Evaluation of Chemical Makeup and Microbiological Parameters of Raw Milk in Hyderabad, Pakistan

Abdul Samil Dahri[1](Corresponding Author), Asim Patrick[2], Asghar Ali[3], Muhammad Aqil[4]

[1, 2] Lecturer, Microbiology Government College University, Hyderabad, Sindh, Pakistan, Email: abdulsamidahri@gcuh.edu.pk
[3] Associate Professor, Biochemistry Government College University, Hyderabad, Sindh, Pakistan Email: dr.asghar.ali@geuh.edu.pk
[4] Lecturer, Department of Botany Government College University, Hyderabad, Sindh, Pakistan Email: Muhammad.aqil@geuh.edu.pk

Abstract

Milk is an essential nutritional source, critical for health across all age groups, yet its quality and safety remain a concern in many developing regions. This study evaluates the Chemical makeup and microbiological condition of milk supplied commercially in Hyderabad, Pakistan, where milk serves as a dietary staple. A total of 44 milk samples were collected from various selling points in Hyderabad's three main zones—Qasimabad, Latifabad, and Hyderabad City—and were analysed for key nutritional parameters including acidity, specific gravity, total solids, solids-not-fat, protein, and fat. The mean protein content of the raw milk samples was found to be 3.38% ± 0.63, fat 2.26% ± 0.40, total solids 8.9 ± 1.96, solids-not-fat 6.83 ± 1.63, acidity 0.21 ± 0.04, and specific gravity 1.025 ± 0.00. Statistical analysis indicated that while the mean percentages of protein, total solids, solids-not-fat, acidity, and specific gravity were not statistically different ($P > 0.05$), the mean fat percentage differed significantly ($P < 0.05$), with fat and other critical nutrients markedly lower than in pure milk. Microbiological analysis revealed that 45.5% of samples contained contaminants such as Staphylococcus aureus, Escherichia coli, and Salmonella spp., indicating significant health risks. These findings emphasize the urgent need for stricter quality control and regulatory measures to improve milk standards in Hyderabad, safeguarding public health and ensuring access to nutritionally adequate milk.

Keywords: Milk Quality, Nutritional Analysis, Microbial Contamination, Public Health, Food Safety, Hyderabad

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Introduction

Various animals, such as camels, goats, sheep, buffaloes, and cows, produce milk through their mammary glands. This nutrient-dense liquid is crucial for the nourishment of infants and young children, providing vital nutrients, factors that promote growth, and components that support the immune system (Amr & Farid, 2024) Milk is considered a whole food which contains all the nutrition needed for the body it is consumed by all the age groups. (Garcia, Osburn, & Cullor, 2019). The global milk consumption has increased during the past thirty years significantly, especially in the developing countries like Pakistan.(Patari, Datta, & Mahapatra, 2022). The protein concentration is best shown around 3.8% in buffalo and bovine milk respectively (Fahmid, Sajjad, Khan, Jamil, & Ali, 2016). The Milk of high quality shows no contamination and is rich in nutrient composition which is essential for optimal health. (Korale-Gedara, Weerahewa, & Roy, 2023). The present study focuses on the physio-chemical composition of milk and the presence of microorganisms present in raw milk sold in Hyderabad markets to assess its quality (Shah et al., 2016). Despite these concerns, there is a lack of comprehensive studies evaluating both the chemical and microbiological quality of raw milk in Hyderabad, Pakistan. Previous research has primarily focused on either chemical composition or microbial contamination in isolation, leaving a gap in understanding the combined impact of both factors on milk quality. Furthermore, systematic sampling and statistical analysis of milk quality in this region is rare, and the studies that have been done are not generalizable. To bridge these gaps, this study uses robust sampling techniques and statistical analysis to evaluate in detail both the nutritional composition and microbial safety of milk, to produce reliable and representative results. This has been addressed in the present study by studying the key nutritional parameters and bacterial contaminants present in the milk samples drawn around respective zones of Hyderabad. This research intends to offer policymakers evidence-based information regarding milk quality and to suggest policy directions to raise the standards of milk safety.

Materials and Methods

The present study was conducted by (Memon, Arain, Kaka, Memon, & Arisar, 2024) randomly chosen stores 12 milk samples were about from three different zones of Hyderabad: Hyderabad city, Latifabad and Qasimabad. 200 ml each. The overall Milk composition was analysed based on protein, fats, total solids, solid-not-fat, acidity, specific gravity and the presence of microbial contamination in the milk sample by using the established methodology. (Shah et al., 2016)

- **Protein** was determined by using the formol titration method (Heath, 1978).
- **Fat** was detected by using Gerber's approach (Khan, 2004).
- **Total Solids** through the oven drying process were calculated (Harding, 1995).
- **Solids-Not-Fat** total solids were calculated by subtracting them from fat (Clark, Barbano, & Dunham, 1989)
- **Acidity** was determined through the titration method (POPESCU & ANGEL, 2009)
- **Specific Gravity** was measured using a device called a lactometer (Mohd Fazla et al., 2023)
- Data were analyzed using SPSS software (version 30). Descriptive statistics, including mean and standard deviation, were calculated for physico-chemical parameters. One-way ANOVA was used to compare the means of protein, fat, total solids, solids-not-fat, acidity, and specific

gravity across samples. A P-value of <0.05 was considered statistically significant.

