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Quality and safety evaluation of cow milk samples from Amreli City, Gujarat, India

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Abstract
A cross-sectional study was conducted to evaluate the compositional quality and preliminary microbiological quality of cow milk sold in Amreli city. Fifty raw cow-milk samples were collected from retail points (milk vendors, dairy shops, and small producers) and analysed for fat content, solids-not-fat (SNF), and methylene blue reduction time (MBRT). Thirteen samples (26%) had fat content below the prescribed FSSAI requirement of 3.2% for cow milk. Eight samples (16%) had SNF below the prescribed standard. Twenty-one samples (42%) showed MBRT ≤ 30 minutes, indicating elevated microbial activity. The results suggest significant non-compliance with compositional standards and frequent microbiological deterioration at the point of sale, pointing to issues in handling, adulteration, or cooling chain lapses. Recommendations include strengthened monitoring, vendor training on hygiene and cold-chain improvements, and follow-up microbiological culture testing.

Keywords: Cow milk, Amreli, FSSAI, fat content, SNF, MBRT, milk quality, dairy safety

Introduction
Milk is universally recognized as a complete food, providing high-quality proteins, essential fatty acids, lactose, and a wide spectrum of micronutrients such as calcium, phosphorus, magnesium, and vitamins A, D, and B-complex (Patil*et al*., 2024) [11]. In India, cow milk holds a special place in the diet and contributes significantly to nutritional security, especially in rural and semi-urban areas where it serves as a primary source of animal protein and energy. With India being the world's largest milk producer, ensuring the quality and safety of milk at both production and retail levels is of paramount importance for public health as well as for sustaining consumer confidence in the dairy sector.

However, raw milk is also highly perishable and susceptible to deterioration and contamination if not handled properly. Issues such as adulteration with water, removal of cream, addition of neutralizers, and improper storage or transportation without refrigeration contribute to variability in milk quality (Reddy *et al* 2017; Singh & Gandhi 2015) [12, 13]. Furthermore, unhygienic milking practices and lack of effective cold-chain infrastructure create conditions conducive to rapid microbial growth, which compromises both the safety and shelf life of milk (Singh & Gandhi 2015) [13]. Several studies from different regions of India have reported that due poor handling as well as practices of adulteration variations in milk compositional parameters, as well as poor bacteriological quality (Dhanalakshmi *et al*., 2020; Jawale & Devangare (2022)) [3, 7]. Such findings highlight the need for regular surveillance at the consumer end. To safeguard consumers, the Food Safety and Standards Authority of India (FSSAI) has prescribed minimum compositional standards for different classes of milk. For cow milk, a fat content of at least 3.2% and a prescribed level of SNF are mandated (FSSAI, 2017). These parameters are crucial not only for nutritional adequacy but also for detecting adulteration and dilution practices. Non-compliance with these values reflects both economic fraud and nutritional compromise. Alongside compositional assessment, methylene blue reduction test (MBRT) is commonly used as a rapid, low-cost method for evaluating bacteriological quality (De Silva *et al*., 2016) [2].

The principle of MBRT lies in the ability of actively respiring bacteria to reduce methylene blue dye; thus, shorter decolourization times indicate higher microbial loads. While it does not identify specific organisms, MBRT is widely applied in field surveys as a practical indicator of hygiene and handling conditions.

A recent study on goat milk quality in Mathura city reported that milk samples frequently failed to meet compositional and microbiological standards, underscoring the persistence of such issues in urban milk supply chains (Ojha *et al*., 2020) [10]. Comparable investigations in other Indian states also point to adulteration, seasonal variation, and weak sanitary practices as recurring challenges (Dhanalakshmi *et al*., 2020; Jawale & Devangare (2022) [3, 7]. Despite this, very limited published data exist for the Amreli region of Gujarat (Kabariya, & Ramani 2018) [8], where dairy farming plays a key role in livelihoods and milk is widely marketed through informal retail channels.

In this context, the present study was undertaken to evaluate the compositional and bacteriological quality of cow milk in Amreli city using standard fat and SNF determination methods and MBRT as a rapid microbial indicator. By documenting the proportion of samples that fail to comply with prescribed standards, the study aims to generate baseline data for this region, provide insight into possible causes of non-compliance, and recommend interventions to improve the safety and nutritional integrity of milk available to consumers.

Materials and Methods

The study was carried out in Amreli city, Gujarat, India**,** which is an important centre of milk production and marketing in the Saurashtra region. Amreli represents a typical urban dairy market where milk is sourced from smallholder farmers and distributed to consumers through street vendors, local dairy shops, and household-level producers. To capture a representative overview of milk quality at the retail level, a cross-sectional survey design was adopted. A total of fifty raw cow-milk samples were collected during the study period. Sampling locations were selected to include different parts of the city so that the variability across marketing channels was reflected. Retail outlets were approached randomly, and samples were obtained directly from vendors, dairy shops, and small producers selling unpasteurized milk. Each sample, approximately 200 ml in volume, was collected in pre-sterilized polypropylene containers that were carefully sealed and labelled with unique identifiers. To minimize changes in composition or bacterial growth during transport, samples were immediately placed in insulated boxes with ice packs, maintaining a temperature of 6-8 °C. All samples were transported to the laboratory of dairy technology and analysed within four hours of collection.

