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COMPOSITIONAL EVALUATION OF FRESH MILK COLLECTED FROM SALE OUTLETS OF NALL, A REMOTE AREA OF BALOCHISTAN, PAKISTAN

Aseya[1], Nadeem Rashid[1]*, Shah Faisal[2], Hafiza Maryum Nadeem[3], Firaj Ahmad[4], Majid[5], Wali Muhammad[6]

[1]**Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB),** University of Balochistan, Quetta, Pakistan
[2]**Pharmacist**, Jhall Magsi, Balochistan, Pakistan
[3]**Department of Physical Therapy**, University of Balochistan, Quetta, Pakistan
[4]**Jhallawan Law College**, Quetta, Pakistan
[5]**Health Department**, Balochistan, Pakistan
[6]**Livestock and Dairy Development Department,** Balochistan, Quetta, Pakistan
***Correspondence Author:** Prof. Dr. Nadeem Rashid. E. mail: nadeemuob@yahoo.com

Abstract
Milk is generally considered a complete diet with high nutritional value for all age groups. The present study focused on evaluating the fresh raw milk sold at outlets to determine its physicochemical composition, adulteration, and mycotoxin contamination. The dairy milk samples (n=20) collected from Nall, a nearby remote area of Khuzdar District, were analyzed using a Lacto-scan, Dairy Milk Adulterant Detection Kit, and Thin Layer Chromatography. The results regarding the physicochemical composition, including Total Fats, Solids-Not-Fat (SNF), Density, Sugar Contents (Lactose), Salt, Protein, Added Water, Freezing Point, Conductivity, and pH, showed a significant (P<0.05) difference among sales outlets. The overall mean percent values were 2.85±1.21, 6.31±1.72, 20.37±5.65, 3.43±0.96, 0.50±0.14, 2.32±0.62, 31.65±19.74, -0.39±0.12, 3.67±0.55, and 6.65±0.19, respectively. The results of the screening for adulterants revealed that the collected milk samples were found to be adulterated with detergent, sugar, and skim milk, with adulteration percentages of 10%, 30%, and 25%, respectively. However, adulteration with hydrogen peroxide, boric acid, starch, urea, carbonate, formalin, hypochlorite, sodium chloride, and pulverized soap was not observed. Aflatoxin M1 contamination above the permissible limits was evident in 5% of samples.

Keywords: *Adulterant screening, Aflatoxin M1, Lacto-scan, Milk composition, Thin layer chromatography*

INTRODUCTION

Milk is widely regarded for its appealing taste and unique nutritious value (1). It is a natural beverage that provides balanced amounts of quality fats, proteins, carbohydrates, minerals, and vitamins (2). Consumed by people of all ages and genders (3), milk from different animal sources may vary in composition, viscosity, odor, and taste, although it retains similar fundamental nutritional properties (4). Any addition or removal of substances from natural milk is termed as adulteration (5), a practice that may reduce milk quality and can introduce harmful substances into the dairy supply chain, posing risks to human health (6).

Mycotoxins are a heterogeneous group of toxic secondary metabolites secreted by fungi. Under favorable conditions, toxigenic fungi (molds) from genera such as *Fusarium*, *Aspergillus*, and *Penicillium* can produce hundreds of mycotoxin variants (7). When livestock ingest mycotoxin-contaminated feed, it not only causes detrimental health effects in animals but also poses significant risks to humans consuming products derived from exposed animals (8). Among mycotoxins, aflatoxins are the most toxic, and among aflatoxins, aflatoxin-B1 (AFB1) is the most potent. Aflatoxin-M1 (AFM1), often referred to as the milk toxin, is a hydroxylated metabolite formed when AFB1 is metabolized in the liver by cytochrome P450 enzymes (9). Approximately 0.3% to 6.2% of ingested AFB1 is converted into AFM1 (10). Classified by the International Agency for Research on Cancer (IARC) as a Group 2B probable human carcinogen (11), AFM1 in milk and dairy products poses a serious worldwide public health threat (12). Its documented effects include hepatocellular carcinoma (HCC), teratogenicity, cytotoxicity, and DNA mutations (13). Regulatory limits reflect this concern: the US Food and Drug Administration (USFDA) sets the maximum level for AFM1 in milk at 0.5 parts per billion (ppb), while the European Union (EU) imposes a stricter limit of 0.05 ppb (14). Milk, a highly nutritious liquid, is the sole food source for infants and a primary dietary component for growth, development, and immune system support across all age groups (2). Consequently, contamination of milk with AFM1 exceeding permissible levels is a significant public health concern.

