Comparative Effect of Cooling on the Physico-chemical-sensory Properties of Ghee from Cow and Buffalo Milk, and Evaluation of the Low-fat Spread Prepared from Cow and Buffalo Milk Ghee

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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 flavor, 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 color than rapidly pre-cooled cow ghee, whereas slowly pre-cooled buffalo ghee has creamish white color and rapidly pre-cooled buffalo ghee had white color. 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 color and appearance, body and texture, spreadability, and overall acceptability, as compared to buffalo ghee LFS. Chemically, it was observed that both the LFS differ in FFA content, while they had similar fat, protein, carbohydrate, ash, and total solids content, as well as pH. Oiling off and wheying off were found higher in cow ghee LFS over buffalo ghee LFS. Color, appearance, and flavor scores were found improved by the addition of butter annatto color and diacetyl flavor respectively. Color and appearance, body and texture, spreadability, and overall acceptability scores were higher for cow ghee LFS when subjected to 35 °C for 10, 20, and 30 min. It was found that after 10 min of exposure to 35 °C, the physical qualities of both LFS were unchanged, but the sensory properties diminished as time passed.

Keywords Low-fat spread · Cow ghee · Buffalo ghee · Pre-cooling · Exposure time

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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 USA, Argentina, and Paraguay (Food and Agriculture Organization of the United Nations – FAO 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 paratha, 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% is water (Patange et al. 2015). 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, 2020).

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 those 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.

To make effective
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 °C and slowly pre-cooled to 20 °C

Fig. 1 Flow diagram for LFS preparation from cow and buffalo ghee

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 twofold muslin cloth, pasteurizing at 72 °C for 15–20 s, and then cooling to 20 °C and storing overnight in the refrigerator.
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 s 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 s 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 Color and Flavor on Qualities of LFS**

The effect of the addition of 0.1% of annatto color and 4 ppm of diacetyl flavor on LFS was evaluated for color and flavor 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.

| Code | Description                                      |
|------|--------------------------------------------------|
| 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                   |
| BS4  | Buffalo 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 contents 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 a 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 were estimated as suggested by deMan and Wood (1958).

**Sensory Evaluation**

Sensory qualities of buffalo and cow ghee, which are commonly utilized in dairy products, were analyzed using the method of sensory profile by a multidimensional approach (Patanje 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 color (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 appearance, spreadability, body and texture, flavor, 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.

**Color Analysis**

Color 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 color 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 Systems, UK) provided with texture expert exceed software. The sample was carefully filled up in a cuboidal polypropylene tub (11.0*6.4*3.5 cm) 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 of 25.0 mm by a 30° PERSPEX CONE PROBE. The other test conditions included measuring force in compression mode, re-test period of 5.0 mm/s, test speed of 2.5 mm/s, and post-test speed of 10 mm/s. 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 $P < 0.05$ level, the Duncan analysis was used.

**Results and Discussion**

**Physico-chemical Properties of Cow and Buffalo Milk Ghee**

The physico-chemical properties of cow and buffalo milk ghee are 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/g 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) contents 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 flavor typified 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 buffalo ghee (38.3 °C). Lakshminarayana and Rama Murthy (1985) reported that 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, and 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 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 cow ghee pre-cooled slowly had shifted towards pronounced yellow color compared to pre-cooled rapidly (Table 2). The observed trend in color of ghee was also reflected in terms of $L^*$, $a^*$, and $b^*$ color 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

| Table 1 | Physico-chemical properties of cow and buffalo ghee used in experiment |
|---------|---------------------------------------------------------------|
| Type of ghee | Properties |
|           | Fat (%) | Iodine value (mg/g) | FFA (% oleic acid) | Slip point (°C) |
| Cow ghee  | 99.50±0.3 | 31.90±0.7 | 2.40±0.06 | 34.4±0.31 |
| Buffalo ghee | 99.50±0.4 | 33.10±0.7 | 2.80±0.07 | 38.3±0.44 |
| Sem       | 0.03     | 0.07     | 0.06    | 0.14    |
| CD        | NS       | 0.15     | 0.37    | 1.33    |

