body and texture score decreased spreadability of the CGS was also decreased. The reason behind this cow ghee had a lower melting point (32.20 °C) than buffalo ghee (33.64 °C), higher viscosity values were recorded with cow ghee (33.89) than buffalo ghee as reported by Changade et al (2006). The average liquid fraction was also more in the cow milk ghee (83%) than buffalo milk ghee (62%) as reported by Frankel et al (1958). The flavor score of CGS was increased up to CS2 and it was more pleasant than CS₁. It might be due to the fact that, the sample removed from refrigeration condition, flavoring compounds were not released in the required amount due to low temperature but in CS2 treatment the flavoring compounds may release in enough concentration and gives typical flavor so the score of ${\rm CS}_2$ is more than ${\rm CS}_1$. It was also observed that the flavor score was significantly (p<0.05) decreased from CS₃ to CS₄. In treatment CS₃ and CS₄, as the exposure time increased, the temperature of the cow ghee spread was increased simultaneously, and due to the increase in exposure time to a higher temperature, the intensity of flavor was decreased and thus flavor score was significantly decreased from CS₃ to CS₄. Similar results were found in the case of BGS. The overall acceptability score was also significantly (P<0.05) affected by the type of ghee used in LFS and exposed

time too. The maximum score for the overall acceptability was recorded to the spread of CS_1 however it was at par with CS_2 indicating that for 10 min the overall acceptability of CGS spread was different statistically. When the time passes the overall acceptability score was below 7 in case BGS. By considering all the sensory attributes it was recorded that spread could be consuming up to 10 min of exposing period after removing from the refrigerator.

Conclusions

Slowly pre-cooled cow ghee was more yellow in colour than rapidly pre-cooled ghee, whereas slowly pre-cooled buffalo ghee was creamy white than rapidly pre-cooled. Rapidly pre-cooled cow ghee had a highly smooth and paste-like texture compared to buffalo ghee, that had been rapidly pre-cooled. The overall acceptability score of cow milk ghee's low-fat spread was higher than buffalo milk ghee. Rapidly pre-cooled buffalo ghee has a higher consistency score than rapidly pre-cooled cow ghee, which may be due to the fat content. There was a non-significant difference in fat, crude protein, carbohydrates, ash and TS percentage in both cow and buffalo ghee LFS and free fatty acid content was more in buffalo ghee LFS than cow ghee LFS.

Declarations

Author Contribution: Patange D D: Project administration, methodology, data curation, formal analysis; Dhanake V V: writing-original draft preparation, writing-reviewing and editing; Deepak Chandran: writing-original draft preparation, writing-reviewing and editing; Radha: writing-original draft preparation, writing-reviewing and editing; Manoj Kumar: formal analysis, writing, supervision, resources, writing-reviewing and editing; Jose M Lorenzo: resources, supervision, writing-reviewing and editing.

Funding: Not available

Acknowledgement: Thanks to GAIN (Axencia Galega de Innovación) for supporting this review (grant number IN607A2019/01).

Conflicts of Interest: In this research, authors are declaring no conflict of interest.

Availability of data and material: All the data used in the manuscript are available in the tables and figures.

Code availability: Not applicable.

Ethics approval: Not applicable.

Consent to participate: All authors has given their full consent to participate.

Consent for publication: All authors has given their full consent for publication.

References

1. Abdeldaiem AM, Jin,Q, Liu R, Wang X (2014) Effects of pH values on the properties of buffalo and cow butter-based low-fat spreads. Grasas Y Aceites 65(3):117. https://dx.doi.org/10.3989/gya.0105141