A cross-sectional descriptive study was conducted in the Hyderabad district, with laboratory work performed at the Microbiology Laboratory, GC University Hyderabad. To ensure the complete representation of the whole region, a multistage sampling strategy was used through which Hyderabad was divided into three different zones. The zones were Hyderabad City (Z1), Latifabad (Z2) and Qasimabad (Z3). Each zone stands for different demographical and environmental characteristics. To study the sample size the WHO sample size formula was used to calculate.

$$n = \frac{z^2 pq}{d^2}$$

A total of 44 raw milk samples were collected aseptically to the selling points in the sterilized glass bottles. For the analysis, the milk samples were sent to the Laboratory of Microbiology at GC University in ice packs. For the processing and analysis, special care was taken to maintain the temperature of the samples at 6-8 C. Over four weeks 11 samples were collected each time once a week. The total plate count method was used for microbiological analysis (Dahri et al., 2020). By using normal saline (NSS) the milk samples were diluted by tenfold technique 1 ml of milk and 9 ml of normal saline. On the pour plate, 0.1 ml of inoculum from 10-3 and 10-4 dilutions were poured and then incubated for 24 hours at 37 C and total plate counts were estimated. Using the following formula:

$$\mathrm{Log}_{10}\,\mathrm{CFU/gm} = \sum C$$

With the factor of dilution, d accounted for. *Staphylococcus aureus* was obtained by adding 1ml of raw milk to each decimal dilution on the pre-solidifiedmanitol salt medium, colony were counted under the microscope by incubating the plates at 37oC for 48 hours. *Salmonella* was discovered by adding 25 mL of raw milk to 225 mL of sterilized buffered peptone water, culturing overnight at 37°C, and counting colonies. Escherichia coli was tested by making serial dilutions of 1 mL raw milk in 9 mL of 0.1% sterile buffered peptone water, plating 10 µL of appropriate dilutions on MacConkey Agar, and incubating at 37°C for 24 hours before colony counting.

Results and Discussion

The milk sample was analysed by using physio-chemical parameters such as protein, fat, total solids, solids-not-fat and specific gravity.

Table 1: Physical-Chemical Characteristics of Hyderabadi Milk Samples

Characteristic	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	Control
Protein (%)	2.59 ± 0.76	3.36 ± 0.74	2.73 ± 0.74	2.56 ± 0.72	3.48 ± 0.63	3.01 ± 0.73	3.06 ± 0.62	3.34 ± 0.69	2.89 ± 0.81	2.71 ± 0.91	3.30 ± 0.68	3.36 ± 0.58	4.09 ±0.41
Fat (%)	2.08 ± 0.32	1.91 ± 0.33	1.83 ± 0.24	2.09 ± 0.26	2.12 ± 0.28	2.11 ± 0.31	2.05 ± 0.51	2.28 ± 0.36	1.29 ± 0.31	1.63 ± 0.24	2.13 ± 0.30	1.90 ± 0.36	6.29 ±0.43
Total Solids (%)	7.53 ± 1.91	7.92 ± 2.03	7.71 ± 1.63	8.79 ± 1.19	9.01 ± 1.83	8.19 ± 1.23	7.96 ± 2.65	8.21 ± 1.94	7.89 ± 2.50	7.34 ± 2.51	8.03 ± 1.95	7.98 ± 2.06	15.34 ±0.81
Solids-Not-Fat (%)	5.53 ± 1.64	6.09 ± 1.73	5.82 ± 1.41	6.74 ± 1.09	6.89 ± 1.72	6.14 ± 1.18	5.79 ± 2.04	5.98 ± 1.58	6.36 ± 2.14	5.73 ± 2.19	**6.82 ± 1.64**	6.09 ± 1.77	9.08 ±0.46
Acidity (%)	0.24 ± 0.06	0.15 ± 0.07	0.18 ± 0.03	0.16 ± 0.04	0.21 ± 0.05	0.17 ± 0.04	0.24 ± 0.07	0.14 ± 0.08	0.18 ± 0.06	0.24 ± 0.06	0.15 ± 0.05	0.14 ± 0.09	0.15 ±0.06
Specific Gravity	1.024 ± 0.000	1.026 ± 0.000	1.021 ± 0.000	1.022 ± 0.000	1.024 ± 0.000	1.021 ± 0.000	1.023 ± 0.000	1.025 ± 0.000	1.021 ± 0.000	1.024 ± 0.000	1.023 ± 0.000	1.024 ± 0.000	1.033 ±0.001

In this study, three bacteria were isolated and identified: *Staphylococcus aureus* (18.2%), *Escherichia coli* (27.3%), and *Salmonella spp.* (4.5%). Of the 44 samples analyzed, 20 (45.5%) were found to be contaminated with these bacteria, with *E. coli* present in 25% of samples, *Staphylococcus aureus* in 16%, and *Salmonella spp.* in 5%.

