

# Physico-chemical characteristics of butter prepared from the milk of selected indigenous and crossbred cows

Sathiya Taherabbas<sup>1</sup>, Akshay Ramani<sup>1</sup>, Raman Seth (✉)<sup>1</sup>, Kamal Gandhi<sup>1</sup>, Rajan Sharma<sup>1</sup>, Bimlesh Mann<sup>2</sup>

Received: 10 January 2024 / Accepted: 04 April 2024 / Published online: 23 December 2024  
© Indian Dairy Association (India) 2024

**Abstract:** This study aimed to investigate the effects of the season in relation to breed and the butter preparation method on chemical composition,  $\beta$ -carotene content, conjugated linoleic acid (CLA) content, color attributes, texture profile, and keeping quality. Fat content showed minor variation in butter types, conventional butter had significantly ( $p < 0.05$ ) lower free fatty acid (FFA) levels than desi butter. Tharparkar butter, especially in summer batches, had the highest  $\beta$ -carotene content. CLA levels varied with breeds and seasons; increased during summer due to outdoor grazing on green fodder. The colour analysis highlighted breed-specific variations, with Tharparkar butter displaying superior visual characteristics. Texture analysis demonstrated that conventional butter was harder, less adhesive, and showed higher cohesiveness compared to desi butter. The study emphasizes the need for careful breed selection and seasonal considerations to ensure the nutritional quality, appearance, texture, and quality of butter products. Monitoring free fatty acid levels is crucial for assessing butter shelf life and quality.

**Keywords:** Desi butter, spreadability, firmness,  $\beta$ -carotene, conjugated linoleic acid (CLA).

## Introduction

The milk production in India for the year 2021-22 was reported to be 221.01 MT, with a CAGR (compound annual growth rate) of approximately 6.2% (NDDDB Report, 2022). Butter is the second most widely consumed fat-rich dairy product in India, accounting for 6.5% of total milk conversion, following ghee (Battula et al. 2020). Butter is a water-in-oil type emulsion, wherein water is dispersed within the oily medium (Patel et al. 2015). In comparison to vegetable oil-based margarine, butter is more expensive, leading to adulteration practices by profit-oriented food business operators. It has been reported that A1 milk protein from exotic cow breeds may have adverse health effects on humans, such as juvenile diabetes, coronary heart disease, irritable bowel syndrome, schizophrenia, and autism (Parashar and Saini 2015; Chia et al. 2017). Indigenous cow milk contains the A2 variant of milk protein, suggesting that focusing on A2 milk and milk products may be beneficial for better health outcomes. Cow milk butter appears more yellowish than buffalo milk due to its higher  $\beta$ -carotene content, which is converted into vitamin A by an intestinal mucosal enzyme ( $\beta$ -carotene-15,152-monooxygenase) present in buffalo (Patel et al. 2015).  $\beta$ -carotene has been reported to play a role in preventing night blindness, xerophthalmia, strengthening the immune system against infections, promoting gastrointestinal function, and supporting proper growth, development, and functioning of the reproductive systems (Grune, 2010). Various flavoring compounds, including diacetyl,  $\delta$ -octalactone,  $\delta$ -decalactone, skatole, and butanoic acid, contribute significantly to the flavor of butter, with each compound having a threshold concentration in relation to butter flavor. Butter primarily consists of fat, serving as a repository for bioactive lipids such as short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA), and phospholipids. Butterfat is a major carrier of phospholipids (such as lecithin and glycoprotein) and fat-soluble vitamins like vitamins A, D, E, and K, which play essential roles in regulating various biological activities, including bone mineralization, tissue differentiation, cell growth, and coenzyme activity (Park et al. 2021). While butter consumption offers health benefits, it is important to consider the potential risks associated with saturated fatty acids, trans fatty acids (TFA), and cholesterol found in butter (Givens, 2017). In India, butter is

<sup>1</sup>Dairy Chemistry Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India

<sup>2</sup>Assistant Director General (EP&HS), ICAR, New Delhi, India

Raman Seth (✉)  
Email: [ramanseth123@yahoo.co.in](mailto:ramanseth123@yahoo.co.in)

commonly used as a cooking medium at the household level, where household butter is typically prepared using the desi method. Exploring the composition and therapeutic value of butter from indigenous cow milk is a relatively new area of research, could provide valuable insights. However, there is limited data available on the composition of butter from selected indigenous cow breeds and crossbreeds, including their  $\beta$ -carotene, CLA content and rheological properties.

## Materials and Methods

### Chemicals and reagents

Fatty acid methyl ester (FAME),  $\beta$ -carotene, and linoleic acid-conjugated reference standards were procured from Sigma-Aldrich Co, St. Louis, USA. All solvents (chloroform, methanol, hexane, diethyl ether, petroleum ether, ethanol absolute, n-butanol, Tetrahydrofuran, acetonitrile and milli-Q water) used were HPLC grade. Oxalic acid, sodium carbonate, sodium chloride, potassium hydroxide pellets, sodium hydroxide pellets, sodium bisulfate, sulphuric acid, potassium dichromate, and phenolphthalein reagent were AR grade and procured from Qualigens Fine Chemicals, Mumbai, India. Starter culture (NCDC 167) was obtained from the National Collection of Dairy Culture, ICAR-NDRI, Karnal.