Compositional Analyses

Fat Content

The compositional analysis of the milk samples included determination of fat content and solids-not-fat (SNF). Fat content was estimated using the Gerber methodin accordance with the procedure outlined in the FSSAI Manual of Methods of Analysis of Foods. In this method, sulphuric acid was added to the butyrometer to digest milk proteins and release fat, after which a measured volume of milk and amyl alcohol was added. The mixture was centrifuged and subsequently placed in a water bath at 65 °C, and the length of the fat column was read directly on the butyrometer scale. Results were expressed as percentage by weight. According to FSSAI standards, cow milk should contain a minimum of 3.2% fat, and samples below this threshold were recorded as non-compliant.

Solids-Not-Fat (SNF)

Solids-not-fat (SNF) were determined by the lactometer method with temperature correction. Milk temperature was first standardized to 27 °C, and the lactometer reading was recorded and corrected accordingly. SNF values were calculated using the corrected lactometer reading and fat percentage in the standard formula. In cases where an electronic milk analyser was available, SNF values were cross-verified. As per FSSAI standards, cow milk must contain a minimum SNF level, generally ≥ 8.3%, depending on regional requirements, and samples below this level were categorized as deficient.

Microbiological Quality Assessment

Methylene Blue Reduction Test (MBRT)

The bacteriological quality of the samples was assessed using the Methylene Blue Reduction Test (MBRT), a widely accepted rapid method for evaluating the microbial load of milk. For this, 10 ml of each milk sample was taken in a sterile test tube, and 1 ml of 0.005% methylene blue dye solution was added. After gentle mixing, the tubes were incubated in a water bath at 37 ± 1 °C, and the time taken for the disappearance of blue colour was noted. MBRT relies on the ability of metabolically active bacteria to reduce the dye, hence shorter decolourization times correspond to higher microbial activity and poor milk quality. Results were classified according to conventional interpretation: decolourization within 30 minutes indicated poor quality with high bacterial load, between 30 and 90 minutes suggested fair quality, 90-120 minutes indicated good quality, and greater than 120 minutes represented excellent bacteriological quality. Although MBRT does not provide exact microbial counts or identify specific organisms, it serves as a reliable indicator of handling practices, hygiene levels, and the effectiveness of cooling during storage and transport.

Statistical Analysis

Data obtained from compositional and MBRT analysis were entered into Microsoft Excel for tabulation and analysis. Results were expressed in terms of absolute counts and percentages of non-compliant samples. Mean values and standard deviations were calculated for fat and SNF where applicable. Since the study was designed as a baseline quality assessment survey rather than an inferential investigation, only descriptive statistics were employed. However, the generated data provide a foundation for future studies where statistical comparisons between vendor categories, seasonal effects, or logistic regression models to identify predictors of non-compliance could be carried out.

Results

A total of 50 raw cow-milk samples were collected from different retail points across Amreli city and analysed for compositional and microbiological quality. The findings are summarized in Table 1.

With respect to fat content, 13 out of 50 samples (26.0%) were found to contain less than the prescribed minimum of 3.2% fat for cow milk under FSSAI regulations. These samples were therefore classified as non-compliant. The observed non-compliance suggests possible dilution practices, partial skimming, or natural variability associated with breed

and feeding conditions (Azad & Ahmed 2016) [1]. Despite this, the majority of samples (74.0%) met the required fat standard, indicating overall fair compliance, though the proportion of deficient samples remains concerning.

Evaluation of solids-not-fat (SNF) revealed that 8 samples (16.0%) fell below the minimum standard requirement. Low SNF values are often associated with adulteration by addition of water, seasonal nutritional stress on dairy animals, etc (Fox *et al*., 1998; Azad & Ahmed 2016) [5, 1]. While the majority of samples (84.0%) complied with FSSAI specifications, the presence of nearly one-sixth of samples with substandard SNF underscores the need for systematic monitoring at retail level.

The methylene blue reduction test (MBRT), used as a rapid indicator of microbiological load, showed that 21 samples (42.0%) exhibited dye decolourization within 30 minutes. This finding reflects a substantial proportion of samples with poor bacteriological quality, likely arising from unhygienic milking practices, contamination during storage, or inadequate chilling during transportation. The remaining samples demonstrated longer MBRT values, indicating fair to good microbial stability, although only a minority achieved the excellent category (>120 minutes).