- 2. Achaya KT (1997) Ghee, vanaspati and special fats in India. In Lipid technologies and applications, eds F.D Gunston and F.B. Padley.Marcel Dekker Inc., New York pp.369-390.
- 3. Ahmad N, Saleem M (2018) Studying heating effects on desi ghee obtained from buffalo milk using fluorescence spectroscopy. PloS One 13(5):p.e0197340. https://doi.org/10.1371/journal.pone.0197340
- 4. Ahmad N, Saleem M (2020) Characterization of cow and buffalo ghee using fluorescence spectroscopy. Int J Dairy Tech 73(1):191-201. https://doi.org/10.1111/1471-0307.12632
- 5. Amerine MA, Pangborn RM, Roesster EB (1965) Principles of sensory evaluation of food. Academic press, INC, New York, USA.
- Antony B, Sharma S, Mehta BM, Ratnam K, Aparnathi KD (2018) Study of Fourier transforms near-infrared (FT-NIR) spectra of ghee (anhydrous milk fat). Int J Dairy Tech 71(2):484-490. http://dx.doi.org/10.1111/1471-0307.12450
- 7. AOAC (2002) Official Methods for the Analysis of the Association of Official Analytical Chemist, 13th Ed., A.O.A.C. Washington, D.C.
- 8. Arumughan C, Narayana KM (1979) Grain formation in ghee (butter fat) as related to structure of triglycerides. J Food Sci Tech 16(2):242-247.
- 9. Bector BS (2002) Chemical characteristics of cow and buffalo milk fat in Lecture compendium on Advances in Fat Rich Dairy Products, Dairy Technology Division National Dairy Research Institute, Karnal, pp.6-10.
- 10. Bhaskar SM, Makker SK, Agrawala SP (2003) Ghee based butter- a technological brake through in dairy industries. Indian Food Ind 22(5):61-62.
- 11. Bhatia P, Sharma V, Arora S, Rao PS (2019) Effect of cholesterol removal on compositional and the physicochemical characteristics of anhydrous cow milk fat (cow ghee). Int J Food Prop 22(1):1-8.
- 12. Bullock DH (1966) A preliminary study of a new low fat dairy spread. Canadian Dairy Ice-Cream J 45(1):26.
- 13. Changade SP, Tambal RV, Kanoje RR (2006) Ghee making from high acidic milk. J Dairy Food Home Sci 25(2):97- 100.
- 14. Carolina PS, Luis Fernando RB (2020) Chemical, physicochemical, microbiological and sensory characterization of cow and buffalo ghee. Food Science Technology, Campinas, 402), 444-450,
- 15. Dalaly BK, Spurgeon KR, Seas SW (1968) Factors in body and water holding capacity of a low-fat spreadtype dairy product. J Dairy Sci 51:938.
- 16. Danthine S, Lefe´bure E, Trinh HN, Blecker C (2014) Effect of palm oil enzymatic interesterification on physicochemical and structural properties of mixed fat blends. J American Oil Chem Soc 91(9):1477–1487.
- 17. DeMan JM, Wood FM (1958) Oiling off properties of butter. J Dairy Sci 41:369-371.
- 18. Deshpande B, Thompkinson DK (2000) Table Spread A Review. Indian J Dairy Sci 53(3):154-168.
- 19. Duhan N, Sahu JK, Naik SN (2018) Temperature dependent steady and dynamic oscillatory shear rheological characteristics of Indian cow milk (Desi) ghee. J Food Sci Tech 55(10), 4059-4066.
- 20. El-Zeini HM (2006) Microstructure, rheological and geometrical properties of fat globules of milk from different animal species. Polish J Food Nutr Sci 15(2):147-154.
- 21. Food and Agriculture Organization of the United Nations FAO. (2019). Food and agriculture data. Rome: FAO. Retrieved from: http://www.fao.org/faostat/en/#compare

