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Comprehensive assessment of pasteurized milk quality in Pune City: A physicochemical and microbiological analysis

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Abstract

This study evaluates the physicochemical and microbiological quality of pasteurized milk from five dairies in Pune: Aarey, Chitale, Katraj, Nandan, and Sane. Fifteen samples (3 per dairy) were analyzed for pH, fat, protein, sugar, ash, water, and total solids, along with Direct Microscopic Count (DMC), Standard Plate Count (SPC), Methylene Blue Reduction Time (MBRT), and Phosphatase activity. Results showed pH values of 6.87-7.10 and specific gravity of 1.03-1.031. Fat ranged from 2.33% (Sane) to 3.80% (Chitale), protein from 2.83% to 3.40%, and sugar from 2.67% (Aarey) to 5.23% (Chitale). Water content was highest in Aarey (88.10%) and lowest in Sane (86.05%), inversely reflecting total solids. Ash ranged from 0.85% to 0.91%. Microbiologically, DMC and SPC were highest in Katraj (176.67×10^3 cells/mL and 170.00×10^3 CFU/mL) and lowest in Aarey and Sane. MBRT exceeded 6 hours for Chitale, Nandan, and Sane, indicating good quality. The Phosphatase test was positive only in Aarey, suggesting inadequate pasteurization. The findings reveal variability in milk quality across dairies and emphasize the need for stringent quality control to ensure consumer safety.

Keywords: Pasteurization effectiveness, milk standards, food safety regulations, public health, consumer protection

Introduction

Milk is widely acknowledged as nature's most nearly perfect food due to its unique composition of essential nutrients (Wright & Huck, 2002) ^[36]. It serves as a dietary cornerstone across all age groups, valued for its nutritional richness and diverse culinary applications (Górska-Warsewicz *et al.*, 2019) ^[12]. In addition to direct consumption, milk is utilized in beverages, baked goods, and as the foundational ingredient in dairy products such as cheese, yogurt, and butter. Its perishable nature, however, necessitates preservation methods such as pasteurization to ensure safety and extend shelf life (Kailasapathy, 2015) ^[19].

Milk contributes significantly to the intake of critical nutrients including calcium, vitamin D, phosphorus, and vitamin B12 often in a highly cost-effective manner (Drewnowski, 2011) ^[6]. Moreover, milk from non-cattle sources such as goat and camel also present unique nutritional benefits (Roy *et al.*, 2020) ^[28]. The nutritional value of milk, however, can be compromised by microbial contamination, which poses serious health risks if left unaddressed.

Pasteurization, the thermal treatment of milk to eliminate pathogenic microorganisms, is a standard process employed globally to safeguard public health. According to regulatory definitions, pasteurization involves heating milk to a specified temperature for a set duration to destroy all human pathogens without recontamination (Grade "A" Pasteurized Milk Ordinance, 2019; Monaci *et al.*, 2006) ^[9, 21]. There are two primary pasteurization methods: High-Temperature Short Time (HTST), where milk is heated to 72°C (161°F) for 15 seconds (Monaci *et al.*, 2006) ^[21], and Ultra-Heat Treatment (UHT), which involves heating milk to 140°C (284°F) for 4 seconds (Grade "A" Pasteurized Milk Ordinance, 2019) ^[9]. Despite its long-standing efficacy, variations in processing conditions, storage, and distribution necessitate ongoing evaluation of pasteurized milk's microbiological and physicochemical quality (Monaci *et al.*, 2006) ^[21].

This research focuses on assessing the safety and quality of pasteurized milk in Pune City, a rapidly growing urban hub with an increasing demand for safe dairy products. The study evaluates the efficacy of pasteurization in eliminating pathogenic microorganisms, its impact on physicochemical parameters such as fat, protein, sugar, and pH, and the microbiological profile of pasteurized milk using methods like SPC, DMC, MBRT, and the phosphatase test. Additionally, it examines the compliance of pasteurized milk with national and international quality standards. Through this multifaceted analysis, the research aims to contribute to food safety efforts, inform regulatory practices, and reinforce consumer confidence in the dairy sector of Pune City.

