



International Journal of Agriculture Extension and Social Development

ISSN Print: 2618-0723
ISSN Online: 2618-0731
NAAS Rating (2026): 5.04
IJAESD 2026; SP-9(10): 36-40
www.extensionjournal.com
Received: 30-07-2026
Accepted: 25-09-2026
Published: 07-10-2026

Nivedita PG

M.Tech Scholar, Department of Dairy Chemistry, ICAR–National Dairy Research Institute, Southern Regional Station, Bengaluru, Karnataka, India

Lakshman Naik

Principal Scientist, Department of Dairy Chemistry, ICAR–National Dairy Research Institute, Southern Regional Station, Bengaluru, Karnataka, India

Balisana Kamyabali

M.Tech Scholar, Department of Dairy Chemistry, ICAR–National Dairy Research Institute, Southern Regional Station, Bengaluru, Karnataka, India

Isolation, quantification and physicochemical characterization of whole casein and individual casein fractions from Deoni and Malnad *Gidda* cattle milk

Nivedita PG¹, Lakshman Naik², Balisana Kamyabali³

DOI: <https://www.doi.org/10.33545/26180723.2026.v9.i10Sa.4386>

ABSTRACT

Casein is the major protein in bovine milk and is largely responsible for its nutritional, functional, and technological properties. Despite the importance of indigenous cattle breeds, information on the composition and physicochemical characteristics of their casein fractions remains limited. In this study, whole casein and its individual fractions were isolated, quantified, and characterized from the milk of Deoni and Malnad *Gidda* cattle. Fresh pooled milk samples collected from healthy lactating cows maintained at the Livestock Research Centre, ICAR–National Dairy Research Institute, Southern Regional Station, Bengaluru, were used for the analysis. Whole casein was isolated by isoelectric precipitation and subsequently separated into α_{s1} -, α_{s2} -, β -, and κ -casein fractions using the differential urea solubility method. The isolated fractions were analysed for protein, moisture, ash, fat, and acid-insoluble ash contents. Malnad *Gidda* milk contained higher protein ($3.48 \pm 0.43\%$) and whole casein (28.05 ± 0.43 g/L) than Deoni milk ($3.35 \pm 0.27\%$ and 25.50 ± 0.54 g/L, respectively). Among the individual fractions, α_{s1} -casein and β -casein were the predominant proteins in both breeds, while Malnad *Gidda* milk showed relatively higher concentrations of α_{s1} - and α_{s2} -casein. Physicochemical analysis revealed that β -casein had the highest protein content, α_{s2} -casein contained the highest moisture content, and κ -casein exhibited the highest ash content. Fat and acid-insoluble ash were negligible in all casein fractions. Overall, the results demonstrate that Malnad *Gidda* milk is a richer source of whole casein and its major fractions than Deoni milk. These findings provide valuable baseline information on the physicochemical characteristics of casein fractions from indigenous cattle breeds and support their potential application in dairy processing and the development of functional dairy ingredients.

Keywords: Casein fractions, Deoni, Indigenous cattle, Malnad *Gidda*, Physicochemical characterization, Whole casein

Introduction

Milk is widely recognized as one of the most nutritionally complete natural foods, providing high-quality proteins, lipids, lactose, vitamins, and minerals that are essential for growth, development, and overall health. Beyond its nutritional value, milk also contains several biologically active components that contribute to its functional and technological properties. The composition of milk is influenced by factors such as species, breed, genetic makeup, feeding practices, stage of lactation, and environmental conditions. Consequently, understanding breed-related differences in milk composition is important for improving dairy production and developing value-added dairy products (Fox *et al.*, 2003; Fox *et al.*, 2015) [1, 2].

Caseins are the predominant proteins in bovine milk, accounting for nearly 80% of its total protein content. They are synthesized exclusively in the mammary gland and naturally exist as colloidal micelles stabilized by calcium phosphate. In addition to supplying essential amino acids, caseins play an important role in transporting calcium and phosphorus to the neonate. They also contribute significantly to the manufacture and quality of dairy products, particularly cheese, fermented milk products, and milk protein concentrates (Holt *et al.*, 1993; Fox *et al.*, 2003) [3, 1].