### Sample collection

Fresh milk samples were collected from individual mid-lactating cattle of various breeds, including Tharparkar, Sahiwal (an indigenous breed), Karan Fries (a crossbred breed), as well as pooled cow milk from all three breeds. These samples were obtained from the Livestock Research Centre (LRC) of the National Dairy Research Institute (NDRI) in Karnal. The collected milk samples were stored at 4°C until further analysis. Before analysis, the samples were warmed to 40°C and analyzed within a 2-hour time frame. To evaluate the effect of seasonal variation, the sampling period was divided into two seasons: Winter (November-January) and Summer (February-April). The dairy animals were maintained under identical feeding and management conditions throughout the study. They were provided with concentrate feed during all seasons, while berseem, oats, and alfalfa were additionally supplied during the winter season.

### Preparation of Butter

Desi and conventional butter samples were prepared using starter culture (NCDC 167), following the unsalted butter preparation technique described by De (2012).

### Chemical parameters

The chemical parameters, including free fatty acids, moisture, fat, and curd content, of both conventional butter samples and desi butter samples were determined using standard methods

outlined in IS 548-1 (1964) for free fatty acid analysis and IS 3507 (1966) for moisture, fat, and curd content determination.

### $\beta$ -carotene measurement

The estimation of  $\beta$ -carotene was performed using the HPLC method described by Dhankhar (2017). The analysis was conducted using an Agilent 1260 infinity system (Agilent Technologies, USA) equipped with a Diode Array Detector (DAD) and a reversed-phase C18 column (ZORBAX 300 SB-C18, 4.6 x 250 mm, 5  $\mu$ m). The wavelength used for detection was set at 450 nm. A mobile phase consisting of a mixture of methanol/THF/water was utilized, and the column flow rate was set at 0.8 ml/min. To ensure the purity of the solvent mixture, proper filtration using a 0.22  $\mu$ m pore size filter and sonication at 40 Hz for 10 minutes were performed prior to the HPLC test.

### CLA measurement

Methyl esters were prepared following the guidelines outlined in ISO/IDF (2002). CLA methyl esters was injected at volumes of 1, 2, 3, 4, and 5  $\mu$ l using an auto sampler (AOC-20i), into a Shimadzu GCMS TQ 8030 gas chromatography system coupled with a flame ionization detector. Chromatographic separation of fatty acid methyl esters (FAMES) was achieved using an SPTM-2560 fused silica capillary column (100 m x 0.25 mm I.D. x 0.20  $\mu$ m film thickness, Supleco). Nitrogen was used as the carrier gas with a flow rate of 0.8 ml/min. The oven temperature program initiated at 140°C with a hold time of 5 min, followed by a ramp to 240°C at a rate of 3°C/min (hold time of 15 min). The injector and ion source temperatures were both maintained at 250°C throughout the analysis. A detailed regression line was plotted based on the peak areas obtained at different concentrations of the FAME mixture. The fragmentation spectra of the standard linoleic acid conjugated methyl ester were compared with those obtained from the sample run.

### Colour measurement

The colour value of butter was determined using the method outlined by Sert and Mercan (2020) in terms of  $L^*$ ,  $a^*$ , and  $b^*$  values. A Hunter Colourflex colourimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) equipped with a xenon flash lamp as the light source was employed for this analysis. Each sample was tempered at 10°C and tested in triplicate in this system,  $L^*$  indicates the position of the sample on the dark-light axis,  $a^*$  represents the position on the green-red axis, and  $b^*$  signifies the position on the blue-yellow axis.

### Texture profile analysis (TPA)

The textural attributes of the butter samples were evaluated using a Texture Analyser TAXT2i (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 25 Kg load cell. A typical two-bite compression test was performed on the product using a P-75 platen compression probe attached to the texture analyser. Three

replicates were conducted for each sample. Before testing, the butter samples were stored at a temperature of 10°C. The parameters measured included hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. The Texture Expert for Windows software version 1.20 (Stable Micro Systems) was used for data analysis.

**Statistical Analysis**

Statistical analysis was performed using SPSS 20 software (IBM Corporation, USA). The two-way ANOVA (Analysis of variance) and Tukey’s HSD post-hoc test (P <0.05) were used for the evaluation of differences in concentration of fatty acids, β-carotene and conjugated linoleic acid of butter between breeds and seasons. The results were expressed as mean with standard deviation.

