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Understanding the effect of milk composition and milking season on quality characteristics of *chhana*

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Abstract

The quality characteristics of *chhana* varied due to the milk composition (cow-, buffalo-, and mixed- milk) which in turn was affected by the milking season (summer and winter). Upon heating and acidification of milk samples water holding phenomena and denatured protein association within and with other components lead to variation in both macroscale properties (color, texture, and rheology) and molecular bonding patterns (FTIR character). Yield, lightness (L^* value), textural firmness, and elastic modulus of *chhana* increased with increasing proportion of buffalo milk in mixed milk due to higher total solids and less moisture content in both the seasons. Total protein, fat, water, and interaction between them and extent of hydrogen bonding significantly affected the rheological and textural properties of *chhana* samples.

KEYWORDS

Chhana, FTIR, heat-acid induced coagulation, rheology, texture

1 | INTRODUCTION

Milk is a biological secretion by the mammals derived from complete milking of dairy animals. It contains all the essential components for growth in the form of six major nutrients in multi-dispersed phases: water, fat, protein, lactose, minerals, and vitamins (Aneja, Mathur, Chandan, & Banerjee, 2002). Composition of milk is of great importance for the dairy industry due to its diverse nature. Compositional variation is also very important for meeting specific processing requirements to suit the processors and consumers requirements. Milk components especially casein, fat, and calcium, influence the product manufacturing process (product yield, composition, and quality) (Fox, Guinee, Cogan, & McSweeney, 2000). Variation in species of milk animal, stage of lactation, feed, and seasonal variations have significant effect on the composition and properties of milk components (Malacarne, Martuzzi, Summer, & Mariani, 2002; Pretto, De Marchi, Penasa, & Cassandro, 2013). Concentrations of protein and fat tend to be higher at the initial and final stages of the lactation period compared to the middle period (Bernabucci, Acetera, & Onchi, 2002). Protein and fat content of milk is generally higher during the winter, fall months and lowest during the spring, and summer months. This variation is mainly due to change in climatic conditions and feeding regime (Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009; Larsen et al., 2010; Ozrenk & Selcuk, 2008). Bertocchi et al. (2014) reported that factors like physiology, individual metabolism of animal, humidity, photoperiod, and somatic cell count (*leukocytes*) also affect milk composition.

In India, milk industry relies mainly on milk obtained from either cow or buffalo or mixed milk obtained by mixing both of them. India is the world's largest producer of milk (165.4 million tons) in which

Abbreviations: a^*, red/green; b^*, yellow/blue; BM, buffalo milk; *Chhana*, Indian cottage cheese analogue; CM, cow milk; FTIR, Fourier Tranform Infrared Spectroscopy; G', storage modulus; G'', loss modulus; G^*, complex modulus; L^*, lightness/darkness; LVE, linear viscoelastic range; MM, mixed milk; tanδ, phase angle; β-LG, beta lactoglobulin; κ-CN, kappa casein.

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buffalo- and cow- milk share are about 49.2 and 46.2%, respectively (NDDB data, 2018). In buffalo milk all the major components are present in higher amount than cow milk (Chandan, 2007a, 2007b; Fox et al., 2000). Almost all dairy products manufactured utilizing cow milk, can be prepared using buffalo milk as well. Variation in composition of buffalo and cow milk subsequently influences the physico-chemical, rheological, and textural properties of the final product (Chandan, 2007a, 2007b). Buffalo milk based coagulated products exhibit greater firmness with elasticity due to the stronger interactions of proteins (caseins and denatured whey proteins; Lucey & Singh, 1997; Lucey, Munro, & Singh, 1998). Higher fat content in buffalo milk results in a product with rich mouth-feel and higher firmness that is mainly due to the interactions between the milk fat globule membrane and the protein matrix in coagulated products (Chandan, 2007a, 2007b). Calcium content in buffalo milk has been estimated to be higher in comparison to cow milk, which influences the processing conditions and yield of certain dairy products (Ahmad et al., 2008; Guo & Hendricks, 2010). The innate natural properties of buffalo milk render it a suitable and appropriate base material to manufacture indigenous dairy products (curd, *khoa*, *kheer*, *paneer*, and clarified butter) in India. On contrary, cow milk yields a soft coagulum adequate for producing *channa* based sweetmeat products (*rasagolla*, *sandesh*, *rasamalai*, and *chumchum*; Chandan, 2007a, 2007b).

Chhana, commonly known as Indian cottage cheese, is referred to as the milk solids obtained on acid induced coagulation of heated whole milk. *Chhana* production comprises of casein coagulation with entrapped water, fat, and water soluble components by adding a suitable coagulant (citric acid, lactic acid, calcium lactate, etc.) to hot milk. Higher total solids, calcium, protein contents, and curd tension in buffalo milk results in hard, chewy, rubbery, and uneven textured *chhana* as compared to cow milk, which renders it unsuitable for the manufacturing of superior quality sweetmeats (Bandyopadhyay, Chakraborty, & Raychaudhuri, 2006). Buffalo milk is commercially more viable as maintenance of buffaloes is cheaper and disease incidence is lower as compared to cows. Hence, it is essential to optimize the extent to which buffalo milk can be mixed with cow milk to produce *chhana* of acceptable physico-chemical and sensorial attributes. There have been a number of attempts to develop and modify the manufacturing process of *chhana* (Kumar, Gupta, & Patil, 2005; Kumar, Kumar, Gupta, & Birendra, 2012; Sen & De, 1984). Systematic research work based on molecular level understanding of the heat-acid induced coagulation process of *chhana* production with cow-, buffalo-, and mixed-milk systems has not yet been reported in scientific literature. The objective of the present work is to understand the correlation between molecular level bonding pattern development and macroscale properties (textural and rheological) for heat-acid induced milk coagulation process using cow, buffalo, and mixed milk. Experiments were carried out in summer and winter to study the influence of climatic conditions on milk as well. Physico-chemical, textural, rheological, and spectral properties were measured to explain the nature of conversion from milk to *chhana*.

2 | MATERIALS AND METHODS

2.1 | Materials

Fresh raw buffalo milk (BM) (Murrah breed, 14–15 L/day production) and fresh raw cow milk (CM) (Jersey breed, 10–12 L/day production) was procured from a local dairy farm from Dhanas, Chandigarh (India) in both the seasons (summer: May–June; winter: January–February). Two kilo gram of milk samples were collected, transported to the laboratory, and processed within 1 hr of milk collection. Mixed milk (MM) samples were prepared by mixing BM and CM in desired proportions, respectively (75:25—MM_1, 50:50—MM_2, 25:75—MM_3 by weight).

1% citric acid (w/v) solution was used as a coagulant. Anhydrous citric acid pellets were supplied by Loba Chemie Pvt. Ltd. (Mumbai) with following specifications: molecular weight—192.13; assay—min. 99.5%.