- 22. Frankel EN, Smith LM, Jack EL (1958) Chromatographic separation of some milk lipids. J Dairy Sci 41(4):483-491. https://doi.org/10.3168/jds.S0022-0302(58)90952-4
- 23. Frede E, Buchheim W (1994) Butter making and churning of blended fat emulsions. J Soc Dairy Tech 47(1):17-27.
- 24. FSSAI (2015). Manual of methods of analysis of foods (Oils and Fat), Manual no 02. Food safety and standards authority of India ministry of health and family welfare Government of India New Delhi.
- 25. Goyal GK (2002) Developments in the packaging of butter and ghee. In Lecture compendium on Advances in fat rich dairy products. Dairy Technology Division, National Dairy Research Institute Karnal, pp.144-148.
- 26. Hemati F (2018) Effect of microbial transglutaminase and gelatin on the rheological and sensory properties of low-fat cream cheese. J Food Process Preserv 10(1):47-60.
- 27. Kapadiya DB, Aparnathi KD (2018) Evaluation of commonly used herbs to enhance shelf life of ghee against oxidative deterioration. J Food Process Preserv 42(7):13658. https://doi.org/10.1111/jfpp.13658
- 28. Kumar A, Tripathi S, Hans N, Pattnaik HSN, Naik SN (2018) Ghee: Its Properties, Importance and Health Benefits. Lipid Univ 6:6-14.
- 29. Kulkarni CV (2017) Ultrasonic processing of butter oil (ghee) into oil-in-water emulsions. J Food Process Preserv 41(5):e13170.
- 30. Kumbhare S, Prasad W, Khamrui K, Wani AD, Sahu J (2021) Recent innovations in functionality and shelf-life enhancement of ghee, clarified butter fat. J Food Sci Tech 1-13.
- 31. Lakshminarayana M, Rama Murthy MK (1985) Cow and buffalo milk fat fractions. Part-I. Yield, physicochemical characteristics and fatty acid composition. Indian J Dairy Sci 38(4):256-264.
- 32. Londhe-Patil PB, Patange DD, Yadav MM, Kamble DK, Gaondhare SJ (2019) Selection of strawberry form for preparation of low-fat spread. Pharma Inn J 8(10):283-288.
- 33. Mehta M (2013) Consumption pattern and fatty acid composition of ghee. Food Sci Res J 4(2): 116-120.
- 34. Mehta BM, Kumar JA, Darji VB, Aparnathi KD (2018) Evaluation of different methods to monitor primary stage of oxidation of heat clarified milk fat (ghee). J Food Process Preserv 42(8):e13688.
- 35. Mor S, Sharma V, Minz PS, Nain N (2018) Effect of Season, Heat Clarification Temperature and Ripening of Cream on Color Parameters of Ghee. Int J Curr Microbiol App Sci 7(10):30-43.
- 36. Munro DS, Cant PAE, Mac GAKH, Illingworth D, Kennett A, Main AJ (1992) Concentrated milk fat products. In the 56 technology of Dairy products, ed. R. Early. Blackie and Sons Ltd, Glasgow, pp. 117-145.
- 37. Panchal B, Bhandari B (2020) Butter and Dairy Fat Spreads. In: Truong T., Lopez C., Bhandari B., Prakash S. (eds) Dairy Fat Products and Functionality. Springer, Cham. https://doi.org/10.1007/978-3-030-41661-4_21
- 38. Patange DD (2005) Studies on manufacture of low-fat spread using ghee. Thesis submitted to Mahatma Phule Krushi Vidhyapeeth Rahuri.
- 39. Patange DD, Patel AA, Kamble DK (2015) Quality of ghee based low fat spread under various processing condition. In souvenir of 6th International conference on emerging technologies in food and nutrition for health management (ICETF), Bangalore.
- 40. Prajapati PS, Gupta SK, Patel AA, Patil GR (1991) Processing of low-fat butter flavoured spread. J Food Sci Tech 28(4):208 211.

- 41. Prasanth KPK, Jeyarani T, Gopala KAG (2016) Physicochemical characteristics of phytonutrient retained red palm olein and butter-fat blends and its utilization for formulating chocolate spread. J Food Sci Tech 53:3060-3072. https://doi.org/10.1007/s13197-016-2279-8
- 42. Tekin-Cakmak ZH, Karasu S, Kayacan-Cakmakoglu S, Akman PK (2021) Investigation of potential use of by-products from cold-press industry as natural fat replacers and functional ingredients in a low-fat salad dressing. J Food Process Preserv 45(8):e15388.
- 43. Reddy YK, Laxminarayana M, Sarma KS, Ranganathan M, Kumar S (2000) Preparation of low-fat dairy spread using skimmed milk, chakka and butter. Indian J Dairy Sci 53(1):11-13.
- 44. Tekin-Cakmak ZH, Karasu S, Kayacan-Cakmakoglu S, Akman PK (2021) Investigation of potential use of by-products from cold-press industry as natural fat replacers and functional ingredients in a low-fat salad dressing. J Food Process Preserv 45(8):e15388.
- 45. Wadhwa BK, Jain MK (1984) Studies on lactone profile of ghee. I. Development of the method for the isolation of lactones. Indian J Dairy Sci 22(4):457-469.