Overall, the results point to compositional non-compliance in nearly one-quarter of samples and microbiological deterioration in almost half, highlighting both economic adulteration risks and public health concerns in the milk marketed in Amreli city.

Table 1: Quality assessment of cow milk samples collected from Amreli city (n = 50)

Parameter	Samples below standard	Percentage (%)	Standard threshold (FSSAI/ Accepted)
Fat content	13	26.0	≥ 3.2%
SNF	8	16.0	≥ 8.3% (regional FSSAI)
MBRT	21	42.0	> 30 min desirable

The present study provides critical insights into the quality and safety of raw cow milk sold in Amreli city, with results indicating both compositional non-compliance and microbiological deterioration in a significant proportion of samples. These findings align with reports from other parts of India, underscoring milk safety as a persistent challenge in informal and semi-formal dairy markets (Minj & Beher 2012) [9].

The analysis showed that 26% of samples contained fat levels below the prescribed 3.2% minimum. Such deviations may arise from deliberate adulteration practices (e.g., dilution with water or partial skimming of cream for ghee or butter production) or from natural causes, such as differences in cow breed, stage of lactation, and seasonal feeding regimes (Fox *et al*., 1998) [5]. Nevertheless, fat deficiency has both economic implications, since consumers are denied value for money, and nutritional implications, as milk fat carries essential fatty acids and fat-soluble vitamins (A, D, E, and K). Similar levels of fat non-compliance have been reported in Mathura for goat milk, where retail samples frequently failed to meet prescribed standards, suggesting that such issues are widespread in urban milk supply chains (Ojha *et al*., 2020) [10]. The detection of SNF deficiency in 16% of samples further emphasizes concerns regarding adulteration or poor production quality. SNF encompasses proteins, lactose, and minerals, all of which are critical for the nutritional integrity of milk. Reduced SNF is a typical indicator of dilution with water, which not only diminishes nutritional value but also increases the risk of introducing contaminants such as pathogens or chemical residues. Studies from Uttar Pradesh and Rajasthan have similarly identified SNF deficiencies in retail milk, linking them to intentional adulteration and poor dairy management practices. Thus, the results from Amreli are consistent with broader trends across India, reflecting systemic challenges in milk handling and monitoring.

The high prevalence of poor MBRT results (42% ≤ 30 min) is particularly alarming. MBRT is a well-established indicator of microbial activity, and rapid dye decolourization suggests high bacterial load in milk samples. Such results indicate either unhygienic milking conditions, including contaminated utensils and poor udder hygiene, or failures in post-harvest handling, particularly the absence of immediate chilling. The informal milk sector in India often relies on room-temperature storage and transport without refrigeration, providing conditions favourable for bacterial multiplication. The Amreli results, therefore, mirror findings from other urban surveys where MBRT indicated poor microbial quality in 35-50% of raw milk samples. The public health significance of these results cannot be overstated. Milk contaminated with high bacterial loads is not only prone to rapid spoilage but also poses a risk of transmission of foodborne pathogens such as Escherichia coli, Staphylococcus aureus, Salmonella, and coliforms. Although MBRT does not provide species-specific information, it serves as a warning indicator requiring confirmatory tests such as standard plate count, coliform enumeration, yeast and mould counts, and pathogen-specific culture or molecular assays. Routine incorporation of such microbiological analyses is essential to fully characterize the risk profile of milk sold in Amreli.

The combined findings of compositional non-compliance and poor microbial quality highlight a dual challenge: economic adulteration on one hand and safety risks on the other. For consumers, this means compromised nutrition, reduced shelf life of milk, and increased risk of gastrointestinal infections. For the dairy sector, recurrent quality failures erode consumer confidence and hinder the promotion of safe, high-value dairy products. The results also underscore the need for regulatory enforcement, including random sampling and penalties for adulteration, as well as capacity building for small-scale vendors, focusing on hygienic milking and the use of cost-effective chilling solutions.

Recommendations for Future Work

Follow-up investigations should expand the dataset to include seasonal variation, vendor categorization, and physicochemical adulteration tests (e.g., detection of neutralizers, detergents, or starch). Furthermore, statistical modelling could identify predictors of poor quality, such as vendor type, time of day, or handling conditions. Strengthening cold-chain infrastructure, encouraging cooperative models for milk collection, and raising awareness among consumers to prefer pasteurized or packaged milk are critical steps toward improving milk quality and safety in Amreli and similar urban centres.

Conclusion

The present surveillance of 50 cow-milk samples from Amreli city found 26% of samples with fat below the FSSAI reference value and 16% with SNF below standards; 42% decolourized methylene blue in 30 minutes or less. These findings indicate a need for targeted interventions, vendor education, stricter compositional checks, improved cooling/ transport, and routine microbiological testing, to ensure milk safety and consumer protection.

Conflict of Interest: Not available.

Financial Support: Not available.

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