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## Comparative study on the impact of seasonal variations on pure cow and buffalo ghee

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### Abstract

The quality and stability of ghee, a clarified milk fat widely used in South Asian cuisine and traditional medicine, are significantly influenced by environmental and processing conditions. This study investigates the effect of seasonal variations on the physico-chemical parameters of pure cow and buffalo ghee. Analytical parameters assessed include Butyro-refractometer (BR) readings, Reichert-Meissl (RM) values, Polenske values, and Kirschner values (IV). Ghee samples were produced using the creamery butter method to ensure consistency. Findings revealed that seasonal variations significantly affected all measured parameters, indicating a strong correlation between climatic factors and ghee composition. Notably, all values remained within the permissible thresholds established by AGMARK (1981) and the Food Safety and Standards Regulations (FSSR, 2011). A key observation was that buffalo ghee consistently exhibited higher Kirschner values (26.84-33.96) than cow ghee (20.74-24.14), underscoring the Kirschner index as a robust marker for distinguishing between the two types.

**Keywords:** Kirschner Value, ghee, cow, buffalo, physico-chemical constant

### Introduction

India stands as the global leader in milk production, contributing an estimated 239.3 million tonnes during the 2023-2024 period (NDDB, 2025). Accounting for nearly one-quarter of the world's total milk output, the Indian dairy sector is predominantly sustained by cow and buffalo milk. While cow milk comprises approximately 83% of global production and buffalo milk around 13% (FAO, 2023), the Indian scenario diverges notably- buffalo milk contributes nearly 40-43% of the national supply (NDDB, 2024). Among the diverse array of dairy products, ghee represents the most significant avenue of milk utilization in India, underscoring the need for rigorous quality assessment.

Ghee, derived from the Sanskrit term *ghrita* meaning "sprinkled," is a clarified form of butter that has held cultural, culinary, and medicinal importance across the Indian subcontinent for centuries. Its appeal lies in its natural origin, rich sensory profile, nutritional density, and functional versatility. Traditionally prepared from cow, buffalo, or mixed milk sources, ghee is valued for its digestibility and health-promoting properties, attributed to its short-chain fatty acids. Estimates suggest that approximately 30-35% of India's milk is converted into ghee annually (Pawar *et al.*, 2012) <sup>[12]</sup>, reinforcing its role as a preferred medium for cooking, frying, and garnishing.

Beyond its culinary applications, ghee is recognized for containing essential fat-soluble vitamins (A, D, E, and K) and bioactive fatty acids such as linolenic and arachidonic acid. Historical accounts and traditional medicine sources have credited ghee with benefits ranging from cognitive enhancement to therapeutic effects on ulcers and ocular health (Rangappa & Achaya, 1974) <sup>[14]</sup>. Given its widespread consumption and cultural significance, it is imperative that ghee production adheres to compositional standards outlined by the Food Safety and Standards Authority of India (FSSAI, 2011).

However, the physico-chemical properties of ghee are not static; they are influenced by multiple variables including the method of preparation, clarification temperature, species of milk origin, feeding practices, and seasonal conditions. In light of these factors, the present study aims to investigate the impact of seasonal variation on the physico-chemical characteristics of pure cow and buffalo ghee.

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## Material and Methods

### Chemicals and reagents

Potassium hydroxide pellets, Sodium hydroxide pellets and Sulphuric acid (AR grade, Qualigens fine chemicals, Mumbai, India), Oxalic acid (Glaxo Laboratories Ltd, Mumbai, India), Barium hydroxide and Silver Sulfate (AR grade Sisco Research Laboratories Pvt. Ltd., Maharashtra, India), Glycerol (AR grade, Ranbaxy Laboratories Ltd., Punjab, India), Phenolphthalein (AR, s.d. fine-chem Ltd., Mumbai, India).

### Preparation of ghee samples

Samples of cow/buffalo ghee were prepared by creamery butter method (De, 2010). Cow and buffalo milks used for the preparation of respective ghee samples were collected every two months up to complete ten months (August-September, October-November, December-January and February-March, April-May) from the local dairies of kurukshetra. Soon after the collection of milk samples, these were warmed to 40 °C and separated into cream using mechanical cream separator. Butter was prepared from cream under standard conditions (9 °C in summer and 13 °C in winter) by churning of cream using hand operated butter churn. The butter was then heated on direct flame in a stainless-steel vessel and clarified into ghee with continuous stirring at temperature of 120 °C/flash. Ghee was then filtered through 6-8-fold muslin cloth followed by further filtration by using Whatman No. 4 filter paper in glass vacuum assembly and finally filled in bottles, cooled to room temperature and kept in a refrigerator (5 to 10 °C) till further analysis.