Review of Literature

Milk safety and quality have long been central concerns in public health, particularly due to the risks associated with raw milk consumption. Numerous studies have documented the transmission of pathogens such as *Escherichia coli* O157:H7, *Campylobacter* spp., *Listeria monocytogenes*, and *Salmonella enterica* through unpasteurized milk (Headrick *et al.*, 1998; Oliver *et al.*, 2009; Mungai *et al.*, 2015) ^[15, 23, 22]. These pathogens can cause severe illness, especially among children and immunocompromised individuals. For instance, an *E. coli* O157:H7 outbreak in 2005 led to 18 infections, including four cases of hemolytic uremic syndrome (Denny *et al.*, 2008).

Further research reinforces these concerns. Raw milk samples from dairy farms frequently harbor *Listeria monocytogenes* and *Salmonella* spp., highlighting microbiological risks (Jayarao & Henning, 2001) ^[18]. Moreover, the detection of antibiotic-resistant *Salmonella* strains in raw milk poses serious concerns for public health (LeJeune & Rajala-Schultz, 2009) ^[20].

A comprehensive review documented multiple milkborne disease outbreaks due to improper pasteurization and raw milk consumption (Robinson *et al.*, 2020) ^[27].

Regulatory frameworks are vital to ensuring milk safety. The U.S. Food and Drug Administration (FDA) outlines pasteurization protocols and contamination control measures in its Milk Guidance Documents (FDA, 2021) ^[9]. Reviews also emphasize quality control mechanisms required to mitigate foodborne risks (Yilmaz & Güneş, 2018) ^[37]. Recent research highlights evolving threats to milk safety, including antimicrobial resistance and supply chain contamination (Garnier *et al.*, 2022) ^[11].

Pasteurization, introduced in the early 20th century, remains a cornerstone of milk hygiene. According to the Grade "A" Pasteurized Milk Ordinance (2019) ^[9], pasteurization is the process of heating milk to a minimum of 72°C for 15 seconds to eradicate human pathogens.

The dual goals of pasteurization are to ensure microbial safety and extend shelf life by reducing spoilage organisms (LeJeune & Rajala-Schultz, 2009) ^[20].

While pasteurization offers significant advantages, there are ongoing concerns about its effects on the nutritional value and sensory characteristics of milk. Minor but measurable changes in color, flavor, and nutrient retention have been reported (Fakir *et al.*, 2017) ^[7]. Moreover, improper handling post-pasteurization can lead to microbial recontamination, reinforcing the need for rigorous quality control (Hudson *et al.*, 2003; Calahorrano-Moreno *et al.*, 2022) ^[16, 3].

Microbiological testing methods such as the Standard Plate Count (SPC), Direct Microscopic Count (DMC), Methylene

Blue Reduction Test (MBRT), and phosphatase activity assays are widely used to assess microbial load and pasteurization adequacy (Hudson *et al.*, 2003; Monaci *et al.*, 2006) ^[16, 21]. These techniques are critical to verifying compliance with regulatory and health standards.

However, localized studies evaluating the implementation and efficacy of pasteurization processes are limited, especially in urban Indian contexts such as Pune. With rapid urbanization and changing supply chain dynamics, understanding how pasteurization is applied and monitored at the city level is crucial for ensuring public health.

This research seeks to bridge that gap by evaluating the physicochemical and microbiological characteristics of pasteurized milk in Pune City. By comparing these findings with regulatory benchmarks, the study offers data-driven insights to guide dairy producers, public health authorities, and regulatory bodies in ensuring the safety and quality of milk for urban consumers.

Materials and Methods

Materials

The materials utilized in this study included Milk Agar (Atlas, 1983), stains, and reagents such as Hucker's Crystal Violet and Solution A (Crystal Violet Methylene Blue) and Solution B (Ammonium oxalate, Isoamyl Alcohol, 90% Ethanol, Concentrated Distilled Water). Quantitative Benedict's reagent, disodium phenyl phosphate substrate, Sodium carbonate powder, Sodium bicarbonate powder, 4-Nitrophenyl phosphate Disodium Salt GR, and necessary chemicals for solution preparation were also employed (Dairy Practices Council, 2014; Hadžić, Bačić, & Ahmetović, 2020) ^[4, 13].

Methods

Collection of Milk Samples

Samples of milk packaged in sterile polyethylene bags were obtained from various dairies in Pune. Only packages labeled as "pasteurized milk" were selected for the study. Three samples from each dairy were collected, including Chitale Dairy, Sane Dairy, Aarey Dairy, Katraj Dairy, and Nandan Dairy.