2.2 | Analysis of raw milk and *chhana*

2.2.1 | Determination of pH, fat, protein, solid-not-fat (SNF), moisture, total solids, and lactose contents of raw milk

pH was recorded using a bench top digital pH meter (LJ-111, Lab Junction). Total solids, fat, protein, solid-not-fat (SNF), lactose, and pH of raw milk were measured according to Bureau of Indian Standards (1981). All measurements were conducted in triplicate, and the chemicals of analytical grade were used.

2.2.2 | Determination of moisture content and total solids of *chhana* and whey

Moisture, fat, and protein contents were measured according to Bureau of Indian Standards (1981). All measurements were conducted in triplicate.

2.3 | *Chhana* making

Chhana was prepared from the BM, CM, and MMs (MM_1, MM_2, and MM_3) following the method described by Kundu and De (1972) with certain modifications. Five hundred gram milk was transferred to 1 L beaker and heated on a gas burner for 7–10 min until temperature reached 90 ± 1°C followed by cooling to 70 ± 1°C in 5–7 min. Seventy gram citric acid (1%, w/v) solution was then added slowly with constant stirring. Coagulation took place immediately and the coagulated mass along with the whey was left undisturbed for 10 min. Whey was separated using muslin cloth and the coagulum was pressed manually and hung for about 10 min until its moisture content reached between 56 and 58% (wet basis). It was then collected in a petridish, wrapped with aluminum foil to prevent moisture loss and was stored in a desiccator for further analysis. *Chhana* manufacturing process was carried out in three different days in both the seasons for exploring the effect of seasonal variations and repeatability. Everyday samples were prepared in triplicate of 0.5 kg batch for each experiment to gather the variability between samples due to manufacturing process and identifying differences between sample formulations. All the experiments (except FTIR) were performed within 3 hr of sample preparation.

2.3.1 | *Chhana* yield

The *chhana* yield was expressed as:

$$\%\text{Yield} = \frac{\text{Mass of chhana produced}}{\text{Mass of milk}} \times 100 \quad (1)$$

2.4 | Visual color

Color of *chhana* samples was measured by using a Hunterlab colorflex spectrophotometer (Hunter Associate Lab, Reston, VI) at room temperature (25 ± 2°C) (Rodriguez-Aguilera, Oliveira, Montanez, & Mahajan, 2011a, 2011b). Freshly prepared *chhana* was scooped into sample cup (5.9 cm internal diameter × 3.8 cm height) and spread evenly on the measuring surface. For all *chhana* samples, triplicate color measurements were performed by rotating the sample cup at three different angles, and the average values are reported.

2.5 | Texture evaluation

Spreadability test was applied to the *chhana* samples using a spreadability rig unit of Texture Analyzer, TA.XT. Plus (Stable Microsystems, U.K; Rodriguez-Aguilera et al., 2011a, 2011b). Before testing, the *chhana* sample was filled in the sample holder of the spreadability fixture using spatula and the holder was fixed into the base holder centered under the corresponding measuring probe. Instrument software (TEE 32) was used to analyze textural data (force vs. time) and textural parameters were recorded (hardness, shear work, stickiness, and adhesion work). All the measurements were replicated three times, and average values were used.

2.6 | Rheology

Small angle oscillatory shear tests were considered for measuring viscoelastic properties of chhana (Gunasekaran & Ak, 2000). Approximately 1 g of freshly prepared *chhana* was scooped out and placed on the measuring plate of rheometer (Anton Paar, MCR-102). Parallel plate geometry (PP25, 25 mm diameter) at a gap of 1 mm was used for carrying out the rheological measurements. Amplitude sweep tests were carried out at room temperature (25° ± 2°C) to establish the linear viscoelastic (LVE) range. Preliminary amplitude sweep tests showed a linear behavior up to 1% strain and hence, a constant strain of 1% was used for testing *chhana* samples for frequency sweep and time sweep test conditions. Frequency sweep tests at 1% strain within frequency range of 1–100 rad/s were conducted at room temperature (25° ± 2°C). Time sweep tests were performed at room temperature (25° ± 2°C) for 500 s at 1% strain and 10 rad/s frequency. All the measurements were replicated three times to observe the repeatability, and best of these three was analyzed further.

2.7 | FTIR analysis

The IR spectra were acquired using spectrophotometer (Spectrum RX, Perkins Elmer). Milk and *chhana* samples were analyzed using the attenuated total reflection (ATR) diamond crystal cell (path length: 1.66 μm). The spectra acquisition was performed in the wavenumber range of 4,000–667 cm^{-1}at 1 cm^{-1} resolution, 0.2 $cm.s^{-1}$ scan speed. Three scans of each sample using 0.01 mg sample were performed and a normalized spectra was further used to analyze. A blank ATR crystal was used to measure the reference spectra (distilled water as background) with an interval of three samples for rationing the sample spectra. After each sample scan, cleaning, and dry wiping of the ATR crystal was done using a tissue paper and the presence of any remains was spectrometrically examined.

2.8 | Statistical analysis

All data (except rheological and FTIR data) were presented as means ± standard error of the means. The analysis of variance (ANOVA) was performed to evaluate the significance of variations in milk and *chhana* samples with the Microsoft Office statistical package (Microsoft Excel, MS Office 7.0). Tukey's posthoc test was used to detect the significance of differences among the treatments at 95% confidence interval. Different superscripts were added to the values to elaborate the significant differences ($p < .05$).

3 | RESULTS AND DISCUSSION

3.1 | Preliminary analysis of raw milk

Moisture content, total solids (TS), fat, solid nonfat (SNF), protein, and lactose contents of raw milk are reported in Table 1. All the major nutrients were in higher proportions in BM than CM. Similar observations were reported by Wahid and Rosnina (2011). Milk properties are significantly impacted by heat stress (Bernabucci et al., 2010). Moisture content was higher in summer milk samples as compared to winter milk samples. Concentration of fat and protein in both the milks were found to be lower by approximately 0.4 and 0.2%, respectively in summer season. Similar observations were made by Renna,

TABLE 1 Composition of buffalo milk (BM), cow milk (CM), and mixed milk (MM) samples (BM:CM ratio in MM_1-75:25; MM_2-50:50; MM_3-25:75) (a) in summer; (b) in winter