**Results and Discussion**

**Chemical composition of butter samples**

The chemical composition of butter prepared through desi and conventional methods was investigated, and the results are presented in Table 1. The fat content of the butter samples showed no significant variations (p ≥ 0.05) in the fat percentage among Karan-Fries, Sahiwal, Tharparkar, and conventional butter. Notably, conventional butter exhibited the highest fat percentage, attributed to lower fat loss in buttermilk during the conventional preparation process, as noted by previous studies (Panchal and Bhandari 2020). The free fatty acid (FFA) content of conventional butter was significantly lower (p ≤ 0.05) compared to desi butter derived from indigenous cow milk. Among the desi cow milk butter samples, non-significant differences (p ≤ 0.05) were observed in the FFA content of Karan-Fries, Sahiwal, and Tharparkar cow butter. Moreover, no significant differences (p ≥ 0.05) were noted in the FFA content among butter samples from Karan-Fries, Sahiwal, and Tharparkar cow milk. The curd content of butter, derived from Karan-Fries, Sahiwal, Tharparkar milk, and conventional sources, exhibited non-significant differences (p ≥ 0.05). These results indicate the uniformity in curd content across the tested butter samples.

**β-carotene content of butter**

The β-carotene content of freshly prepared butter using desi and conventional methods is presented in Fig. 1(a). Statistical analysis revealed significant differences (p ≤ 0.05) in the β-carotene content among the butter samples. Butter derived from Tharparkar milk exhibited the highest β-carotene content, followed by conventional butter and Sahiwal butter, with the lowest content found in Karan Fries-derived butter. This observation aligns with the findings of Kumar et al. (2022). Notably, the β-carotene content in Karan-Fries butter differed significantly (p<0.05) from all other types. Furthermore, a significant increase in β-carotene content was noted in butter prepared during the summer season, attributed to outdoor grazing on green fodder (Antone et al. 2015). The average β-carotene content in butter derived from conventional, Karan Fries, Sahiwal, and Tharparkar milk was 5.90 μg/g fat, 4.63 μg/g fat, 5.8 μg/g fat, and 6.11 μg/g fat, respectively. β-carotene content of butter during summer season (5.3 – 6.77 μg/g fat) was significantly (p≤0.05) higher than butter in winter season (3.95-5.44 μg/g fat). This seasonal variation in β-carotene content is consistent with previous studies (Marino et al. 2012), which reported a 25% to 68% decrease in β-carotene content in milk samples collected during the winter season compared to those collected in the summer season. Additionally, the shift from a summer diet to a winter diet led to a rapid decrease in β-carotene content, as demonstrated by Noziere et al. (2006).

The decrease in β-carotene content in milk fat with the changing seasons is well-documented in the literature (Noziere et al. 2006, Marino et al. 2012). These findings underscore the importance of seasonality in influencing the nutritional quality of butter, particularly concerning β-carotene content.

**CLA content of butter**

The conjugated linoleic acid (CLA) content in butter samples derived from various breeds is presented in Fig. 1(b). Statistical analysis revealed significant variations in CLA content among the different breeds. Butter prepared from Karan Fries milk exhibited the highest CLA content (15.7 mg/g fat), followed by Tharparkar (12.91 mg/g fat), and Sahiwal (12.12 mg/g fat) breeds.

**Table 1** Chemical composition of butter prepared from indigenous and crossbred cow milk

Butter	Moisture %	Fat %	FFA %	Curd %
CBPM	15.69 ± 0.172 <sup>ab</sup>	82.25 ± 0.69 <sup>a</sup>	0.14 ± 0.002 <sup>b</sup>	1.11 ± 0.06 <sup>a</sup>
DBKF	15.57 ± 0.073 <sup>b</sup>	82.09 ± 0.38 <sup>a</sup>	0.27 ± 0.05 <sup>a</sup>	1.07 ± 0.08 <sup>a</sup>
DBSW	15.79 ± 0.111 <sup>a</sup>	82.07 ± 0.34 <sup>a</sup>	0.27 ± 0.03 <sup>a</sup>	1.15 ± 0.07 <sup>a</sup>
DBTP	15.76 ± 0.098 <sup>b</sup>	82.01 ± 0.27 <sup>a</sup>	0.29 ± 0.01 <sup>a</sup>	1.13 ± 0.05 <sup>a</sup>

Results are expressed as means ± S.D (n=3). a-b means within columns with different superscript are significantly different (p ≤ 0.05) from each other.

CBPM- conventional butter from pooled milk ; DBKF- desi butter from Karan Fries ; DBSW- desi butter from Sahiwal; DBTP- desi butter from Tharparkar