Tables

Table 1. Physico-chemical properties of cow and buffalo ghee used in experiment

Type of ghee	Properties			
	Fat (%)	lodine value (mg/ g)	FFA (% oleic acid)	Slip point (°C)
Cow ghee	99.50±0.3	31.90°±0.7	2.40 ^a ±0.06	34.4°±0.31
Buffalo ghee	99.50±0.4	33.10 ^b ±0.7	2.80 ^b ±0.07	38.3 ^b ±0.44
Sem	0.03	0.07	0.06	0.14
CD	NS	0.15	0.37	1.33

Note: * mean ± SE of five replications within column followed by same letter were no significantly different at p< 0.05; NS= non-Significant

Table 2. Effect of method of rapid and slow pre-cooling on sensory properties of cow and buffalo milk ghee

Type of ghee and method of pre-cooling	Colour (score)*	Texture (score)*	Consistency (score)*
CG1	67.31 ^c ±0.26	5.28 ^a ±0.12	85.12 ^c ±0.80
CG2	73.92 ^d ±0.16	50.12 ^c ±0.10	67.75 ^a ±0.32
BG1	22.44 ^a ±0.70	11.88 ^b ±0.11	94.16 ^d ±0.71
BG2	30.36 ^b ±0.54	72.60 ^d ±0.73	71.45 ^b ±0.10
Sem	0.05	0.04	0.14
CD (5%)	0.15	0.13	0.26

Note: * mean \pm SE of five replications within column followed by same letter were no significantly different at p< 0.05.

Table 3. Effect of method of rapid and slow pre-cooling on CIELAB Color values and texture properties of cow and buffalo milk ghee

Type of ghee and method of precooling	L*	a*	b*	Hardness (g)	Work of shear (g/s)	Stickiness (g)	Work of adhesion (g/s)
CG1	43.99 ^a ±52	2.82°±0.05	16.49 ^c ±0.29	3024.8 ^c ±88.4	6723.0 ^c ±341.8	-481.6 ^c ±11.65	-103° ±3.30
CG2	49.50 ^b ±83	2.57 ^b ±0.05	18.65 ^d ±0.40	2175.0 ^a ±67.9	3222.6 ^a ±77.4	-202.4ª ±6.84	-39 ^a ±1.10
BG1	60.50° ±79	2.88 ^c ±0.06	6.52 ^a ±0.34	3480.6 ^d ±30.5	7919.0 ^d ±111.1	-588.6 ^d ±4.15	-147 ^d ±2.91
BG2	68.77 ^d ±28	1.73 ^a ±0.04	8.82 ^b ±0.25	2318.2 ^a ±103	3954.0 ^b ±73.5	-270.8 ^b ±6.84	-52 ^b ±1.06
Sem	0. 57	0.06	0.28	77.16	134.60	8.40	3.16
CD (5%)	1.76	0.17	0.85	237.76	414.75	25.88	9.75

Note: * mean \pm SE of five replications within column followed by same letter were no significantly different at p< 0.05.

Table 4. Effect of type of ghee on CIELAB color values, texture and physical properties of LFS

Type of ghee	L*	a*	b*	Hardness (g)	Work of shear (g/s)	Stickiness (g)	Work of adhesion (g/s)	Oiling off (%)	Wheying off (%)	
CGS	77.61	1.09	32.67	643.8	2035.4	-81.6	-17.8 ±0.17	4.07	7.63 ±0.05	
	±0.62	± 0.03	±0.27	±4.94	±35.83	±0.54		±0.02		
BGS	82.35	1.86	21.89	791.4	3130 ± 26.96	-97.6	-27.1 ±0.32	3.70	6.93	
	±0.40	±0.06	±0.38	±7.06	20.70	± 0.77		±0.06	±0.02	
p value	0.020	0.001	6.60E- 06	5.95E-05	4.397E- 06	6.247E-05	2.962E- 06	0.0282	0.00024	
(p<0.05)			00		00		00			

Table 5. Effect of addition of annatto color on color and appearance score of LFS

Sample	Treatment	Mean score*	SEm	CD	CV
Without addition of annatto	Cow ghee spread	7.70 ^b ±0.05 0.06 0.		0.20	1.85
	Buffalo ghee spread	7.13°±0.07			
With addition of annatto	Cow ghee spread	8.50 ^d ±0.01			
	Buffalo ghee spread	8.10 ^c ±0.03			

Note: * mean \pm SE of five replications within column followed by same letter were no significantly different at p< 0.05