	SUMMER				
(a) Constituent (%)	**BM**	**MM_1**	**MM_2**	**MM_3**	**CM**
Moisture content	84.95 ± 0.54^{e}	85.07 ± 0.16^{d}	85.42 ± 0.32^{c}	85.83 ± 0.33^{b}	86.12 ± 0.17^{a}
Total solids	14.67 ± 0.34^{a}	14.49 ± 0.22^{b}	13.96 ± 0.31^{c}	13.52 ± 0.4^{d}	12.92 ± 0.13^{e}
Fat	5.88 ± 0.22^{a}	5.72 ± 0.19^{b}	5.23 ± 0.31^{c}	4.96 ± 0.26^{d}	4.55 ± 0.29^{e}
SNF	8.82 ± 0.14^{a}	8.76 ± 0.22^{b}	8.65 ± 0.15^{b}	8.53 ± 0.27^{c}	8.36 ± 0.31^{d}
Protein	4.51 ± 0.29^{a}	4.45 ± 0.15^{b}	4.37 ± 0.12^{bc}	4.29 ± 0.25^{c}	4.18 ± 0.24^{d}
Lactose	3.80 ± 0.11^{d}	3.83 ± 0.18^{c}	3.85 ± 0.26^{b}	3.87 ± 0.29^{ab}	3.89 ± 0.25^{a}
	WINTER				
(b) Constituent (%)	**BM**	**MM_1**	**MM_2**	**MM_3**	**CM**
Moisture content	84.18 ± 0.3^{e}	84.54 ± 0.31^{d}	84.94 ± 0.34^{c}	85.63 ± 0.1^{b}	85.89 ± 0.47^{a}
Total solids	15.23 ± 0.25^{a}	14.88 ± 0.4^{b}	14.42 ± 0.47^{c}	14.05 ± 0.3^{d}	13.71 ± 0.33^{e}
Fat	6.30 ± 0.12^{a}	5.92 ± 0.27^{b}	5.62 ± 0.19^{c}	5.31 ± 0.21^{d}	5.07 ± 0.10^{e}
SNF	8.89 ± 0.11^{a}	8.83 ± 0.27^{b}	8.76 ± 0.42^{c}	8.68 ± 0.39^{d}	8.62 ± 0.12^{e}
Protein	4.61 ± 0.21^{a}	4.48 ± 0.14^{ab}	4.40 ± 0.19^{b}	4.32 ± 0.26^{c}	4.27 ± 0.10^{d}
Lactose	3.69 ± 0.14^{e}	3.85 ± 0.2^{d}	3.91 ± 0.12^{c}	3.95 ± 0.18^{b}	3.99 ± 0.16^{a}

Note: Results represented as mean values with their standard deviation; means with different superscripts in rows differ significantly ($p < .05$).

Lussiana, Malfatto, Mimosi, and Battaglini (2010). They reported a decrease in milk yield, fat, and protein contents during the summer months. Buffalo milk had maximum TS, protein and fat content in both the seasons. Milking season affects feed availability and grazing area for the milch animals, which in turn affects milk composition and production (Bernabucci, Lacetera, Ronchi, & Nardone, 2002). The compositional differences influenced the physico-chemical properties. The changes in milk composition (mainly protein and fat) content with season may be due to the changes in protein synthesis and blood plasma lipids, which also vary with animal's feeding regime (Bernabucci et al., 2015; Bertocchi et al., 2014).

3.2 | Yield

Composition of milk along with other factors such as extent of heat treatment, type and strength of coagulant, whey protein and lactose losses incurred after coagulation and percentage of moisture present in the final product affected the yield of *chhana* (Bhattacharya, Mathur, Srinivasan, & Samlik, 1971; Chandan, 2007a, 2007b; Sachdeva & Singh, 1988a, 1988b). MM_1 sample derived highest yield of *chhana* in both summer and winter season compared to other milk samples, while CM sample showed lowest yields in both seasons (Table 2). It can be due to (a) higher amount of denatured whey proteins- beta lactoglobulin (β-LG) content due to presence of CM proteins and subsequent interaction with casein micelle (Sindhu & Arora, 2011), (b) higher BM protein percentage hence, maximum number of the kappa casein (κ-CN)-denatured β-LG complex assembly sites (Singh, 2004; Singh & Fox, 1987), and (c) surface area for BM fat globules (fat globule size) was maximum leading to maximum yield (Ménard et al., 2010). Hence, interaction sites for denatured CM whey proteins in the MM samples increased. As CM whey proteins (β-LG) denaturation occurs at a faster rate (Singh, 2004), partial substitution with CM (25%) in MM_1 sample may be responsible for higher yield and protein recovery. Hence, the chance of adsorption of denatured CM whey proteins, followed by acidic denaturation of the casein micelle and subsequent entrapping with the fat globule occurs faster in partially substituted milk samples. Surface area available for adsorption of denatured whey proteins on fat globule and casein micelles decreased with decreasing proportion of BM in the mixtures. This affected the protein recovery and hence, the final yield. It may therefore be inferred that these bindings are of physical – nature which depend upon the surface area and the steric forces involved (Chakraborty et al., 2020).

Higher yield of *chhana* can also be explained with the higher percentage of total solids in BM for better moisture retention as compared to CM in the mixture. Casein hydration affects water holding properties of the *chhana*. Temperature, pH, surface hydrophobicity, and number of exposed polar groups number affect the water–protein interaction (amount of water bound to protein). Two types of water–protein interaction have been proposed by Kneifel, Paquin, Abert, and Richard (1991): (a) part of water bound to molecule and is not available as a solvent (absorbed water) and (b) part of water entrapped in the protein matrix (retained water). Apart from soluble globular milk proteins, casein micelles can bind relatively large amount of water. Protein molecules of fresh milk are unfolded and their water-binding capacity may increase when milk is heated. Nature of the protein is responsible for the amount of bound water in denatured milk protein (Bech, 1980). The more the protein recovery, the more entrapped water content due to moisture retention and hence, it can be concluded that MM_1 had the maximum moisture content (Table 2).

TABLE 2 Effects of type of milk and seasonal variation on composition of *chhana* and whey from buffalo milk (BM), cow milk (CM), and mixed milk (MM) (BM:CM ratio in MM_1-75:25; MM_2-50:50; MM_3-25:75)

Type of milk used	Summer				Winter			
	Chhana		Whey		*Chhana*		Whey	
	Yield (%)	Moisture (%)	Yield (%)	Total solids (%)	Yield (%)	Moisture (%)	Yield (%)	Total solids (%)
BM	15.45 ± 0.67^b	54.07 ± 0.22^a	80.74 ± 0.38^c	4.42 ± 0.30^c	15.82 ± 0.40^b	55.50 ± 0.24^b	80.22 ± 0.30^c	5.44 ± 0.21^d
MM_1	15.66 ± 0.20^a	54.24 ± 0.26^a	79.63 ± 0.52^e	4.51 ± 0.22^b	16.61 ± 0.23^a	55.72 ± 0.21^a	79.46 ± 0.14^d	5.87 ± 0.33^c
MM_2	15.52 ± 0.51^b	53.93 ± 0.31^b	80.51 ± 0.48^d	4.59 ± 0.42^b	15.90 ± 0.32^b	55.09 ± 0.50^c	80.16 ± 0.42^c	5.94 ± 0.15^b
MM_3	14.87 ± 0.32^c	53.41 ± 0.40^c	80.95 ± 0.27^b	4.77 ± 0.34^a	15.03 ± 0.27^c	54.73 ± 0.36^d	80.39 ± 0.38^b	6.03 ± 0.17^a
CM	14.02 ± 0.30^c	53.63 ± 0.33^c	81.43 ± 0.11^a	4.15 ± 0.53^d	14.28 ± 0.11^d	53.11 ± 0.17^e	81.02 ± 0.26^a	5.29 ± 0.31^e

Note: Results represented as mean values of three replicates with their standard deviation; Means with different superscripts in column differ significantly ($p < .05$).