Mean±SE of five replications within column followed by same letter were not 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 | Color (score)* | Texture (score)* | Consistency (score)* |
| CG1     | 67.31±0.26 | 5.28±0.12 | 85.12±0.80 |
| CG2     | 73.92±0.16 | 50.12±0.10 | 67.75±0.32 |
| BG1     | 22.44±0.70 | 11.88±0.11 | 94.16±0.71 |
| BG2     | 30.36±0.54 | 72.60±0.73 | 71.45±0.10 |
| Sem     | 0.05     | 0.04     | 0.14    |
| CD (5%) | 0.15     | 0.13     | 0.26    |

*Mean±SE of five replications within column followed by same letter were not significantly different at $P < 0.05$
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 color. 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, 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 scores of cow ghee and buffalo ghee were significantly ($P < 0.05$) affected by the method of pre-cooling (Table 2). The texture of cow ghee that had been rapidly pre-cooled (score 5.28) was smoother and pastier than that of 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 coarse and bigger than cow milk ghee. Elzein et al. (2006) also reported much average globule size of buffalo milk fat (8.7 µm) as compared 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 were 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 than the score of buffalo ghee LFS (7.13). It was recorded that cow ghee-based LFS had more scores due to light yellow to pale yellow color and appearance. Naturally, butter spread has an occurrence of pale yellow color which was desired (Patange 2005). Further, cow milk ghee contains a higher amount of carotene pigment which was lacking in the buffalo milk ghee (Achaya 1997; Kumbhare et al. 2021). Color units of the cow ghee (15.90) were about five times higher than those of buffalo ghee (3.56) as reported by Changade et al. (2006). From 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

### 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 pre-cooling | $L^*$ | $a^*$ | $b^*$ | Hardness (g) | Work of shear (g/s) | Stickiness (g) | Work of adhesion (g/s) |
|---------------------------------------|------|------|------|--------------|------------------|--------------|---------------------|
| CG1                                   | 43.99±52 | 2.82±0.05 | 16.49±0.29 | 3024.8±88.4 | 6723.0±341.8 | −481.6±11.65 | −103±3.30 |
| CG2                                   | 49.50±83 | 2.57±0.05 | 18.65±0.40 | 2175.0±67.9 | 3222.6±77.4 | −202.4±6.84 | 39±1.10 |
| BG1                                   | 60.50±79 | 2.88±0.06 | 6.52±0.34 | 3480.6±30.5 | 7919.0±111.1 | −588.6±4.15 | −147±2.91 |
| BG2                                   | 68.77±28 | 1.73±0.04 | 8.82±0.25 | 2318.2±103 | 3954.0±73.5 | −270.8±6.84 | −52±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 |

Mean ± SE of five replications within column followed by same letter were not significantly different at $P < 0.05$.
texture and spreadability than that 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 which 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 contributes 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 that from the cow milk ghee (score 6.70). Cow ghee LFS has a more pleasant flavor than buffalo ghee LFS (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. Spread created from cow ghee had a more prominent ghee flavor, resulting in a fall in ghee flavor score, while spread made from buffalo ghee had an increase in ghee flavor. The overall acceptability score of LFS prepared from cow ghee (score 7.90) was more than that of 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 flavor, 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 color of buffalo color milk fat, the L* value of BGS was higher than that of GGS. Similarly, all the textural properties were significantly varied for both the spread. The

| Type of ghee | L*       | a*     | b*     | Hardness (g) | Work of shear (g/s) | Stickiness (g) | Work of adhesion (g/s) | Oiling off (%) | Whacking off (%) |
|--------------|----------|--------|--------|--------------|--------------------|----------------|------------------------|---------------|-----------------|
| CGS          | 77.61±0.62 | 1.09±0.03 | 32.67±0.27 | 643.8±4.94 | 2035.4±35.83 | -81.6±0.54 | -17.8±0.17 | 4.07±0.02 | 7.63±0.05 |
| BGS          | 82.35±0.40 | 1.86±0.06 | 21.89±0.38 | 791.4±7.06 | 3130±26.96 | -97.6±0.77 | -27.1±0.32 | 3.70±0.06 | 6.93±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             |