Determination of Physical Characteristics of Milk

Milk's physical characteristics are influenced by its composition and various external factors. As an emulsion, milk consists of fat globules dispersed in an aqueous phase containing proteins, lactose, and minerals, which collectively determine its physical state (Prasad *et al.*, 2018; Walstra & Jenness, 1984) ^[25, 35]. The color of milk varies due to factors such as breed, feed, and fat percentage, with carotenoids from feed contributing to a yellowish hue, while fat content affects opacity (Prasad *et al.*, 2018; Singh, McCarthy, & Lucey, 1997) ^[25, 31]. Additionally, milk's flavor results from a combination of sweet lactose, salty minerals, and other compounds, with aspects like feed, processing, and storage conditions playing a significant role in taste development (Prasad *et al.*, 2018; Sherbon, 1988) ^[25, 30].

Determination of chemical characteristics of milk

The composition of milk is assessed through various analytical methods to determine its key components. Protein content is evaluated using the precipitation method, which isolates proteins from milk samples for quantification (Fox *et al.*, 2015) ^[10]. The fat content is measured through Gerber's butyrometer method, a widely used technique that

involves acid digestion and centrifugation for precise fat estimation (Sharma & Singh, 2018) ^[29]. The solids content is determined by weighing the milk sample before and after drying, allowing for the measurement of total solids present in the sample (Walstra *et al.*, 2006) ^[34]. Sugar content is assessed using the titration method with Benedict's reagent, which reacts with milk sugars to provide an accurate concentration reading (Tamime, 2009) ^[33]. Lastly, ash content is evaluated by incinerating milk samples and weighing the residual minerals, offering insights into the inorganic composition of the milk (Panse *et al.*, 2021) ^[24].

Microbiological Tests

Various analytical methods were employed to assess the physical, chemical, and microbiological characteristics of pasteurized milk samples collected from different dairies. Direct Microscopic Count (DMC) was utilized to examine milk smears stained with methylene blue, allowing for microscopic evaluation of microbial presence (Jay *et al.*, 2005) ^[17]. Standard Plate Count (SPC) enabled bacterial

enumeration using sterile milk agar plates to determine microbial load (Richter *et al.*, 2019) ^[26]. Methylene Blue Reduction Time (MBRT) test was conducted to evaluate microbial activity based on the rate of methylene blue reduction, offering insights into the overall milk quality (Farkye, 2016) ^[8]. The phosphatase test, both qualitative and quantitative, was performed to assess the efficiency of pasteurization by detecting residual enzyme activity (Adams & Moss, 2017) ^[1]. Additionally, Cornell's Method employed 4-Nitrophenyl phosphate Disodium Substrate solution to determine phosphatase activity, ensuring proper thermal processing (Haug *et al.*, 2007) ^[14]. Lastly, a quantitative analysis was conducted using optical density measurements to quantify phenol concentration in milk samples, following a standardized protocol for precise evaluation (Singh *et al.*, 2020) ^[32]. These methods were meticulously executed to ensure accurate assessment of milk quality and safety parameters.

Results

Table 1: Physical parameters of pasteurized milk from five dairies in Pune

Parameter	Aarey (A)	Chitale (C)	Katraj (K)	Nandan (N)	Sane (S)
Colour	Cream white	Cream white	Cream white	Cream white	Cream white
pH	6.93	6.90	6.90	7.10	6.87
Temperature (°C)	15.3	9.0	11.0	16.3	10.0
Flavor/Smell	Salty	Sweet	Sweet	Salty	Salty
Consistency	Normal	Normal	Normal	Normal	Normal
Specific Gravity	1.030	1.031	1.031	1.031	1.031

Legend: This table displays the average physical characteristics of pasteurized milk samples from Aarey, Chitale, Katraj, Nandan, and Sane dairies. Parameters such as pH, temperature, flavor, color, consistency, and specific gravity were measured to evaluate the sensory and physicochemical stability of the milk. *Performed in triplicates, and the average was calculated.*

Table 2: Chemical composition of pasteurized milk from five dairies in Pune

Parameter	Aarey (A)	Chitale (C)	Katraj Dairy (K)	Nandan (N)	Sane (S)
% Water	88.10	88.02	86.87	87.76	86.05
% Total Solids	13.57	12.31	14.12	12.59	14.28
% Fat	2.73	3.80	3.40	3.00	2.33
% Protein	3.40	2.83	3.00	3.14	2.90
% Sugar	2.67	5.23	4.80	4.80	5.07
% Ash	0.85	0.91	0.90	0.91	0.88