Table 6. Effect of diacetyl flavor on flavor score of LFS

Sample	Treatment	Mean score*	SEm	CD	CV
Without addition of diacetyl flavor	Cow ghee spread	6.70 ^a ±0.02	0.02	0.06	0.63
	Buffalo ghee spread	7.18 ^b ±0.01			
With addition of diacetyl flavor	Cow ghee spread	7.30 ^c ±0.03			
	Buffalo ghee spread	8.15 ^d ±0.01			

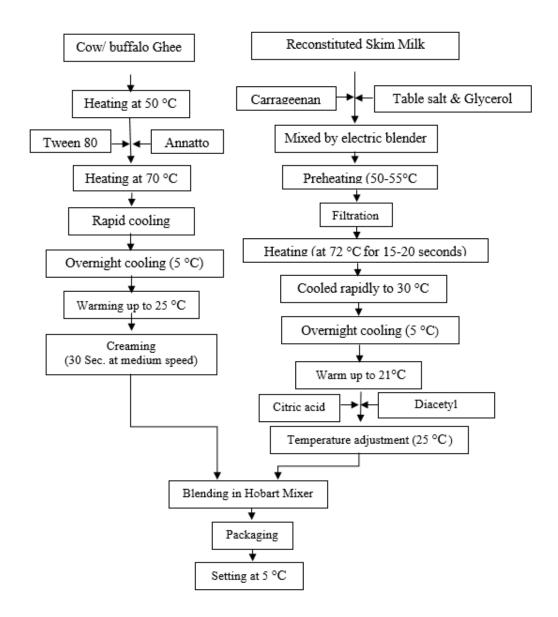
Note: * mean \pm SE of five replications within column followed by same letter were no significantly different at p< 0.05.

Table 7. Effect of exposure time on sensory properties of LFS prepared from cow milk ghee and buffalo milk ghee

Treatment	Color and appearance	Body and texture	Spreadability	Flavor	Overall acceptability
CS ₁	7.70 ^f ±0.05	7.90 ^g ±0.07	7.98 ^g ±0.02	6.70 ^c ±0.09	7.95 ^h ±0.05
BS ₁	7.13 ^d ±0.01	7.35 ^e ±0.06	7.55 ^d ±0.03	7.18 ^f ±0.04	7.23 ^e ±0.03
CS ₂	7.45 ^e ±0.02	7.79 ^f ±0.06	8.10 ^h ±0.07	6.76 ^d ±0.02	7.80 ^g ±0.09
BS ₂	7.00 ^c ±0.11	7.10 ^d ±0.11	7.65 ^e ±0.10	7.74 ^g ±0.10	7.00 ^d ±0.10
CS ₃	7.00 ^c ±0.08	7.10 ^d ±0.04	7.92 ^f ±0.03	6.11 ^b ±0.08	7.60 ^f ±0.08
BS ₃	6.90 ^b ±0.11	6.70 ^c ±0.07	7.50 ^c ±0.14	6.85 ^e ±0.01	6.25 ^b ±0.02
CS ₄	6.55 ^a ±0.04	5.25 ^a ±0.01	6.15 ^a ±0.07	6.00 ^a ±0.11	6.55 ^c ±0.03
BS ₄	6.75 ^a ±0.02	6.25 ^b ±0.09	7.25 ^b ±0.07	6.66 ^d ±0.9	6.00 ^a ±0.01
CD	0.21	0.19	0.22	0.22	0.18
SEm	0.07	0.07	0.08	0.08	0.06
CV	2.17	2.10	2.33	2.56	1.94

Note: * mean ± SE of five replications within column followed by same letter were no significantly different at p< 0.05.

Figures



Flow diagram for LFS preparation from cow and buffalo ghee

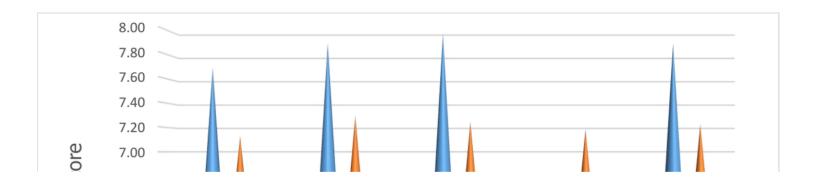


Figure 2

Effect of type of ghee on sensory attributes (score) of low-fat spread