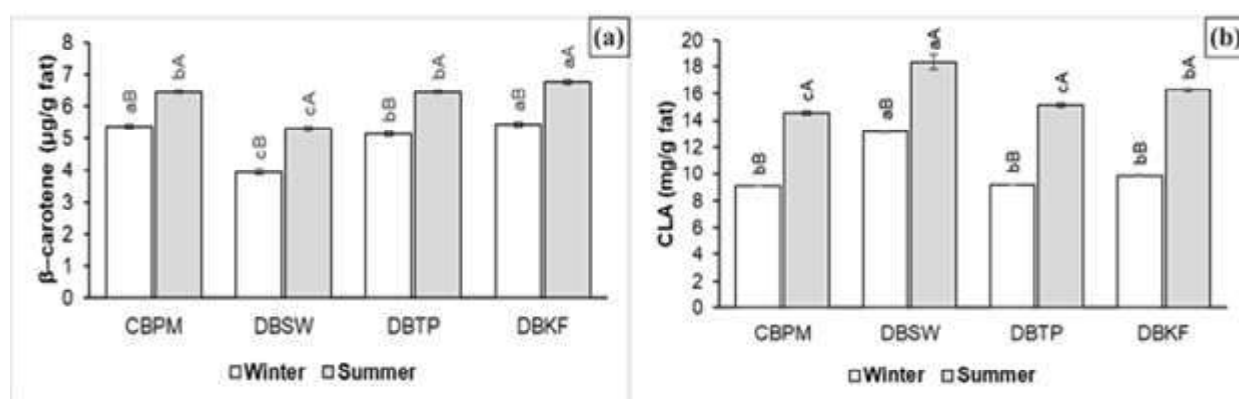


Fig. 1 Effect of breed and season on  $\beta$ -Carotene and CLA content in butter

(a)  $\beta$ -carotene content of butter (b) CLA content of butter

Results are expressed as means  $\pm$  S.D (n=3). A-B Means within a season and a-b means within treatment (CBPM, DBKF, DBSW, and DBTP) with different superscripts were significantly different ( $p \leq 0.05$ ) from each other.

**Table 2** Effect of season on colour values of butter prepared from indigenous and cross bred cow milk

Breeds	L*		a*		b*	
	Winter	Summer	Winter	Summer	Winter	Summer
CBPM	78.35 $\pm$ 0.25 <sup>bA</sup>	75.47 $\pm$ 0.15 <sup>cB</sup>	5.61 $\pm$ 0.06 <sup>aA</sup>	3.79 $\pm$ 0.06 <sup>aB</sup>	26.67 $\pm$ 0.19 <sup>cB</sup>	31.42 $\pm$ 0.13 <sup>bA</sup>
DBKF	81.06 $\pm$ 0.32 <sup>aA</sup>	77.99 $\pm$ 0.33 <sup>aB</sup>	3.89 $\pm$ 0.06 <sup>dA</sup>	2.01 $\pm$ 0.06 <sup>cB</sup>	23.69 $\pm$ 0.29 <sup>dB</sup>	30.84 $\pm$ 0.20 <sup>cA</sup>
DBSW	79.14 $\pm$ 0.29 <sup>bA</sup>	76.31 $\pm$ 0.16 <sup>bB</sup>	4.08 $\pm$ 0.06 <sup>cA</sup>	3.68 $\pm$ 0.05 <sup>aB</sup>	29.71 $\pm$ 0.18 <sup>bB</sup>	31.68 $\pm$ 0.20 <sup>bA</sup>
DBTP	76.88 $\pm$ 0.94 <sup>cA</sup>	75.91 $\pm$ 0.33 <sup>bA</sup>	4.45 $\pm$ 0.09 <sup>bA</sup>	3.47 $\pm$ 0.08 <sup>bB</sup>	30.24 $\pm$ 0.1 <sup>aB</sup>	37.18 $\pm$ 0.13 <sup>aA</sup>

Results are expressed as means  $\pm$  S.D (n=3). A-B Means within rows and a-b means within columns with different superscript were significantly different ( $p \leq 0.05$ ) from each other.

CBPM- conventional butter from pooled milk; DBKF- desi butter from Karan Fries ; DBSW- desi butter from Sahiwal; DBTP- desi butter from Tharparkar