3.3 | Color analysis

Chhana contains proteins, fat, and carotenoids that are responsible for reflecting specific wavelengths of light. Lightness (L^*) value was maximum (90.84 in summer; 90.4 in winter) for BM *chhana*, and decreased with increasing proportion of CM in the MM *chhana* samples and was minimum (89.06 in summer; 89.36 in winter) for CM *chhana* (Figure 1). This may be due to the micelle aggregation phenomenon as casein micelles have been reported to scatter light (Ahmad, Piot, Rousseau, Grongnet, & Gaucheron, 2009; Sinaga, Bansal, & Bhandari, 2017). Casein micelles, when coagulated, aggregate and form clusters. These opaque clusters reflect all light and hence, the coagulum is white (Johnson, 1999). Hence, it can be concluded that the more the casein content, the more will be the L^* value. Another reason of higher L^* value may be due to the presence of more saturated fat content which is higher in buffalo milk (Ménard et al., 2010) and hence, buffalo milk *chhana* produced a higher degree of light scattering. CM *chhana* had minimum a^* value (−1.23 in summer; −1.57 in winter) which indicated more greenish nature. a^* values for BM and BM containing samples were more due to the presence of the biliverdin pigment, which is present in BM and absent in CM (Salam & Shibiny, 2011). It increased with increasing BM in the MM samples leading to maximum a^* value (−0.32 in summer; −0.52 in winter) for BM *chhana*. The yellowness (b^*) values increased with increasing proportion of CM in the MM samples and was maximum for CM *chhana* in both the seasons (Figure 1).

3.4 | Texture profile analysis

In this study, firmness was maximum in case of MM_1 *chhana* in both the seasons (Figure 2). The hardness values of chhana samples were higher in winter season as compared to summer season. Firmness is influenced by milk composition (type of protein present, variation in moisture, and fat contents), processing conditions, and so on. Since the process factors were standardized, variations in firmness of *chhana* samples can be attributed to milk composition. Firmness of *chhana* decreased with decreasing proportion of BM content in the admixtures. Minimum firmness was observed for CM *chhana*. Sindhu (1996) reported that *chhana* from buffalo milk was harder and had chewy texture due to higher concentration of casein in the miceller state with bigger size, and higher content of total and colloidal calcium. Denatured whey proteins from cow milk in MM_1 lead to the enhancement of protein content following more protein–protein interactions and hence, a firmer protein network (Dimitreli & Thomareis, 2007). Also, higher calcium content in BM milk affects the casein's isoelectric precipitation by increasing the curd tension, resulting in the development of abundant close knit linkages between micelles leading to close network of proteins with greater hardness (Ahmad et al., 2009). This mechanism plays a major role during manufacturing of *chhana* with higher yield and firmer texture from BM as compared to CM. Greater hardness of *chhana* made from BM requires more amount of force to bring about the deformation and thus the work of shear is more in case of BM *chhana* (data not shown) as compared to other samples in both the seasons. MM_1 exhibited maximum work of shear value as compared to other samples.

Stickiness of CM *chhana* was higher as compared to other *chhana* samples (Figure 2) in both the seasons. Decrease in stickiness can be explained by increase in moisture content of the BM *chhana* and MM *chhana* samples. Higher moisture and fat content BM and MM_1 *chhana* resulted in higher firmness and lower stickiness as compared to CM *chhana*. Olson and Johnson (1990) also reported similar observations and stated that low-fat cheeses exhibit a higher degree of stickiness (higher adhesive character) when masticated as compared to high-fat cheeses. This is evident in CM *chhana* samples with low fat content. Protein content is another major factor influencing the adhesive and sticky characteristics of cheese varieties with varying composition (Chen, Larkin, Clark, & Irwin, 1979). The type and nature of the protein matrix and its properties along with the degree of fat dispersion may contribute to stickiness and adhesiveness. In BM *chhana*, the protein matrix is altered by increased protein content, making it more compact and therefore less adhesive and sticky. Work of adhesion

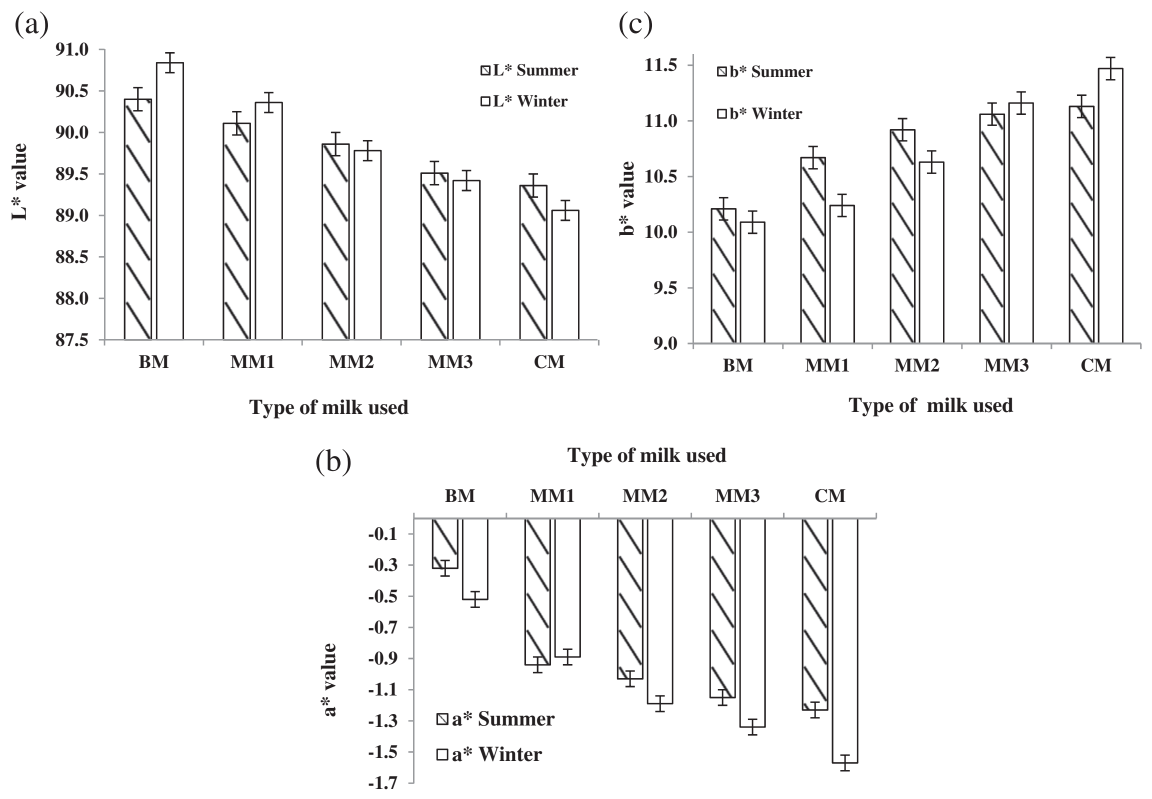

FIGURE 1 Effect of composition and season on color profile (a) L^* value; (b) b^* values of *Chhana*; BM-buffalo milk; CM-cow milk; MM-mixed milk (BM:CM ratio in MM_1-75:25; MM_2-50:50; MM_3-25:75)