Bovine casein consists of four major fractions— α_{s1} -casein, α_{s2} -casein, β -casein, and κ -casein—each with distinct physicochemical and functional characteristics. Together, these fractions influence important properties of milk, including coagulation, micellar stability, emulsification, water-holding capacity, and overall processing performance. Among them, β -casein is the most hydrophobic fraction, whereas κ -casein is the only glycosylated casein and is essential for maintaining the stability of casein micelles. Variations in the proportions of these fractions can affect the technological properties of milk and its suitability for producing high-quality dairy products and functional ingredients (Swaigood, 2003; Fox *et al.*, 2015) [4, 2].

India possesses a rich diversity of indigenous cattle breeds that are well known for their adaptability, disease resistance, and ability to produce milk with desirable nutritional characteristics. Among these, Deoni and Malnad *Gidda* are two important indigenous breeds of southern India. Deoni cattle are valued for their dual-purpose utility and comparatively higher milk production, whereas Malnad *Gidda* cattle are well adapted to

Corresponding Author:

Nivedita PG

M.Tech Scholar, Department of Dairy Chemistry, ICAR–National Dairy Research Institute, Southern Regional Station, Bengaluru, Karnataka, India

the hilly regions of *Karnataka* and are recognized for producing milk with relatively higher protein content and favourable functional attributes. Previous studies have also reported that milk proteins from these indigenous breeds possess desirable nutritional characteristics compared with those from several exotic breeds (Das *et al.*, 2011; Thimmesh, 2018) [5, 6].

Although previous studies have described the general composition of milk from indigenous cattle, detailed information on the isolation, quantification, and physicochemical characteristics of whole casein and its individual fractions in Deoni and Malnad *Gidda* cattle is still limited. A better understanding of these casein fractions is important because they directly influence the nutritional quality, functional properties, and processing behaviour of milk. Such information would also help in assessing the potential of these indigenous breeds for the development of specialised dairy products and functional ingredients. Therefore, the present study aimed to isolate whole casein from the milk of Deoni and Malnad *Gidda* cattle, quantify the major casein fractions (α_1 -, α_2 -, β -, and κ -casein), and evaluate their physicochemical characteristics. The findings of this study are expected to provide baseline information on the casein profile of these indigenous breeds and contribute to their effective utilisation in dairy processing and the development of value-added dairy ingredients.

Materials and methods

Experimental location and milk sample collection

The study was conducted at the Livestock Research Centre (LRC), Southern Regional Station (SRS), ICAR–National Dairy Research Institute (NDRI), Bengaluru, *Karnataka*, India. Fresh pooled milk samples were collected from healthy lactating Deoni ($n = 10$) and Malnad *Gidda* ($n = 5$) cows maintained under identical feeding and management conditions. Milk was collected immediately after the morning milking and transported to the laboratory under refrigerated conditions. Before casein isolation, the samples were analysed for their physicochemical composition.

Chemicals, reagents and solvents

All chemicals and reagents used in the study were of analytical reagent (AR) grade. Hydrochloric acid, sodium hydroxide, urea, and other chemicals required for milk analysis, casein isolation, fractionation, and physicochemical characterization were obtained from Sigma-Aldrich and HiMedia Laboratories Pvt. Ltd., Mumbai, India. Petroleum ether, diethyl ether, and absolute ethanol were procured from Qualigens Fine Chemicals (Mumbai, India), Merck Specialities Pvt. Ltd. (Mumbai, India), and Jiangsu Huxari International Trade Co. Ltd. (China), respectively. Distilled water was used throughout the study, and all reagents were freshly prepared following standard laboratory procedures.

Glassware and plasticware

Volumetric flasks, measuring cylinders, beakers, pipettes, conical flasks, and test tubes were obtained from Borosil and Schott Duran. Before use, all glassware was thoroughly cleaned with detergent, rinsed with tap water followed by distilled water, and dried in a hot-air oven. Plasticware, including centrifuge tubes, storage vials, and sample containers, was procured from Tarsons Products Pvt. Ltd., Kolkata, India.

Dialysis membrane

Dialysis of whole casein and the isolated casein fractions was carried out using a dialysis membrane with a molecular weight cut-off of 12 kDa obtained from Sigma-Aldrich.