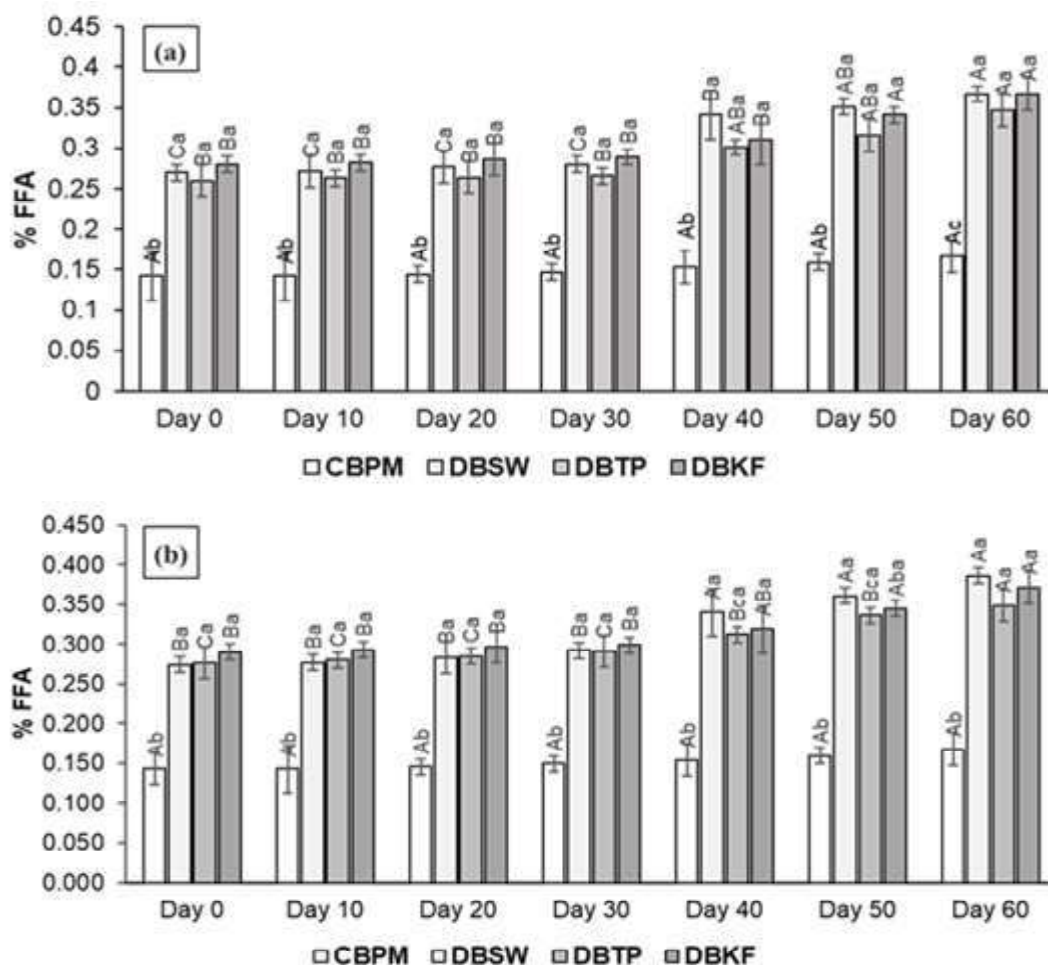
Conventional butter significantly differed from both Karan Fries and Tharparkar milk butter. The average CLA content in butter derived from conventional, Karan Fries, Sahiwal, and Tharparkar milk was 11.78, 15.7, 12.12, and 12.91 mg/g fat, respectively. These values fall within the range reported in the literature (2 to 37 mg/g fat) (Stanton et al. 2020).

Significant seasonal differences ( $p \leq 0.05$ ) were observed in the CLA content of butter, with higher levels during the summer (14.54-18.34 mg/g fat) compared to the winter season (9.1-13.14 mg/g fat). This finding is consistent with previous studies that reported higher CLA levels in summer months compared to winter (Frelich et al. 2009). Ahmad et al. (2019) noted elevated CLA content (22.1 mg/g fat) in milk from pasture-fed cows compared to cows with one-third (8.9 mg/g fat) or two-thirds (14.3 mg/g fat) of their diet as pasture. The increase in CLA during pasture feeding is attributed to the effects on biohydrogenation and the provision of  $\alpha$ -linolenic acid as a lipid substrate, leading to the formation of cis-9, trans-11 CLA in the mammary gland (Indu and

Jayaprakasha 2021, Stanton 2020). These observations underscore the impact of both breed variation and seasonal factors on CLA content in butter, emphasizing the need for comprehensive understanding and careful management in dairy practices.

**Colour value of butter**

The colour characteristics of butter prepared from selected indigenous and crossbred cow milk, employing both conventional and desi methods, are detailed in Table 2. The colour attributes, namely lightness (L\*), redness (a\*), and yellowness (b\*), were analyzed. During the winter season, Karan-Fries butter displayed the highest lightness value (81.06), whereas Tharparkar butter recorded the lowest value (76.88). There was a significant ( $p < 0.05$ ) difference in lightness values between butter from Karan-Fries, Sahiwal and Tharparkar milk. Notably, there was statistically significant higher lightness values ( $p < 0.05$ ) observed during the winter season compared to the summer season.



**Fig. 2** Effect of breed on self-life of butter prepared from indigenous and crossbred cows (a) Butter prepared in winter season (b) Butter prepared in summer season Results are expressed as means  $\pm$  S.D ( $n=3$ ). *A-B* Means within storage (days) and *a-b* means within treatment (CBPM, DBKF, DBSW, and DBTP) with different superscripts were significantly different ( $p \leq 0.05$ ) from each other.

Butter conventionally prepared from pooled cow milk consistently exhibited significantly higher ( $p < 0.05$ ) redness values in both seasons. Among the various breeds analyzed, Tharparkar butter showcased the highest redness, whereas Karan-Fries butter displayed the lowest. Moreover, a significant and consistent difference in redness values ( $p < 0.05$ ) was observed among butter from Karan-Fries, Sahiwal, and Tharparkar milk throughout various seasons. However, a significant difference in yellowness values was noted between Sahiwal, Tharparkar and Karan-Fries butter.