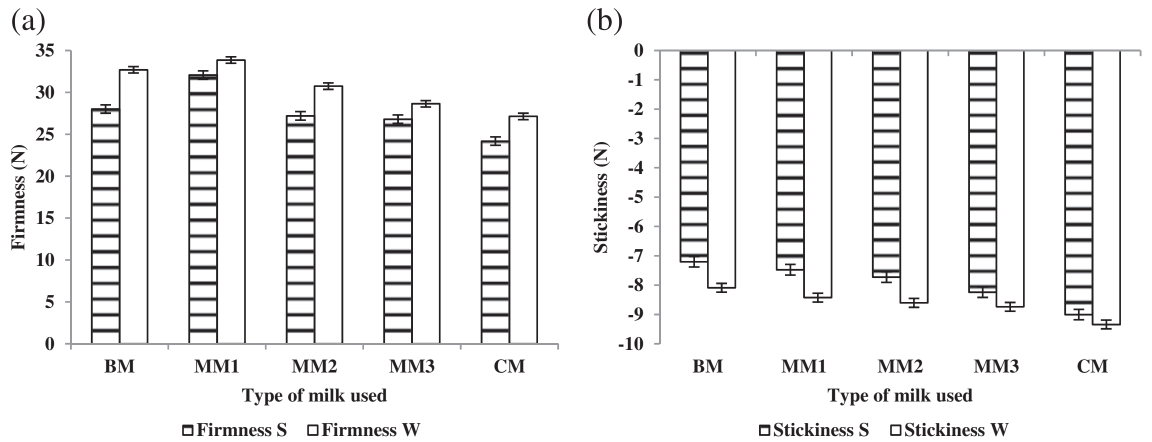

FIGURE 2 Effect of composition and season on textural parameters of chhana samples (a) firmness, (b) stickiness; BM-buffalo milk; CM-cow milk; MM-mixed milk (BM:CM ratio in MM_1-75:25; MM_2-50:50; MM_3-25:75)

was maximum (data not shown) in case of CM *chhana* as it exhibited the highest stickiness value. Due to lower stickiness and higher firmness, BM *chhana* had more elastic, firm, crumbly and chewy texture as compared to CM *chhana* which had soft, smooth, pasty, and sticky texture. Adhikari, Mathur, and Patil (1992, 1993) also reported similar results about hardness, springiness, gumminess and chewiness in CM *chhana* as compared to BM *chhana*.

3.5 | Rheology

Results of the small angle oscillatory shear (SAOS) tests were used to understand the effect of composition and seasonal variation on rheological properties of *chhana* samples. Figure 3 shows the effect of frequency on G' and G'' values of *chhana* samples. G' and G'' depend on the spatial distribution of casein particles and also, on the number of

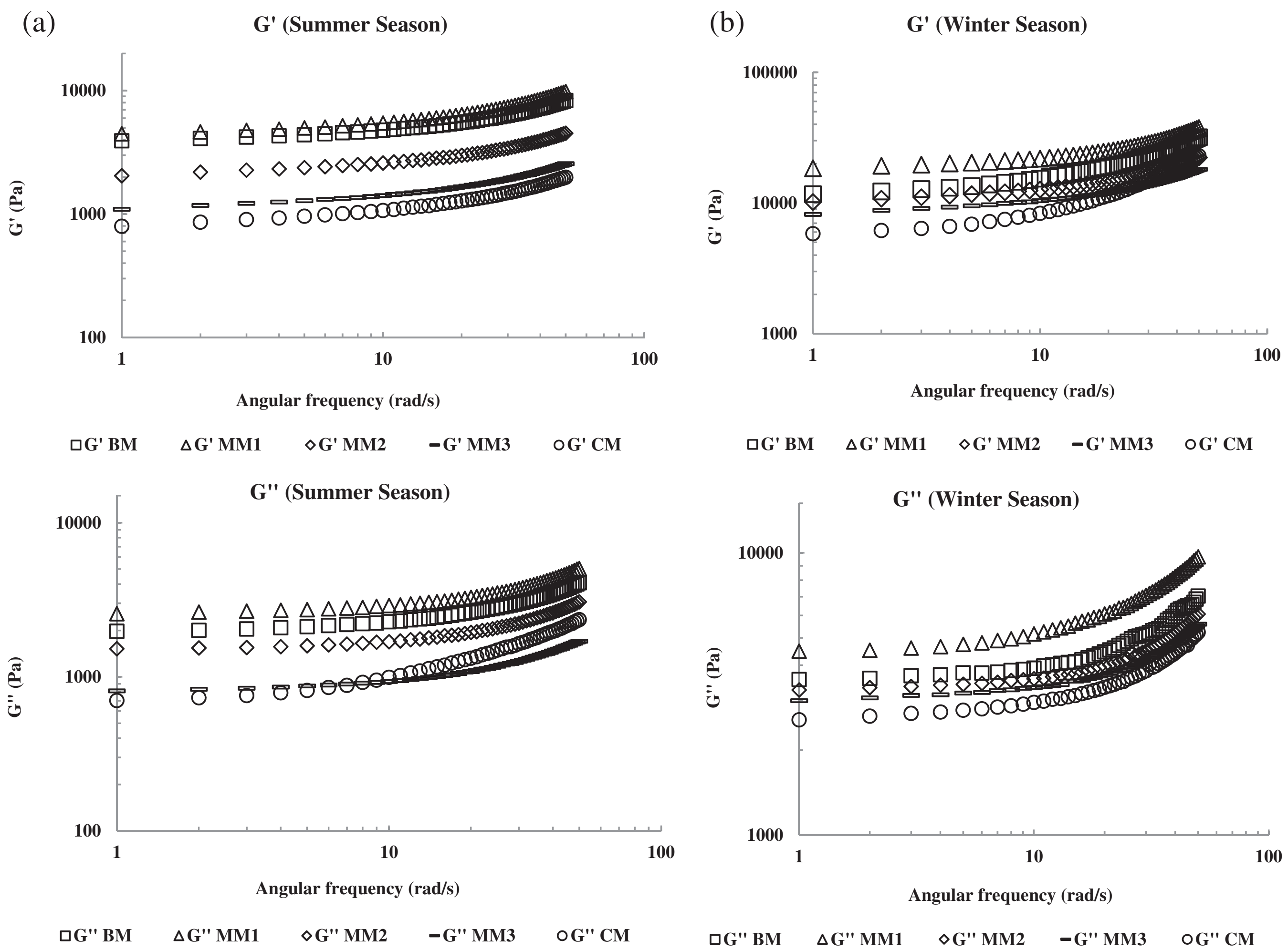

FIGURE 3 Frequency sweep tests of *chhana* samples: elastic modulus (G') and viscous modulus (G'') with respect to compositional and seasonal variation (a) summer; (b) winter; BM-buffalo milk; CM-cow milk; MM-mixed milk (BM:CM ratio in MM_1-75:25; MM_2-50:50; MM_3-25:75)