Physicochemical analysis of milk

Milk samples were analysed for fat, protein, lactose, ash, solids-not-fat (SNF), and total solids using standard analytical methods. Fat content was determined by the Gerber method (IS:1224, Part I, 1977), protein by the Kjeldahl method (AOAC, 2005) [7], lactose by the Lane and Eynon method (ISI, 1981) [8], ash by incineration in a muf-

file furnace according to AOAC (2005) [7], and total solids by the gravimetric method (ISI, 1981) [8]. All measurements were carried out in triplicate, and the results were expressed as mean $\pm$ standard deviation.

Isolation of whole casein

Whole casein was isolated from skim milk using the isoelectric precipitation method described by Davies *et al.* (1977) [9]. Fresh milk was first centrifuged to obtain skim milk, after which the pH was adjusted to 4.6 using dilute hydrochloric acid with continuous stirring to precipitate casein. The precipitated casein was recovered by centrifugation, washed several times with distilled water to remove residual lactose and soluble minerals, dialysed using a 12 kDa molecular weight cut-off membrane, and freeze-dried to obtain purified whole casein.

Fractionation of casein proteins

Whole casein was separated into α_1 -, α_2 -, β -, and κ -casein fractions following the differential urea solubility method described by Hipp *et al.* (1952) [10]. Separation was achieved through sequential precipitation under controlled urea concentrations and pH conditions. The isolated fractions were purified by dialysis to remove residual salts and other low-molecular-weight compounds, freeze-dried, and stored at -20°C until further analysis.

Quantification of whole casein and individual casein fractions

Whole casein and the isolated α_1 -, α_2 -, β -, and κ -casein fractions were quantified after freeze-drying and expressed as g/L of milk. Quantification was based on the dried weight of the purified fractions, and all values were calculated from three independent determinations.

Physicochemical characterization of casein fractions

Whole casein and the isolated casein fractions were analysed for protein, moisture, fat, ash, and acid-insoluble ash using standard AOAC (2005) [7] methods. Protein was determined by the Kjeldahl method, moisture by oven drying to constant weight, ash by muffle furnace incineration, and fat by the standard gravimetric method. Acid-insoluble ash was estimated according to standard dairy analytical procedures. All analyses were performed in triplicate, and the results were expressed on a percentage (w/w) basis.

Statistical analysis

The experimental data were analysed using one-way analysis of variance (ANOVA) with SAS software (Version 9.3; SAS Institute Inc., Cary, NC, USA). Results are presented as mean $\pm$ standard deviation (SD) of three independent experimental determinations ($n = 3$), and differences were considered statistically significant at $P < 0.05$.

Results and discussion

Physicochemical composition of milk

The physicochemical composition of milk from Deoni and Malnad *Gidda* cattle is presented in Table 1. Significant ($P < 0.05$) differences were observed between the two breeds for most of the compositional parameters. Malnad *Gidda* milk contained a higher protein content ($3.48 \pm 0.43\%$) than Deoni milk ($3.35 \pm 0.27\%$). Since protein is one of the major determinants of nutritional value and dairy processing efficiency, the higher protein level in Malnad *Gidda* milk suggests its greater suitability for producing cheese, casein, and milk protein concentrates. Similar observations have been reported for indigenous cattle breeds with superior milk protein characteristics (Fox *et al.*, 2015; Thimmesh, 2018) [2, 6].

Deoni milk, on the other hand, contained relatively higher fat and total solids, whereas Malnad *Gidda* milk had higher lactose and solids-not-fat (SNF) contents. The ash content was comparable between the two breeds and did not differ significantly. These differences are likely associated with inherent breed characteristics and genetic variation, as both breeds were maintained under similar feeding and man-

agement conditions. Comparable breed-related differences in milk composition have also been documented by Fox *et al.* (2003) [1] and Thimmesh (2018) [6].

The relatively higher protein and SNF contents of Malnad *Gidda* milk are advantageous for dairy processing because they contribute to im-

proved product yield, enhanced nutritional value, and better functional properties. These characteristics make Malnad *Gidda* milk a promising raw material for the manufacture of value-added dairy products and milk proteins.