Yellowness is a crucial attribute in butter due to its naturally yellowish hue, mainly due to  $\beta$ -carotene content (O’Callaghan et al. 2016). The yellowness values of butter increased as summer advanced, as indicated by the lower  $b^*$  values in winter (23.69 to 30.24) compared to summer (30.84 to 37.18). These colour analyses provided valuable insights into the visual characteristics

of butter, demonstrating the influence of breed, season, and preparation methods on its appearance. Consequently, butter from Tharparkar milk displayed the highest yellowness, whereas the lowest yellowness was observed in Karan-Fries butter. In comparison, Ponnal (2021) reported  $L^*$  value of 85.76,  $a^*$  value of -4.00, and  $b^*$  value of 32.07 for salted butter. Significant differences ( $p \leq 0.05$ ) were observed in the lightness ( $L^*$ ) and redness ( $a^*$ ) values between butter prepared in winter and summer seasons, with higher values noted in winter and lower values in summer. Conversely, in terms of yellowness values ( $b^*$ ), the trend was reversed, with higher values observed in summer and lower values in winter.

**Texture profile analysis (TPA)**

**Table 3** Effect of breed on texture profile of butter prepared from indigenous and crossbred cows

	CBPM	DBKF	DBSW	DBTP
Hardness	11.69 ± 1.20 <sup>a</sup>	8.64 ± 0.91 <sup>b</sup>	3.94 ± 0.30 <sup>d</sup>	6.03 ± 0.47 <sup>c</sup>
Fracturability	4.58 ± 0.89	-	-	-
Adhesiveness	-322.73 ± 36.60 <sup>c</sup>	-287.66 ± 27.57 <sup>b</sup>	-178 ± 8.29 <sup>a</sup>	-198.12 ± 12.46 <sup>a</sup>
Springiness	0.20 ± 0.07 <sup>a</sup>	0.19 ± 0.015 <sup>b</sup>	0.16 ± 0.010 <sup>d</sup>	0.18 ± 0.007 <sup>c</sup>
Cohesiveness	0.020 ± 0.002 <sup>c</sup>	0.025 ± 0.005 <sup>bc</sup>	0.035 ± 0.005 <sup>a</sup>	0.029 ± 0.002 <sup>ab</sup>
Gumminess	0.23 ± 0.018 <sup>a</sup>	0.21 ± 0.026 <sup>a</sup>	0.14 ± 0.01 <sup>c</sup>	0.17 ± 0.003 <sup>b</sup>
Chewiness	0.046 ± 0.014 <sup>a</sup>	0.039 ± 0.004 <sup>ab</sup>	0.022 ± 0.0008 <sup>c</sup>	0.031 ± 0.001 <sup>bc</sup>
Resilience	0.006 ± 0.0009 <sup>a</sup>	0.005 ± 0.0013 <sup>c</sup>	0.005 ± 0.0006 <sup>d</sup>	0.005 ± 0.001 <sup>b</sup>

Results are expressed as means ± S.D (n=3). a-b means within rows with different superscript are significantly different ( $p \leq 0.05$ ) from each other.

CBPM- conventional butter from pooled milk; DBKF- desi butter from Karan Fries ; DBSW- desi butter from Sahiwal; DBTP- desi butter from Tharparkar

The texture profile of butter prepared from selected indigenous and crossbred cow milk by conventional and desi method is illustrated in Table 3. Statistical analysis revealed significant differences ( $p < 0.05$ ) in hardness, adhesiveness, cohesiveness, gumminess, and chewiness within breeds. Notably, butter prepared through the conventional method in winter exhibited a fracture point, whereas no fracturability was observed in butter prepared using the desi method. Conventional butter was found to be significantly harder ( $p < 0.05$ ) than butter prepared through the desi method, with Sahiwal butter displaying the lowest hardness.

During the double-byte test, conventional butter exhibited higher adhesiveness compared to butter prepared using the desi method. The hardness values for butter prepared in winter were significantly higher than those prepared in summer, a phenomenon attributed to differences in fatty acid composition as reported by Blaško et al. (2010). Additionally, Sert and Mercan (2020) highlighted that butter made through continuous methods was harder than conventionally churned butter. Summer butter displayed significantly higher adhesiveness values compared to winter butter, possibly due to the presence of more unsaturated fatty acids, promoting softer texture and enhanced adhesive properties, a finding corroborated by Pădureț (2021). Cohesiveness values remained relatively constant for Sahiwal butter across both seasons, whereas cohesiveness increased in summer for conventional, Karan Fries, and Tharparkar butters. The cohesiveness value for winter and summer butter were 0.019 to 0.035 and 0.021 to 0.035, respectively. Gumminess exhibited a slight increase in Karan Fries and Tharparkar butter, while a slight decrease was observed in conventional and Sahiwal butter; however, these differences were non-significant ( $p \geq 0.05$ ).