bonds and bond strength between casein particles (Roefs, de Groot-Mostert, & van Vliet, 1990). At a given frequency, the value of G' was significantly greater than G'', indicating a dominant elastic character of all the *chhana* samples in both the seasons. G' exceeded G'' approximately by four times in winter samples; and by three times in summer samples as protein, fat, and moisture contents varied with season, and thus affecting the rheological behavior of *chhana*. Elastic nature (G') was dominant in the BM containing *chhana* samples than the respective G'' values. However, reduction in values of G' and G'' was observed with decreasing the content of BM and increasing content of CM in MM samples. Protein, fat, and calcium content are lower in CM (Chandan, 2003). According to Joshi, Muthukumarappan, and Dave (2004), reduction in calcium concentration weakens the coagulated protein matrix due to differences in protein hydration and fat particles rearrangement.

The SAOS results indicated relatively weak casein matrix structure in CM containing *chhana* samples, which is related to the low protein and fat content. The MM_1 *chhana* sample indicated stronger protein matrix with higher elastic behavior. tanδ values for MM_1 *chhana* samples varied between 0.17 and 0.35, indicating solid-like, strong elastic nature of the samples followed by BM *chhana*. Lower tanδ values indicate the partial loosening of bonds within and between casein molecules as colloidal calcium phosphate (CCP) gets solubilized altering the balance between elastic and viscous components in the network (Lucey & Singh, 1997). At lower pH values (≤5.0), the net negative charge on the casein surface decreases, which reduces the electrostatic repulsion among casein molecules and particles while increasing casein-casein interaction, thorough hydrophobic interactions. Hence, lower tanδ value in milk gel sample (e.g., BM *chhana*) is indicative of more elastic character. tanδ values for CM *chhana* samples varied between 0.45 and 0.58 indicating more viscous character or weak gel like behavior (Figure 4). The tanδ values of all the winter *chhana* samples were lower as compared to summer samples which revealed that summer samples showed weaker gel characteristics. This phenomenon was due to compositional difference with seasonal variation. Similar results are reflected in the textural data obtained for *chhana* samples (Figure 2).

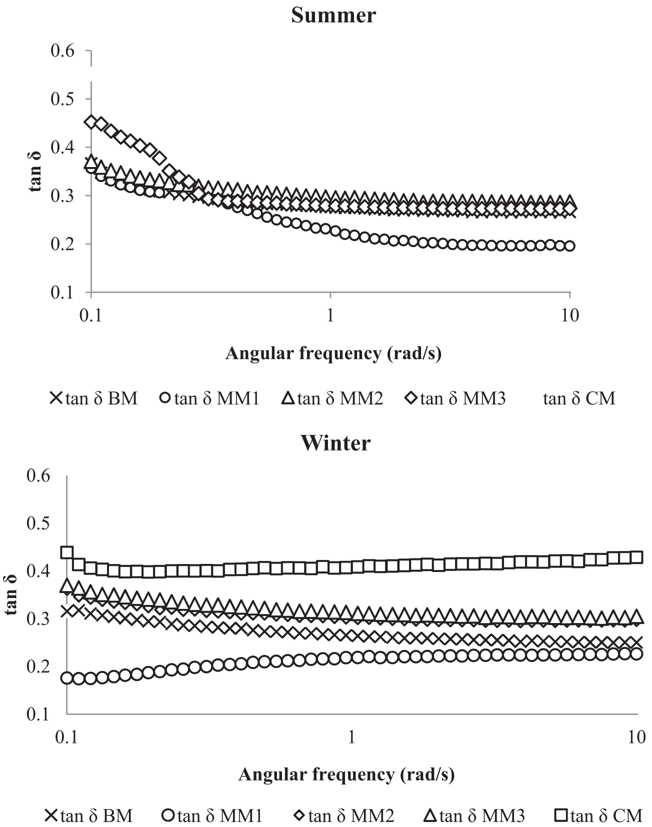

FIGURE 4 Dynamic rheological behavior of *chhana* samples at summer and winter season (strain = 1%); BM-buffalo milk; CM-cow milk; MM-mixed milk (BM:CM ratio in MM_1-75:25; MM_2-50:50; MM_3-25:75)

3.6 | FTIR spectral analysis

3.6.1 | FTIR spectral analysis of milk samples

Representative FTIR spectra of whole milk samples of CM, BM, and MM and their respective *chhana* samples in the region 4,000–900 cm^{-1} are shown in Figure 5. Five major regions were studied in various milk samples (Salleh et al., 2019): (a) C—O stretching vibration band of polysachharides (1,160–1,050 cm^{-1}); (b) stretching vibration of C═O and N—H band of amide I band (proteins) at 1,680 cm^{-1}-1,645 cm^{-1}; (c) stretching vibrational shoulder band of carbonyl and esters (fats) at 1,900 cm^{-1}–1742 cm^{-1}; (d) stretching vibrational band for saturated/ unsaturated (C—H) functional groups present (3,000-2,800 cm^{-1}); and (e) stretching vibrational bands for O—H region around 3,625–3,150 cm^{-1} (water) along with stretching band of N—H (Figure 5).

The region from 4,000 to 3,100 cm^{-1} (Figure 5) comprises of transmittance from N—H and O—H stretching vibrations of amide and hydroxyl groups of amino acids and polypeptides, respectively (Woodcock, Downey, & O'Donnellk, 2008). The higher IR transmittance value in BM when compared with CM and MM samples indicates lower water content in BM. The N—H stretching is masked by the presence of strong and broad O—H stretching band. The C—H stretching vibrations of —CH_3 and >CH_2 (methyl and methylene) fatty acids functional groups present in all milk samples appear between 3,000 and 2,800 cm^{-1} (Figure 5). Two consecutive peaks were obtained at 2,926 and 2,853 cm^{-1} which showed the presence of fatty acid. The >CH_2 band at approximately 3,000–2,900 cm^{-1} is related to the acyl chain on fatty acids (Salleh et al., 2019). As expected, the degree of transmittance for this band correlates with the fat quantity in both milk samples, with a higher fat content resulting in lower IR transmittance. Raw BM contained 6% fat approximately, whereas raw CM contained 4.5% fat approximately, and this was reflected in the >CH_2 bands. The other visual comparison between milk types appeared on the transmittance bands related to the remaining milk components, fatty acids (1,900–1,742 cm^{-1}), protein (at 1,654 and 1,544 cm^{-1} for amide I and II bands, respectively) and lactose (at 1,158 and 1,082 cm^{-1}). Two major peaks at 1,645 and 2,135 cm^{-1} were observed corresponding to the secondary protein structure primarily due to stretching vibrations of the carbonyl groups and carboxyl group from fatty acids and esters. The BM displayed sharper peak at 1,645 cm^{-1} than CM and MM samples. At 2,135 cm^{-1}, BM had almost flat peak whereas CM and MM samples showed a broad band at this frequency. A stretching band at 1645 cm^{-1} is more intense in case of BM which shows presence of stronger protein structures (O═C—NH) when compared to CM and MM samples. These components displayed higher transmittance value and appeared to be present in higher quantities in BM (Figure 5).