Legend: This table presents the average chemical composition of milk samples from each dairy. Parameters include water content, total solids, fat, protein, sugar, and ash percentages, which are critical for assessing nutritional quality and compliance with food standards. *Performed in triplicates, and the average was calculated.*

Table 3: Microbiological quality of pasteurized milk from five dairies in Pune

Test	Aarey (A)	Chitale (C)	Katraj (K)	Nandan (N)	Sane (S)
DMC ($\times 10^3$ cells/mL)	87.67	165.00	176.67	138.33	125.00
SPC (CFU/mL $\times 10^3$)	156.00	77.67	170.00	160.00	53.00
MBRT (hours)	< 6.2	> 7.3	< 6.0	> 7.0	> 6.3
Phosphatase Test	Positive	Negative	Negative	Negative	Mostly Negative (1+ 2-)

Legend: This table summarizes the microbiological test results of pasteurized milk samples from five dairies. Parameters assessed include Direct Microscopic Count (DMC), Standard Plate Count (SPC), Methylene Blue Reduction Time (MBRT), and phosphatase activity. These indicators provide insight into microbial load, milk freshness, and pasteurization effectiveness. *Performed in triplicates, and the average was calculated.*

Discussion

The evaluation of pasteurized milk samples from five major dairies in Pune Aarey, Chitale, Katraj, Nandan, and Sane provided significant insights into their quality, safety, and processing efficiency. All samples displayed a uniform cream-white color and normal consistency, reflecting standardized dairy processing methods and the absence of visible contaminants, which are crucial for consumer acceptance. The pH values ranged from 6.87 to 7.10,

aligning with the standard range for pasteurized milk (6.6-6.9), indicating effective microbial control and preservation of milk quality. However, Nandan's slightly elevated pH (7.10) might suggest early spoilage or differences in processing techniques. Temperature variations at sampling, ranging from 9.0°C to 16.3°C, indicated some inconsistencies in cold chain management. While all samples remained below the critical spoilage threshold of

20°C, stricter temperature control is recommended to prevent microbial growth after pasteurization.

Chemical composition analysis showed variations in fat content, with values ranging from 2.33% in Sane to 3.80% in Chitale, likely due to differences in milk sources (cow vs. buffalo), seasonal changes, or standardization practices. Protein levels remained within acceptable limits (2.83%-3.40%), ensuring adequate nutritional value. Sugar content was highest in Chitale and Sane, exceeding 5%, potentially due to higher lactose content or added sugars for taste enhancement. Water content varied slightly across samples but remained within standard limits (<88.5%), with Katraj and Sane showing more concentrated formulations. Ash content, indicating mineral balance, remained consistent across all samples, suggesting no adulteration.

Microbiological analysis revealed considerable variation in bacterial counts. Katraj and Aarey exhibited elevated microbial levels ($DMC > 170 \times 10^3/\text{ml}$), hinting at post-pasteurization contamination or inadequate processing. In contrast, Chitale and Sane maintained lower microbial counts, reflecting superior hygiene and cold chain management. MBRT values supported these findings, with Chitale, Nandan, and Sane showing $MBRT > 6.3$ hours, indicating lower bacterial activity, while Aarey and Katraj had reduced MBRTs (< 6.2 hours), signifying higher bacterial loads and an increased risk of spoilage. The phosphatase test results further validated pasteurization efficiency, with all dairies except Aarey testing negative. Aarey's positive result suggests either under-pasteurization or enzyme reactivation from microbial contamination, raising significant safety concerns and necessitating regulatory intervention to ensure compliance with established safety standards.

Conclusion

While physical and chemical parameters largely adhered to national standards, microbiological assessment exposed disparities in hygiene and pasteurization effectiveness among the dairies. Chitale and Sane demonstrated the highest microbial safety and nutritional integrity, whereas Aarey and Katraj showed signs of microbial contamination and inadequate processing. The phosphatase-positive result for Aarey underscores the need for stricter thermal processing controls. These findings emphasize the importance of continuous surveillance, cold chain maintenance, and stringent adherence to pasteurization guidelines to safeguard public health. Consumer awareness, regulatory enforcement, and periodic quality audits are essential to ensure the consistent delivery of safe and nutritious milk in urban markets like Pune. This study offers a replicable model for city-wide milk quality monitoring and establishes baseline data for guiding future interventions, policy formulations, and consumer protection initiatives in India's expanding dairy sector.

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