Chewiness, calculated as the multiplication of gumminess and springiness, representing the energy needed to chew solid food until it is ready for swallowing. It remained same for both the winter and summer season in case of Sahiwal and Tharparkar butter but variation found in butter prepared from Karan fries and butter by conventional method, respectively. Chewiness values for butter in winter and summer ranged from 0.022 to 0.048 and 0.022 to 0.044, respectively. Resilience, a measure of how well a product regains its original shape and size, did not exhibit a significant difference ( $p \geq 0.05$ ) between butter prepared in winter and summer seasons.

Texture analyses provided valuable insights into the physical properties of butter, the impact of breed, season, and preparation methods on its texture characteristics.

#### Assessment of butter keeping quality

The keeping quality of butter is characterized by changes in % FFA. Butter lipolysis, the process of breaking down triglycerides into fatty acids, may increase % free fatty acids with storage time, as illustrated in Fig. 2 (a, b). Karan Fries butter exhibited higher initial free fatty acid levels than conventional butter. By the 60<sup>th</sup> day of storage, conventional butter showed no significant difference in %FFA, while desi butter displayed appreciable differences between trial days. On the 60<sup>th</sup> day, the FFA released from both conventional and desi butter was below the regulatory limits outlined in the Food Safety and Standard Regulations (2017).

Fig. 2 (a, b), illustrates how storage duration affects free fatty acids in winter and summer butter. At the beginning of storage, the levels of free fatty acids were 0.142%, 0.270%, 0.260%, and 0.280% for conventional, Karan Fries, Sahiwal, and Tharparkar

butter prepared during the winter season, respectively. By the end of storage, these levels had changed to 0.166%, 0.367%, 0.347%, and 0.367%. For summer butter, the initial free fatty acid levels were 0.143%, 0.274%, 0.277%, and 0.290%, increasing to 0.168%, 0.386%, 0.349%, and 0.371% by the end of storage for conventional, Karan Fries, Sahiwal, and Tharparkar butter, respectively.

After 30 days at 4°C, the FFA content of desi butter increased rapidly, unlike conventional butter, which remained relatively stable. This rapid increase in FFA in desi butter might be attributed to lipolytic enzymes secreted by the starter culture, leading to the breakdown of triglycerides into di- or mono-glycerides, releasing free fatty acids. In contrast, conventional butter, with its inherent lipase activity, showed a more controlled release of free fatty acids. From Fig. 2 (a, b), it is evident that FFA% significantly impacts butter shelf life. Karan Fries butter exhibited the highest increase in FFA content, followed by Tharparkar, Sahiwal, and conventional butter. These findings underscore the importance of monitoring free fatty acid levels for assessing butter quality and shelf life.

### Conclusion

In conclusion, the findings of the present study highlight significant potential for the promotion of milk and milk products derived from indigenous breeds within the dairy sector. Butter prepared from indigenous breeds demonstrates favourable characteristics, including lower hardness, excellent spreadability, appealing colour values, and notable content of  $\beta$ -carotene and CLA. These attributes not only enhance the organoleptic qualities of the butter but also contribute to its nutritional value. The results underscore the importance of preserving and promoting indigenous breeds in the dairy industry, emphasizing the diverse benefits they offer in the production of high-quality dairy products. This research provides valuable insights for stakeholders, policymakers, and dairy industry professionals, supporting informed decision-making to foster the sustainable development of the dairy sector. Future studies could further explore specific genetic factors and breeding techniques to optimize these desirable traits in dairy products, ensuring the continued growth and success of indigenous breeds in the dairy market.

### Acknowledgment

The authors are thankful to the Director of the ICAR-National Dairy Research Institute, Karnal for granting access to the facilities necessary to conduct the research work.

### Conflict of Interest

The authors declare no conflict of interest in the presented research work.