3.6.2 | FTIR spectral analysis of *chhana* samples

All five samples of *chhana* prepared from BM, CM, and MM showed various intense bands (Figure 6). This suggests that the absorption frequencies in *chhana* occur with higher probability of transition (higher transmittance values) in contrast to bands in IR spectra of milk.

The combination band of O—H stretching and asymmetric stretching of water, carbohydrates and carboxylic acids besides other hydroxylic group present in proteins show bands in the region of 3,400–3,200 cm^{-1} for both milk and *chhana* samples. In both the seasons, less broad and sharper peaks were observed in *chhana* samples as compared to milk samples due to loss of moisture in the manufacturing process. The reason for CM *chhana* sample showing weakest band in this region is due to low moisture content. The cluster of IR peaks at 2,853 and 2,926 cm^{-1} and some merged in H-bond region in *chhana* samples are due to different C—H stretching vibrations present in differently substituted chains or side chains of protein. This variation may occur due to the reorganization of water molecules and fat occurring throughout the coagulation process. In order to maintain the native structure, casein and whey protein along with fat experience structural modification. During coagulation, fat and water interactions are essential to maintain a stable and steady whey protein network, thus preventing the coagulum structure disintegration (Sinelli, Barzaghi, Giardina, & Cattaneo, 2005). Due to removal of water, protein proportion increased in the coagulum leading to a clear and sharper peak in both BM and CM *chhana* samples. In

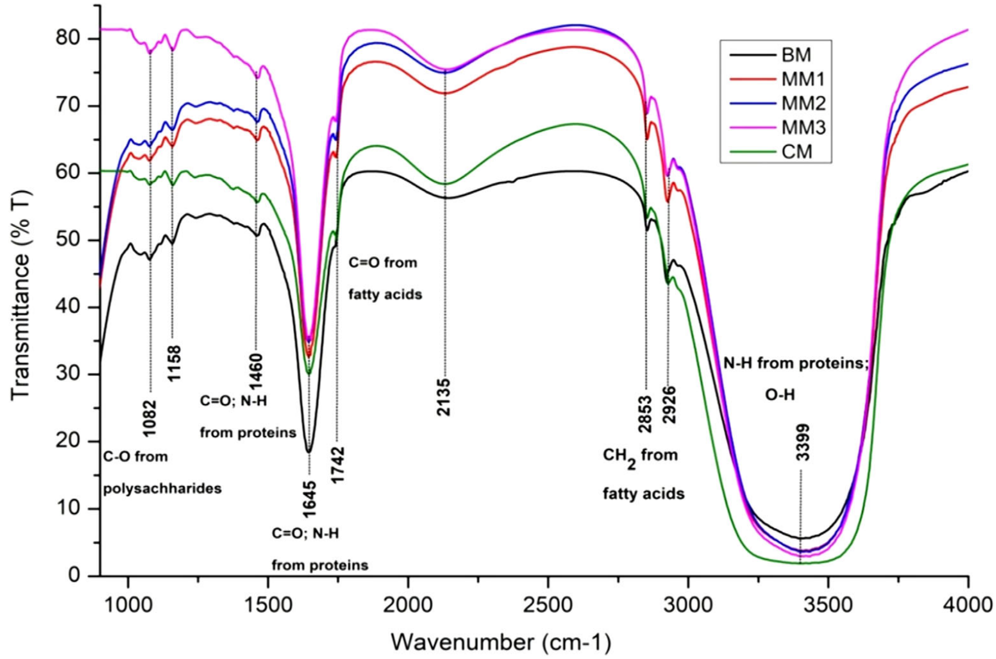

FIGURE 5 FTIR transmittance spectra of raw cow milk (CM), buffalo milk (BM) and various mixed milk samples (BM: CM ratio in MM_1-75:25; MM_2-50:50; MM_3-25:75)

both the seasons, BM *chhana* samples had lower transmittance value as compared to CM *chhana* samples due to higher protein content. The peak at 2,136 cm^{-1} had become flat and the peak value shifted to 2,122 cm^{-1}in case of *chhana* samples as compared to milk samples in both the seasons. It indicates the disappearance or absence of triple bond during *chhana* formation due to lower pH during acidification. The region 1,800–1,710 cm^{-1} is associated with C═O stretching of carbonyl group esters (Chen, Irudayaraj, & Mcmahon, 1998; Koca, Rodriguez-Saona, Harper, & Alverez, 2007; Rodriguez-Saono, Koca, Harper, & Alvarez, 2006).Unlike milk samples, *chhana* samples exhibited variation with a peak value between 1,750–1,650 cm^{-1} showing the presence of C═O stretching band esters of fatty acids in this region. This shift can be attributed to differences in the rate of hydrolysis of *chhana* samples at different stages of coagulation process. The peaks are much sharper as compared to milk samples, which indicate the higher fraction of fat content in the *chhana* samples. BM *chhana* samples had lower transmittance value as compared to CM *chhana* samples in both the seasons as BM contains higher percentage of long chain saturated fatty acids as compared to CM. The transmittance pattern of these bands is similar in all samples. MM_1 *chhana* samples have the lowest transmittance value. The double bond region (1,750–1,550 cm^{-1}) and the single bond region (1,550–1,000 cm^{-1}) depicted more number of peaks with relatively sharp and lower transmittance. The shoulders at 1,744 cm^{-1} transformed into a sharp peak due to the presence of ester carbonyl group. The important amide I band at 1,635 cm^{-1} is present in all the samples and is stronger in MM_1*chhana* sample. The intensity at 1,635 cm^{-1} can be represented in terms of the protein amount (peptide linkages) in *chhana* samples. These sharp peaks are formed if the primary structures contain a sufficient proportion of amino acids with highly hydrophobic side groups and differently substituted chain or side chain of protein in BM, CM, and MM *chhana* samples. This implies that structural folding of proteins occurs significantly with difference in composition. Both the structures I and II of the peptide bonds contribute the occurrence at higher frequency (1,635 cm^{-1}), which shows the presence of saturated structural unit of nitrogen side of peptide bond (Susi & Byler, 1988). It is present in larger amount in *chhana* samples when compared with milk samples due to the removal of water from *chhana* samples during manufacturing. This increases insoluble proteins relatively in *chhana* samples. The region 1,500–1,250 cm^{-1} represents O—C—H, C—CH and C—O—H bending vibrations. These bending vibrations are associated with amino acids and amide III band. Amide III band usually appears around 1,300 cm^{-1} (Dufour, 2009). In our study, the presence of amide III band was around 1,250–1,200 cm^{-1} with a peak value of 1,242 cm^{-1} in MM_1*chhana* samples. MM_1*chhana* samples had lower transmittance value in all the amide regions indicating higher total protein content. The region 1,200–800 cm^{-1} corresponds to C—C and to C═O links (Subramanian, Alvarez, Harper, & Rodriguez-Saona, 2011). This region in our study was in the range of 1,163–1,090 cm^{-1} and contained several small peaks with variable intensities representing lactose. Some flavor components also displayed their bands in the *chhana* samples. BM *chhana* contains more lactose and due to the presence of more saturated fatty acid components certain characteristic flavor components were also present. The bands at lower frequencies (1,090, 1,163, and 1,242 cm^{-1}) certainly belong to C—O bending of alcohols, ethers and esters. The bands at 1542, 1452, 1,401, 1,242, 1,163 and 1,090 cm^{-1} are relatively stronger in *chhana* samples as compared to raw milk samples. This can be explained by the fact that *chhana* samples contain either less amount of water or the relative amount of protein increased. In milk, the samples have more amount of water, and the higher intensity of H-bond does not allow the double bond stretching region (C═O of esters and amide), bending vibrations (fingerprint region) and other mixed molecular vibrations to appear strongly in FTIR spectra of samples.