### Physico-chemical constants of ghee

The physico-chemical constants such as Reichert-Meissl (RM) value, Polenske value (PV) and Butyro-refractometer reading of above said samples (Pure cow ghee samples and buffalo ghee samples) were determined by the methods as described in SP: 18 (Part XI) -1981 (BIS 1981). Kirschner

Value (K) was determined by the method of Ghatak and Bandyopadhyaya (2007).

### Statistical Analysis

The data obtained in the present study was subjected to one-way analysis of variance (ANOVA) for the significant difference in the samples via Duncan's Multiple Comparison test performed at 95% confidence interval with SAS software (version 9.3 for windows), San Diego California, USA.

### Results and Discussion

Physico-chemical constants of pure ghee samples

#### Effect of season on Butyro-refractometer (B.R) reading of pure cow and buffalo ghee

The seasonal variation in Butyro-refractometer (B.R.) readings for pure cow and buffalo ghee is summarized in Table 1. The data indicate that cow ghee exhibited its highest B.R. value during February-March ( $42.89 \pm 0.004$ ) and the lowest during December-January ( $42.18 \pm 0.004$ ). Similarly, buffalo ghee showed a peak B.R. reading in February-March ( $42.22 \pm 0.004$ ) and a minimum in August-September ( $41.29 \pm 0.004$ ). Statistical analysis (ANOVA) confirmed significant monthly variation in B.R. readings for both types of ghee ( $p < 0.05$ ).

All observed values conformed to the FSSAI-prescribed range for ghee in northern India (40–43 at 40 °C). Notably, cow ghee consistently demonstrated slightly higher B.R. readings than buffalo ghee, which may be attributed to its relatively greater proportion of unsaturated fatty acids and lower saturated fat content (Kumar, 2003; Blasi *et al.*, 2008; Menard *et al.*, 2010) [6, 2, 10]. These findings align with previous reports by Kumar (2013) [8], suggesting that seasonal factors such as ambient temperature and humidity may influence animal feeding behavior and digestion, thereby affecting milk fat composition.

**Table 1:** Butyro-refractometer (B.R.) reading of pure cow ghee and buffalo ghee

Temperature of Clarification	Sample Intervals					
	AUG-SEP	OCT-NOV	DEC-JAN	FEB-MAR	APR-MAY	CD value ( $P \leq 0.05$ )
Cow ghee	$42.84 \pm 0.004^b$	$42.27 \pm 0.004^c$	$42.18 \pm 0.004^d$	$42.89 \pm 0.004^a$	$42.85 \pm 0.004^a$	0.0108
Buffalo ghee	$41.29 \pm 0.004^d$	$41.48 \pm 0.00^c$	$41.91 \pm 0.00^b$	$42.22 \pm 0.00^a$	$41.86 \pm 0.004^a$	0.0108

Data presented is mean  $\pm$  SE of three determinations

Values bearing different superscripts in each row differ significantly

#### Effect of season on Reichert-Meissl (RM) value of pure cow and buffalo ghee

Seasonal fluctuations in the Reichert-Meissl (RM) value of pure cow and buffalo ghee are summarized in Table 2. For cow ghee, the highest RM value was recorded during February-March ( $30.45 \pm 0.31$ ), while the lowest occurred in August-September ( $28.18 \pm 0.22$ ). Statistical analysis revealed significant differences ( $p < 0.05$ ) in RM values between samples prepared from ripened cream during August-September and October-November, compared to those from December-January and February-March.

In the case of buffalo ghee, RM values peaked in December-January ( $31.78 \pm 0.09$ ) and were lowest in October-November ( $32.01 \pm 0.08$ ). Although significant monthly variation was observed ( $p < 0.05$ ), no statistical

difference was found between December-January and February-March. Overall, buffalo ghee exhibited higher RM values than cow ghee, which may be attributed to its relatively greater concentration of short-chain fatty acids such as butyric and caproic acids (Kumar, 2008; Lal & Narayanan, 1984; Ramamurthy & Narayanan, 1971) [7, 9, 13]. These findings suggest that seasonal conditions particularly temperature and humidity, may influence animal feeding behavior and digestion, thereby affecting milk fat composition. The results align with previous studies (Kumar, 2013; Rangappa & Achaya, 1974) [8, 14] and confirm that RM values for both cow and buffalo ghee remained within the FSSAI prescribed limits for northern India, i.e., not less than 28.