Zone	Staphylococcus aureus	Escherichia coli	Salmonella spp.	Total (%)
Z1 (City)	02	03	00	05
Z2 (Latifabad)	01	01	01	03
Z3 (Qasimabad)	03	04	01	08
Total	08 (18.2%)	12 (27.3%)	02 (4.5%)	20 (45.5%)

Table 2: The table reflects the bacterial distribution in the three zones of Hyderabad, summarizing the presence of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella spp.* across Z1 (City), Z2 (Latifabad), and Z3 (Qasimabad). The "Total (%)" column provides the overall percentage of each bacterium across all zones

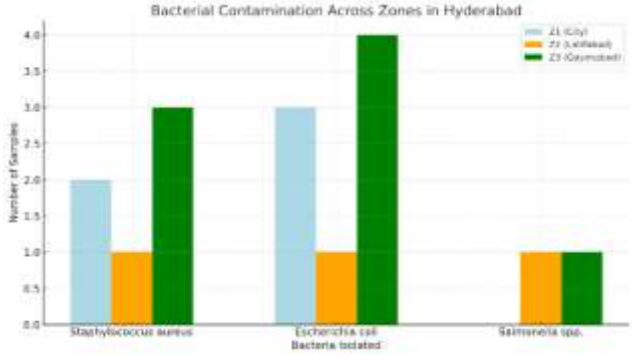

Figure 01: Data chart shows bacterial contamination across the three zones (Z1, Z2, Z3) in Hyderabad. The chart compares the number of samples contaminated with *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella spp.* in each zone.

Milk Selling Point	Positive Samples	Average Bacterial Count of Samples	Total Plate Count (CFU/ml)	Log CFU/ml	Total Staph Count (CFU/ml)	Log CFU/ml	Total E. coli Count (CFU/ml)	Log CFU/ml	Total Salmonella Count (CFU/ml)	Log CFU/ml
Z1 (City)	07	613,114	613,114	5.79	498.00	2.70	238.50	2.38	112.50	2.05
Z2 (Latifabad)	11	489,222	489,222	5.69	420.00	2.62	199.50	2.30	121.00	2.08
Z3 (Qasimabad)	07	589,348	589,348	5.77	452.00	2.65	225.50	2.35	108.00	2.03

Table 03: Average Bacterial Count of Samples

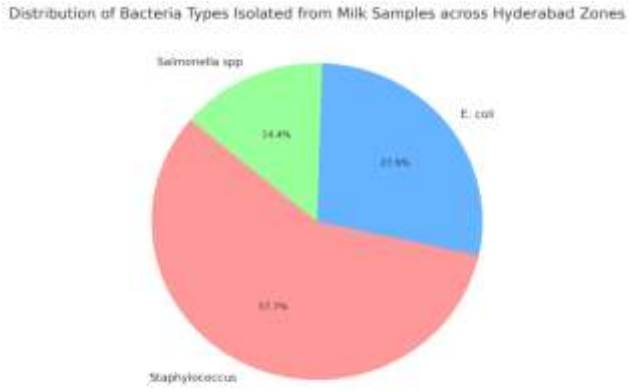

Figure 02: A pie chart showing the distribution of *Staphylococcus, E. coli, and Salmonella spp* isolated from milk samples across Hyderabad's zones, based on their total counts.

The study finds out that the milk sold in Hyderabad has a lower concentration of important nutritional components when compared with unadulterated milk. The study also revealed that the presence of many microorganisms poses serious health concerns for human health. The study revealed the need for effective regulatory protocol and quality assurance and measures so that consumers can have access to milk which is nutritionally balanced and free from all biological contaminations.

Conclusion

This study emphasized serious health issues related to the safety and quality of raw milk marketed in Hyderabad, Pakistan. Physicochemical analyses show that essential nutrients like protein, fat, and total solids

were highly found at a lesser level relative to pure milk, hence, of poor nutritional content. Moreover, the evaluation of microbial presence was found to be dangerous for the health and 50% of the milk samples could be infected with pathogenic bacteria like Staphylococcus aureus, Escherichia coli, and Salmonella spp. These findings reinforce the call for the relevant authorities to act now on the urgent need for regulation.

To resolve these critical problems in terms of quality milk production, the regulatory bodies would rather enforce stringent quality control protocols at all stages of milk production, storage and distribution. In addition, sanitary handling practices among dairy farmers and milk vendors should be encouraged and avoid microbial contamination by education and training programs. This should lead to mandatory routine testing for all milk samples for nutritional content and microbial load. It is also necessary to raise public awareness about the dangers of consuming raw milk, so consumers they agree to boiling or pasteurizing milk before use. Finally, and importantly, while cold chain systems and storage facilities are not reliable, investing in improved infrastructure for milk management will help ensure quality during transportation. Such measures enable authorities to ensure consumers do not only have access to milk but also in a safe and high-quality form that will contribute to a better health outcome in the region.

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Author contribution

All the authors planned the research. Created a technique for this goal. Conducted analysis and authored the article. All the authors have read and agreed to the published version of the work.

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Declarations

Conflict of Interest Declaration

The authors disclose no conflicts of interest.

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