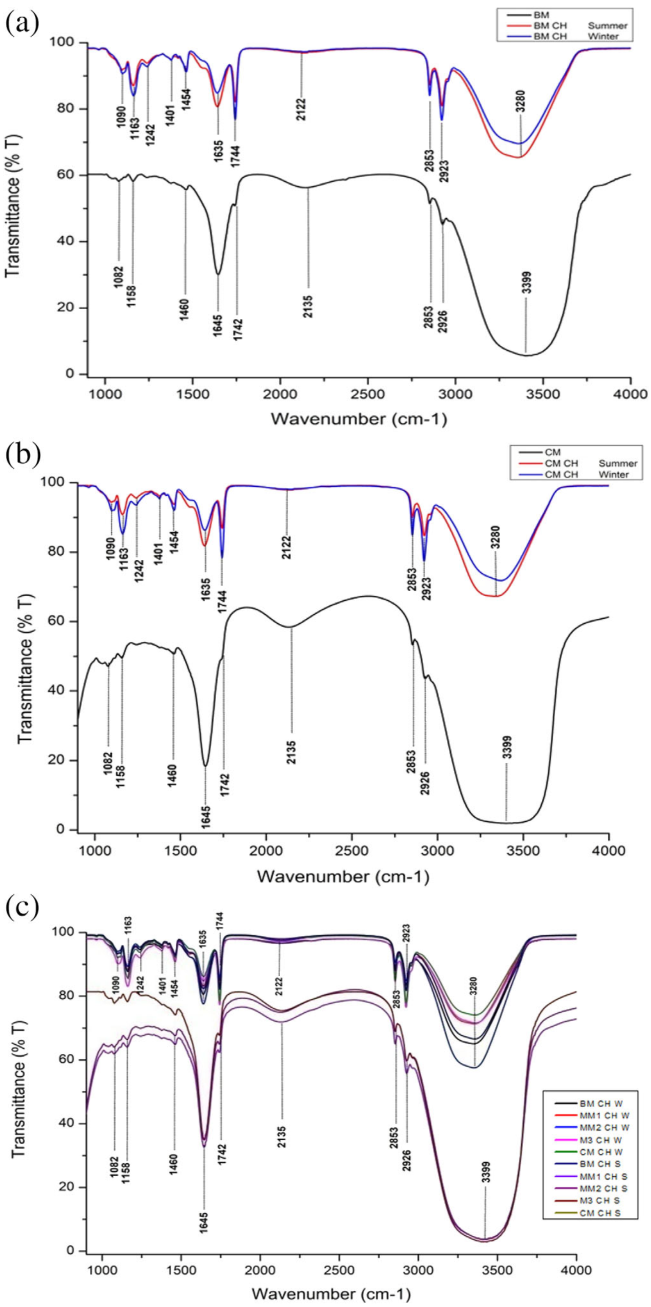

FIGURE 6 FTIR transmittance spectra of *chhana* samples in summer (S) and winter (W) season in comparison with raw milk (a) BM, (b) CM, and (c) MM (BM:CM ratio in MM_1-75:25; MM_2-50:50; MM_3-25:75

4 | CONCLUSION

Chhana is a heat-acid induced milk gel mainly composed of a casein matrix associated with denatured whey protein, with embedded fat globules and water. From whole milk to *chhana* manufacturing, the transformation of the major components (water, proteins, and fat) was observed through FTIR and was corroborated with textural and rheological data. Fat–protein network assembly, state and water hydration phenomena of denatured proteins and their association within and with other components in *chhana* lead to variation in molecular scale (FTIR spectroscopy) as well as macroscale (texture, color, and rheological attributes) properties. Less stronger and narrower O—H vibrations, sharper and stronger amide I and II bands (more primary proteins) and $>CH_2$ bands (long chain saturated fatty acids) in *chhana* samples depicted lower water content, and higher protein and fat contents in all the *chhana* samples as compared to whole milk samples in both the seasons. MM_1 had the highest yield due to higher total solid content (fat and protein) and water hydration phenomena into the casein matrix. Higher protein and fat content in BM lead to higher firmness and lower stickiness in *chhana* samples as compared to CM samples in both the seasons. Firmness decreased and stickiness increased with increasing CM content in the MM samples. The dynamic moduli of BM and MM_1 *chhana* were affected due to the interactions between fat and protein matrix which resulted in firm, elastic, and consistent textured gels. With increase in CM content in the MM samples, the gels became softer and weaker. As BM had higher percentage of protein and saturated fats, the BM *chhana* had highest L^* value and lowest a^* and b^* values when compared to CM *chhana*. Milk composition and milking season significantly influenced quality characteristics of *chhana*. When blended, the interactive effects between milk mixtures increased the brightness and reduced the yellow color intensity of BM *chhana*. The firmness and elasticity of the protein matrix increased in the presence of BM proteins. High tanδ and low b^* were indicative of high CM concentrations in the admixtures. It can be concluded that MM_3 behaved more like CM and the *chhana* prepared from it had similar macroscale and microscale attributes irrespective of seasonal variation.

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AUTHOR CONTRIBUTIONS

Purba Chakraborty: Data curation; formal analysis; investigation; methodology; writing-original draft. **Tejvir Singh:** Formal analysis; visualization; writing-review and editing. **Uma Shankar Shivhare:** Formal analysis; project administration; writing-review and editing. **Santanu Basu:** Conceptualization; data curation; formal analysis; funding acquisition; methodology; project administration; resources; supervision; visualization; writing-review and editing.

ETHICAL STATEMENT

Conflict of Interest: The authors declare that they do not have any conflict of interest.

Ethical Review: This study does not involve any human or animal testing.

Informed Consent: The present study did not have any human or animal trials or testing. So there was no need for taking the informed consent.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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