Table 4: Effect of type of ghee on CIELAB color values and texture and physical properties of LFS.
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 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 that oiling off of LFS ranged from 3.1 to 42.03% (Bullock 1966; Prajapati et al. 1991; Reddy et al. 2000; Bhaskar et al. 2003). Wheying off (%) of the CGS was higher than that of 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 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 Flavor of LFS

The effect of addition of annatto on color and appearance of LFS and effect of diacetyl on flavor of LFS are 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 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 LFS prepared without addition of the annatto color. The LFS prepared with the addition of diacetyl flavor had obtained significantly ($P < 0.05$) additional flavor 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 into spread had more acceptability.

### Effect of Exposure Time on Sensory Attributes of LFS Prepared from Cow Milk Ghee and Buffalo Milk Ghee at 35 ± 1 °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 CS1 followed by CS2 (score = 7.45). The color and appearance score was significantly ($P < 0.05$) decrease for both the spread with increasing exposure 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 scores 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 which 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 treatments as compared to cow ghee spread at CS1 (7.90) and CS2 (7.79). It was also observed that body and texture

| Sample                  | Treatment       | Mean score* | SEm  | CD   | CV  |
|-------------------------|-----------------|-------------|------|------|-----|
| Without addition        | Cow ghee spread | 7.70±0.05   | 0.06 | 0.20 | 1.85|
|                         | Buffalo ghee spread | 7.13±0.07 | | |
| With addition           | Cow ghee spread | 8.50±0.01   |      |      |     |
|                         | Buffalo ghee spread | 8.10±0.03 | | |

*Mean±SE of five replications within column followed by same letter were not significantly different at $P < 0.05$
score in cow milk ghee spread from the CS₁ 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 scores 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 showed 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 is 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 with buffalo ghee as reported by Changade et al. (2006). The average liquid fraction was also more in the cow milk ghee (83%) than in buffalo milk ghee (62%) as reported by Frankel et al. (1958). The flavor score of CGS was increased up to 10 min, the CGS showed appreciable results statistically at par with each other. As the time passes, the overall acceptability score 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 color 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, which had been rapidly pre-cooled. The overall acceptability score of cow milk ghee’s low-fat spread was higher than that of buffalo milk ghee. Rapidly pre-cooled buffalo ghee has a higher consistency score than rapidly pre-cooked 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 in cow ghee LFS.

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### 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;

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**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 |
|-----------|----------------------|-----------------|---------------|--------|-----------------------|
| CS₁       | 7.70 ± 0.05          | 7.90 ± 0.07     | 7.98 ± 0.02   | 6.70 ± 0.09 | 7.95 ± 0.05          |
| BS₁       | 7.13 ± 0.01          | 7.35 ± 0.06     | 7.55 ± 0.03   | 7.18 ± 0.04 | 7.23 ± 0.03          |
| CS₂       | 7.45 ± 0.02          | 7.79 ± 0.06     | 8.10 ± 0.07   | 6.76 ± 0.02 | 7.80 ± 0.09          |
| BS₂       | 7.00 ± 0.11          | 7.10 ± 0.11     | 7.65 ± 0.10   | 7.74 ± 0.10 | 7.00 ± 0.10          |
| CS₃       | 7.00 ± 0.08          | 7.10 ± 0.04     | 7.92 ± 0.03   | 6.11 ± 0.08 | 7.60 ± 0.08          |
| BS₃       | 6.90 ± 0.11          | 6.70 ± 0.07     | 7.50 ± 0.14   | 6.85 ± 0.01 | 6.25 ± 0.02          |
| CS₄       | 6.55 ± 0.04          | 5.25 ± 0.01     | 6.15 ± 0.07   | 6.00 ± 0.11 | 6.55 ± 0.03          |
| BS₄       | 6.75 ± 0.02          | 6.25 ± 0.09     | 7.25 ± 0.07   | 6.66 ± 0.09 | 6.00 ± 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                  |

Mean ± SE of five replications within column followed by same letter were not significantly different at *P < 0.05*
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