### Funding- Not applicable

### References

- Ahmad N, Khan M K, Imran M, Suleman M N, Afzal S (2019) Bovine Feed Manipulation, Enhancement of Conjugated Linoleic Acid and Its Bioavailability. *Bovine Science-A Key to Sustainable Development*. IntechOpen. doi: 10.5772/intechopen.79306
- Battula SN, Laxmana Naik N, Sharma R, Mann B (2020) Ghee, Anhydrous milk fat and butteroil, *Dairy Fat Products and Functionality*, New York, NY: Springer 399–430.
- Blaško J, Kubiniec R, Górova R, Fabry I, Lorenz W, Sojak L (2010) Fatty acid composition of summer and winter cows' milk and butter. *J Food Nutr Res* 49 169–177
- Calderon F, Chauveau-Duriot B, Martin B, Graulet B, Doreau M, Noziere P (2007) Variations in carotenoids, vitamins A and E, and colour in cow's plasma and milk during late pregnancy and the first three months of lactation. *J Dairy Sci* 90: 2335-2346.
- Chia J S J, McRae J L, Kukuljan S, Woodford K, Elliott R B, Swinburn B, Dwyer K M (2017) A1 beta-casein milk protein and other environmental pre-disposing factors for type 1 diabetes. *Nutrition & Diabetes* 7(5): e274-e274
- De S (2012) Indian dairy products. In S De (Ed.), *Outlines of dairy technology* (pp. 382–466). New Delhi: Oxford University Press
- Dhankhar J, Sharma R, Mann B (2017) Optimization of various steps for RP-HPLC determination of  $\beta$ -carotene in milk fat. *Int Food Res J* 24(4): 1393-1398
- Givens D I, Lovegrove J A (2020) Dairy fats and health. In *Milk and Dairy Foods* (pp. 29–49). Academic Press
- Grune T, Lietz G, Palou A Ross AC, Stahl W, Tang G, Thurnham D, Yin S, Biesalski HK (2010)  $\beta$ -Carotene is an important vitamin A source for humans. *J Nutr* 140(12): 2268S-2285S
- Antone U, Zagorska J, Sterna V, Jemeljanovs A, Berzins A, Ikaunieca D (2015) Effects of dairy cow diet supplementation with carrots on milk composition, concentration of cow blood serum carotenoids, and butter oil fat-soluble antioxidative substances. *Agron Res* 13(4): 879–891
- Indu B, Jayaprakasha HM (2021) Conjugated Linoleic Acid-The Natural Trans Fat: A Review. *Asian J Dairy Food Res* 40(4): 351–357
- IS 3507 (1966) Method of sampling and test for butter. <https://law.resource.org/pub/in/bis/S06/IS.3507.1966.pdf>
- IS 548-1 (1964) Methods of sampling and test for oils and fats, Part I: Methods of sampling, physical and chemical tests. <https://archive.org/details/gov.in.is.548.1.1964>
- ISO 15884 (2002)/IDF 182: 2002 Milk fat- Preparation of fatty acid methyl esters. International Organization for Standardization, Geneva
- Kumar S, Banakar P S, Tyagi A K, Sharma H (2022) Intra-species variation in fatty acid profile and nutritional indices of cattle (*Bos indicus*), buffalo (*Bubalus bubalis*) and goat (*Capra hircus*) ghee deciphered using GC-FID and FT-IR spectroscopy. *Int Dairy J* 129: 105342
- Marino VM, Schadt I, La Terra S, Manenti M, Caccamo M, Licitra G, Carpino S (2012) Influence of season and pasture feeding on the content of  $\alpha$ -tocopherol and  $\beta$ -carotene in milk from Holstein, Brown Swiss and Modicana cows in Sicily. *Dairy Sci Technol* 92(5): 501–513
- Milovanovic B, Djekic I, Micinovic J, Djordjevic V, Lorenzo JM, Barba FJ, Mörlein D, Tomasevic I (2020) What is the colour of milk and dairy products and how is it measured?. *Foods* 9(11): 1629
- NDDB report. (2022) Milk production in India. <https://www.nddb.coop/information/stats/milkprodindia> (Accessed on 23.12.2023)
- Noziere P, Graulet B, Lucas A, Martin B, Grolier P, Doreau M (2006) Carotenoids for ruminants, from forages to dairy products. *Anim Feed Sci Technol* 131(3): 418-450.

- O'Callaghan TF, Faulkner H, McAuliffe S, O'Sullivan MG, Hennessy D, Dillon P, Kilcawley KN, Stanton C, Ross RP (2016) Quality characteristics, chemical composition, and sensory properties of butter from cows on pasture versus indoor feeding systems. *J Dairy Sci* 99(12): 9441–9460
- Pădureț S (2021) The Effect of Fat Content and Fatty Acids Composition on Colour and Textural Properties of Butter. *Molecules* 26(15): 4565
- Panchal B, Bhandari B (2020) Butter and dairy fat spreads. *Dairy Fat Products and Functionality: Fundamental Science and Technology* 509-532.
- Parashar A and Saini RK (2015) A1 milk and its controversy-a review. *Int j bioassays* 4(12): 4611–4619
- Park J, Choi J, Kim DD, Lee S, Lee B, Lee Y, Kim S, Kwon S, Noh M, Lee MO (2021) Bioactive lipids and their derivatives in biomedical applications. *Biomol Ther* 29(5): 465.
- Patel A A, Sharma P, Patel H (2015) Butter and fat spreads: Manufacture and quality assurance. *Dairy Processing and Quality Assurance* 266-286
- Ponnal RP, Wood J E, Gill BD, Bergonia CA, Longstaff WM, Slabbert V, Bainbridge-Smith LC, Crawford RA (2021) Colourimetry of dairy products. *Int Dairy J* 113:104886.
- Sert D, Mercan E (2020) Microbiological, physicochemical, textural characteristics and oxidative stability of butter produced from high-pressure homogenisation treated cream at different pressures. *Int Dairy Journal*, 111: 104825
- Stanton C, Murphy J, McGrath E, Devery R (2020) Animal feeding strategies for conjugated linoleic acid enrichment of milk. In *Advances in conjugated linoleic acid research*. pp 123–145 AOCS Publishing