**Table 2:** Reichert-Meissl (RM) value of pure cow ghee and buffalo ghee

Temperature of Clarification	Sample Intervals					
	AUG-SEP	OCT-NOV	DEC-JAN	FEB-MAR	APR-MAY	CD value ( $P \leq 0.05$ )
Cow ghee	28.18±0.22 <sup>b</sup>	28.64±0.31 <sup>b</sup>	29.82±0.17 <sup>a</sup>	30.45±0.31 <sup>a</sup>	28.45±0.31 <sup>b</sup>	0.705
Buffalo ghee	31.21±0.146 <sup>b</sup>	32.01±0.08 <sup>c</sup>	31.78±0.09 <sup>a</sup>	31.55±0.04 <sup>a</sup>	31.56±0.04 <sup>a</sup>	0.267

Data presented is mean± SE of three determinations

Values bearing different superscripts in each row differ significantly

### Effect of season on Polenske value of pure cow and buffalo ghee

The average of Polenske value in different periods of the year of pure cow and buffalo ghee are presented in the tables 3. It can be seen from the table 3 that Polenske value of pure cow ghee was highest in the month of December-January (1.73±0.04) and lowest in month of August-September (1.37±0.04). Analysis of variance of the data (Table 3) revealed that Polenske value of pure cow ghee in August-September did not differ significantly ( $p < 0.05$ ) from Polenske value in October–November and Polenske value in December-January did not differ significantly ( $p < 0.05$ ) from Polenske value in February-March but Polenske value in August-September and October–November differs significantly from December-January and February-March. This may be attributed to the effect of diet on Polenske value. It can be seen from the table 3 that Polenske value of pure buffalo ghee was highest in the month of December-January (1.37±0.041) and lowest in month of August-September and February-March (1.23±0.041). Analysis of variance of the data (Table 3) revealed that Polenske value

of pure buffalo ghee differed significantly ( $p < 0.05$ ) in all months whereas no significant difference in Polenske value was observed in August-September and February-March. Analysis of variance of the data (Table 3) revealed that Polenske value of pure buffalo ghee differed significantly ( $p < 0.05$ ) in all months. Perusal of the data also revealed that average Polenske value of pure cow ghee was higher than the Polenske value of pure buffalo ghee as cow ghee (Table 3) contained slightly higher amount of caprylic acid and capric acid as compared to buffalo ghee (Kumar, 2013; Kumar, 2008; Lal and Narayanan, 1984; Ramamurthy and Narayanan, 1971) [8, 7, 9, 13].

Results obtained in the present study; on the Polenske value of cow ghee is in general agreement with those reported by Kumar (2013) [8], Kumar (2008) [7], Rangappa and Achaya (1974) [14]. This may be due to the change in feeding as well as some seasonal variations like heat and humidity, wherein behavior of animals with respect to feed intake and digestion was affected. In both the cases Polenske value was meeting the AGMARK standards of ghee i.e. 1 to 2.

**Table 3'** Polenske value of pure cow ghee and buffalo ghee

Temperature of Clarification	Sample Intervals					
	AUG-SEP	OCT-NOV	DEC-JAN	FEB-MAR	APR-MAY	CD value ( $P \leq 0.05$ )
Cow ghee	1.37±0.04 <sup>b</sup>	1.47±0.04 <sup>b</sup>	1.73±0.04 <sup>a</sup>	1.67±0.04 <sup>a</sup>	1.59±0.04 <sup>ab</sup>	0.108
Buffalo ghee	1.23±0.041 <sup>b</sup>	1.27±0.041 <sup>ab</sup>	1.37±0.041 <sup>a</sup>	1.23±0.041 <sup>b</sup>	1.24±0.041 <sup>b</sup>	0.108