Table 1. Physicochemical composition of milk from Deoni and Malnad *Gidda* cattle

Parameter	Deoni	Malnad <i>Gidda</i>
Fat (%)	4.10 ± 0.10	3.90 ± 0.52
Protein (%)	3.35 ± 0.27	3.48 ± 0.43
Lactose (%)	4.50 ± 0.10	4.70 ± 0.17
Ash (%)	0.75 ± 0.01	0.68 ± 0.04
SNF (%)	8.47 ± 0.12	8.82 ± 0.15
Total solids (%)	12.85 ± 0.10	12.73 ± 0.14

Values are expressed as mean ± SD of three independent laboratory determinations performed on pooled milk samples.

Quantification of whole casein and individual casein fractions

The concentrations of whole casein and its individual fractions in Deoni and Malnad *Gidda* milk are presented in Table 2. Significant ($P < 0.05$) differences were observed between the two breeds for whole casein and selected casein fractions. Whole casein concentration was higher in Malnad *Gidda* milk (28.05 ± 0.43 g/L) than in Deoni milk (25.50 ± 0.54 g/L). This result is consistent with the higher protein content observed in Malnad *Gidda* milk and indicates its greater potential for the production of casein-rich dairy products.

Among the individual fractions, α_1 -casein was the predominant fraction in both breeds, followed by β -casein. Malnad *Gidda* milk also contained significantly higher α_2 -casein than Deoni milk. In contrast, only slight differences were observed in β -casein and κ -casein concentrations between the two breeds. These results suggest that the

higher total casein content of Malnad *Gidda* milk is mainly due to its greater α_1 - and α_2 -casein concentrations.

The distribution of casein fractions observed in this study agrees with previous reports describing α_1 -casein and β -casein as the major components of bovine casein, while α_2 - and κ -casein occur in comparatively smaller amounts. Fox *et al.* (2015) [2] reported that α_1 -casein and β -casein largely determine the nutritional and technological properties of milk, whereas κ -casein plays a key role in maintaining casein micelle stability. Amalfitano *et al.* (2018) also reported a similar distribution of casein fractions in Brown Swiss cattle, supporting the observations made in the present work.

The higher concentration of whole casein and the major casein fractions in Malnad *Gidda* milk highlights its potential for dairy applications requiring high casein recovery, including cheese manufacture, edible casein production, and milk protein concentrates. The results also provide valuable baseline information on the casein profile of these two indigenous cattle breeds.

Table 2. Quantification of whole casein and individual casein fractions in Deoni and Malnad *Gidda* milk

Casein fraction (g/L)	Deoni	Malnad <i>Gidda</i>
Whole casein	25.50 ± 0.54	28.05 ± 0.43
α_1 -casein	10.30 ± 0.43	11.27 ± 0.05
α_2 -casein	2.41 ± 0.51	3.48 ± 0.86
β -casein	9.70 ± 0.54	9.90 ± 0.002
κ -casein	3.10 ± 0.81	3.40 ± 0.24

Values are expressed as mean ± SD ($n = 3$ independent laboratory determinations).

Physicochemical characterization of whole casein and individual casein fractions

The physicochemical characteristics of the isolated α_1 -, α_2 -, β -, and κ -casein fractions are presented in Table 3. Differences in protein, moisture, ash, fat, and acid-insoluble ash contents were observed among the fractions. Overall, the casein fractions isolated from Malnad *Gidda* milk showed slightly higher protein contents than those obtained from Deoni milk. Among all the fractions, β -casein recorded the highest protein content ($90.00 \pm 0.62\%$ in Deoni and $91.60 \pm 0.95\%$ in Malnad *Gidda*), whereas α_2 -casein showed comparatively lower values. The high protein content of β -casein reflects the effectiveness of the isolation and purification procedure and is in agreement with previous reports identifying β -casein as one of the principal proteins in bovine milk.

Moisture content ranged from 6.30 to 8.60% among the isolated fractions. The highest moisture content was observed in α_2 -casein from both breeds, which may be related to its greater water-binding capacity and minor differences in freeze-drying efficiency. Nevertheless,

the moisture content of all fractions remained within the acceptable range reported for edible acid casein.