Data presented is mean± SE of three determinations

Values bearing different superscripts in each row differ significantly

### Kirschner value

The average of Kirschner value in different periods of the year of pure cow and buffalo ghee are presented in the tables 4. It can be seen from the table 1.4 that Kirschner value of pure cow ghee was highest in the month of August-September (22.96±0.23) and lowest in month of December-January (21.37±0.09). Analysis of variance of the data (Table 4) revealed that Kirschner value of pure cow ghee in August-September and October-November differed significantly ( $p < 0.05$ ) from Kirschner value in December-January and February-March but remained unaffected in period from August-September and October–November. It can be seen from the table 4 that Kirschner value of pure buffalo ghee was highest in the month of August–September (29.34±0.056) and lowest in month of December-January (27.23±0.156). Analysis of variance of the data (Table 1.4)

revealed that Kirschner value of pure buffalo ghee differed significantly ( $p < 0.05$ ) in all months whereas no significant difference in Kirschner value was observed in October–November and February-March. Perusal of the data also revealed that average Kirschner value of pure buffalo ghee was higher than the Kirschner value of pure cow ghee (Tables 4) as buffalo ghee contained slightly higher amount of butyric acid as compared to cow ghee (Kehar, 1956) [5]. Perusal of the data revealed that in both the cases i.e. ghee the effect of season in Kirschner value was visible. However, Kehar (1956) [5] reported high Kirschner value in winter season and lower in summer season for cow ghee samples. He attributed this change to the diminution in amount of secretion of butyric acid due to paucity of green fodder in summer.

**Table 4:** Kirschner value of pure cow ghee of pure cow and buffalo ghee

Temperature of Clarification	Sample Intervals					
	AUG-SEP	OCT-NOV	DEC-JAN	FEB-MAR	APR-MAY	CD value ( $P \leq 0.05$ )
Cow ghee	22.96±0.23 <sup>a</sup>	22.87±0.06 <sup>a</sup>	21.37±0.09 <sup>b</sup>	21.59±0.43 <sup>b</sup>	21.89±0.43 <sup>b</sup>	0.663
Buffalo ghee	29.34±0.056 <sup>a</sup>	28.11±0.019 <sup>b</sup>	27.23±0.156 <sup>c</sup>	28.14±0.122 <sup>b</sup>	28.79±0.122 <sup>ab</sup>	0.275

Data presented is mean± SE of three determinations

Values bearing different superscripts in each row differ significantly

### Physico-chemical constants to characterize cow ghee and buffalo ghee

As presented in Table 5, the Butyro-refractometer (B.R.) readings for cow ghee ranged from 41.87 to 43.62, while buffalo ghee exhibited a slightly broader range of 40.01 to 43.23. Due to this overlap, it is challenging to establish a distinct B.R. value range that reliably differentiates between the two types of ghee. A similar pattern was observed for the Polenske values, where the ranges for cow and buffalo ghee samples intersected, making it impractical to use this parameter for species-specific identification.

In contrast, the Kirschner value demonstrated a clear and consistent distinction. Buffalo ghee samples consistently recorded higher values, with even the lowest readings surpassing the upper limit observed in cow ghee. This pronounced separation suggests that the Kirschner value may serve as a reliable marker for distinguishing buffalo ghee from cow ghee. Establishing species-specific thresholds for this parameter could enhance the characterization and authentication of ghee in regulatory and commercial contexts.

**Table 5:** Physico-chemical constants of pure cow and buffalo ghee irrespective of seasonal variations

Physico-chemical constant	Cow ghee		Buffalo ghee	
	Range	Average	Range	Average
B.R. reading	41.87-43.62	42.55±0.052	40.01-43.23	41.48±0.105
RM Value	27.56-31.13	29.12±0.109	31.91-39.99	31.42±0.174
Polenske Value	1.30-1.90	1.58±0.017	1.10-1.50	1.28±0.011
Kirschner Value	20.74-21.14	22.12±0.106	26.84-33.96	28.34±0.190

Data presented is mean±SE of 15 determinations

### Conclusion

The findings of this study indicate that Butyro-refractometer (B.R.) readings and Polenske values cannot be reliably used to distinguish between cow and buffalo ghee, as the observed ranges for both types overlapped considerably. In contrast, the Kirschner value demonstrated a consistent and significant difference: buffalo ghee samples exhibited higher values across all seasons, with the lowest recorded Kirschner value still exceeding the highest value observed in cow ghee. This distinct separation suggests that species-specific thresholds for Kirschner value could be established, offering a practical tool for differentiating cow ghee from buffalo ghee.

Seasonal variation was evident in all ghee samples, likely influenced by changes in ambient temperature, humidity, and animal feeding behavior, which in turn affect milk composition. Despite these fluctuations, all measured parameters remained within the permissible limits defined by AGMARK (1981) and the Food Safety and Standards Regulations (FSSR, 2011), affirming the regulatory compliance of the ghee samples throughout the study period.

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