Ash content varied between 2.22 and 3.24%, with κ -casein showing the highest values in both breeds. This may be attributed to the stronger association of κ -casein with colloidal calcium phosphate and minerals present within the casein micelle. Similar observations have been reported for bovine milk caseins.

Fat content was negligible in all fractions, indicating efficient removal of milk fat during casein isolation from skim milk. Likewise, acid-insoluble ash values were very low, confirming the absence of significant inorganic impurities in the purified preparations. Lactose was not detected in any fraction because dialysis effectively removed soluble low-molecular-weight components before freeze-drying. The composition of the isolated fractions agrees well with the specifications for edible acid casein reported by FSSAI (2017) [11] and with earlier studies by Fox *et al.* (2003) [1] and Brunda (2019) [12].

Overall, the isolation and purification procedures produced highly purified casein fractions from both Deoni and Malnad *Gidda* milk. The compositional characteristics observed in this study are consistent with published reports and provide valuable baseline information on

the quality and functional properties of casein fractions from these indigenous cattle breeds.

Table 3. Physicochemical composition of isolated casein fractions from Deoni and Malnad *Gidda* cattle milk

Fraction	Protein (%)	Moisture (%)	Ash (%)	Fat (%)	Acid-insoluble ash (%)
Deoni α 1-casein	88.90 $\pm$ 2.40	6.80 $\pm$ 0.01	2.63 $\pm$ 0.42	0.067 $\pm$ 0.92	0.082 $\pm$ 0.02
Deoni α 2-casein	80.00 $\pm$ 0.92	8.60 $\pm$ 0.025	2.54 $\pm$ 0.01	0.029 $\pm$ 0.10	0.084 $\pm$ 0.75
Deoni β -casein	90.00 $\pm$ 0.62	6.68 $\pm$ 0.04	2.22 $\pm$ 0.05	0.040 $\pm$ 0.63	0.093 $\pm$ 0.78
Deoni κ -casein	89.70 $\pm$ 1.20	7.56 $\pm$ 0.02	3.24 $\pm$ 0.89	0.040 $\pm$ 0.33	0.045 $\pm$ 0.54
Malnad Gidda α 1-casein	90.25 $\pm$ 0.76	6.80 $\pm$ 1.50	2.50 $\pm$ 0.04	0.121 $\pm$ 0.45	0.260 $\pm$ 0.67
Malnad Gidda α 2-casein	87.00 $\pm$ 1.25	8.20 $\pm$ 0.06	2.60 $\pm$ 0.19	0.0015 $\pm$ 0.13	0.110 $\pm$ 0.56
Malnad Gidda β -casein	91.60 $\pm$ 0.95	6.30 $\pm$ 0.20	2.60 $\pm$ 0.86	0.0083 $\pm$ 0.09	0.070 $\pm$ 0.34
Malnad Gidda κ -casein	90.10 $\pm$ 0.43	6.40 $\pm$ 0.78	2.90 $\pm$ 1.43	0.0015 $\pm$ 0.76	0.075 $\pm$ 0.78

Values are expressed as mean $\pm$ SD ($n = 3$ independent laboratory determinations).

Conclusion

This study successfully isolated, quantified, and characterized whole casein and its major fractions from the milk of Deoni and Malnad *Gidda* indigenous cattle breeds. Significant differences were observed in both the physicochemical composition of milk and the distribution of casein fractions between the two breeds. Malnad *Gidda* milk contained higher protein and whole casein contents than Deoni milk, suggesting its greater potential for casein recovery and the production of protein-rich dairy products.

Among the individual casein fractions, α 1-casein and β -casein were the predominant proteins in both breeds, whereas α 2- and κ -casein were present in comparatively lower amounts. Physicochemical evaluation showed that β -casein had the highest protein content, α 2-casein exhibited the highest moisture content, and κ -casein contained the highest ash content. The negligible levels of fat and acid-insoluble ash in all fractions indicate that the isolation and purification procedures produced highly purified casein preparations.

Overall, this study provides valuable baseline information on the casein profile and physicochemical characteristics of milk from two important indigenous cattle breeds of southern India. The relatively higher casein content of Malnad *Gidda* milk highlights its potential as a suitable raw material for the manufacture of functional dairy ingredients, casein-based products, and other value-added dairy products. These findings also contribute to a better understanding of the dairy potential of indigenous cattle breeds and support their effective utilization in dairy processing, product development, and future research.

Acknowledgment

The authors gratefully acknowledge the Director, ICAR–National Dairy Research Institute (NDRI), Karnal, and the Head, Southern Regional Station, ICAR–NDRI, Bengaluru, *Karnataka*, for providing the necessary facilities and infrastructure to carry out this research. The authors sincerely thank the Department of Dairy Chemistry and the Livestock Research Centre, Southern Regional Station, ICAR–NDRI, Bengaluru, for their technical support and assistance in sample collection and laboratory analyses. The guidance and encouragement provided by the faculty and staff throughout the course of this study are also gratefully acknowledged.

References

1. Fox PF, McSweeney PLH. *Advanced Dairy Chemistry. Volume 1: Proteins*. Kluwer Academic/Plenum Publishers; 2003.
2. Fox PF, Uniacke-Lowe T, McSweeney PLH, O'Mahony JA. Milk proteins. In: *Dairy Chemistry and Biochemistry*. Springer, Cham; 2015:145–239.
3. Holt C, Sawyer L. Caseins as rheomorphic proteins: interpretation of primary and secondary structures of the α S1-, β - and κ -caseins. *Journal of the Chemical Society, Faraday Transactions*. 1993;89(15):2683–2692.
4. Swaisgood HE. Chemistry of the caseins. In: Fox PF, editor. *Advanced Dairy Chemistry. Volume 1: Proteins*. Boston, MA: Springer; 2003:139–201.
5. Das DN, Katakaltware MA, Ramesha KP, Reddy AO. Productive and reproductive performances of Deoni cattle under intensive management system. *Indian Journal of Animal Sciences*. 2011;81(11):1186–1188.
6. Thimmesha HB. *Elucidation of Physico-Chemical Quality of Deoni and Malnad Gidda Cattle Milk*. M.Sc. Thesis. Bengaluru, India: ICAR–National Dairy Research Institute, Southern Regional Station; 2018.
7. AOAC. *Official Methods of Analysis*. 18th ed. Association of Official Analytical Chemists, Washington, DC, USA; 2005.
8. ISI. *ISI Handbook of Food Analysis, Part XI: Dairy Products*. Indian Standards Institution, New Delhi; 1981.
9. Davies DT, Law AJR. An improved method for the quantitative fractionation of casein mixtures using ion-exchange chromatography. *Journal of Dairy Research*. 1977;44(2):213–221.
10. Hipp NJ, Groves ML, Custer JH, McMeekin TL. Separation of α -, β - and γ -casein. *Journal of Dairy Science*. 1952;35(3):272–281.
11. FSSAI. *Food Safety and Standards Regulations. Part III, Section 4*. Gazette of India, Ministry of Health and Family Welfare, Government of India; 2017.
12. Brunda S. *Bioactive Peptides from Deoni Cow Milk: Production, Characterization and Elucidation of Functional Properties*. Doctoral Dissertation, ICAR–National Dairy Research Institute, Bengaluru, India; 2019.
13. Amalfitano N, Cipolat-Gotet C, Cecchinato A, Malacarne M, Summer A, Bittante G. Milk protein fractions strongly affect the patterns of coagulation, curd firming, and syneresis. *Journal of Dairy Science*. 2019;102(4):2903–2917.
14. Aneja RP. *Technology of Indian Milk Products*. Dairy India Yearbook; 2002.
15. Boland M, Singh H. *Milk Proteins: From Expression to Food*. Academic Press; 2019.
16. Ganguli NC. *Milk Proteins*. Indian Council of Agricultural Research (ICAR), New Delhi; 1974.

17. Korhonen H. Milk-derived bioactive peptides: From science to applications. *Journal of Functional Foods*. 2009;1(2):177–187.

How to cite this article: PG N, Naik L, Kamyabali B. Isolation, quantification and physicochemical characterization of whole casein and individual casein fractions from Deoni and Malnad Gidda cattle milk. *International Journal of Agriculture Extension and Social Development*. 2026;SP-9(10):36-40. doi: <https://www.doi.org/10.33545/26180723.2026.v9.i10Sa.4386>