Keeping in view the critical nutritional value of milk and its vulnerability to adulteration and contamination with hazardous substances such as AFM1, this study aimed to assess the quality of raw milk supplied in a remote area of Balochistan, Pakistan. It specifically focused on physicochemical characteristics, adulteration, and AFM1 contamination. The findings address a key research gap for an underserved region and provide a foundation for developing future intervention strategies.

METHODOLOGY

A baseline, cross-sectional study was conducted from November 2022 to February 2023, focusing on determining the physicochemical characteristics, screening for adulterants, and evaluating mycotoxin contamination in raw milk samples.

MATERIAL PREPARATION

All the materials & equipment used to collect the samples were sterilized. Preparation of Sabouraud Dextrose Agar was done according to standard microbiological procedure and autoclaved at 121°C for 20 minutes. Another 150ml of autoclaved Sabouraud Dextrose Agar was taken in test tubes for slide culture method.

AREA OF STUDY AND SAMPLE COLLECTION

Twenty fresh raw milk samples (500 ml each; n=20) were randomly collected from milk outlets located in Nall (rural area near Khuzdar city). Samples were collected using sterilized, screw-capped glass containers and transported under cold chain conditions to CASVAB within 24 hours for subsequent analysis.

SAMPLE ANALYSIS

Sample analysis was performed at the Toxicology Laboratory of the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan. The study employed standardized analytical protocols to: (i) characterize physicochemical properties, (ii) screen for milk adulterants, and (iii) evaluate Aflatoxin-M1 contamination levels. Detailed methodologies for each analysis are described below.

PHYSIOCHEMICAL COMPOSITION ANALYSIS

The analyzed physicochemical parameters included fat content, solids-not-fat (SNF), lactose, density, mineral salts, protein content, added water detection, freezing point, conductivity, and pH using a LactoScan™ milk analyzer according to the manufacturer's operational protocols. This instrument utilizes advanced Fourier Transform Infrared (FTIR) spectroscopy technology for comprehensive milk composition analysis.

MILK ADULTERANT SCREENING

Potential adulterants were screened using a commercially available milk adulteration test kit (PCSIR, Lahore). The test panel included detection of: detergents, hydrogen peroxide (H_2O_2), hypochlorite, boric acid (H_3BO_3), formalin (CH_2O), starch, urea, carbonates, sodium chloride (Na Cl), sugars, skim-milk powder, pulverized soap, and starch. All tests were performed following the manufacturer's standardized procedures with appropriate quality controls.

EVALUATION OF AFLATOXIN-M1 CONTAMINATION LEVELS

Modified methodology of Rashid *et al*. (2012) using thin-layer chromatography (TLC) was followed to perform Aflatoxin-M1 screening. The procedure commenced with activation of silica gel TLC plates by oven-heating at 110°C for 1 hour. A baseline was marked 1 inch above the plate's lower margin using a pencil. Applied 100 µl of 50 ppb Aflatoxin-M1 standard solution followed by duplicate test sample spots in an alternating pattern across the plate, ensuring consistent spotting technique for both standards and samples. Chromatographic separation was achieved using a mobile phase comprising chloroform, acetone, and water (88:12:1.5 v/v) in a developing tank. Special attention was given to maintain the mobile phase level below the baseline. Development continued until the solvent front reached approximately 1 inch from the plate's upper edge. After marking the solvent front, plates were air-dried and examined under both short (254 nm) and long (365 nm) wavelength UV light for fluorescence visualization of AFM1 spots (15).

RESULTS & DISCUSSION

The physicochemical characteristics of milk samples from Nall are presented in Table I. Significant variations (P<0.05) were observed across all measured parameters among different sale outlets. The samples showed considerable added water content (31.65±19.74%), potentially explaining the observed dilution effects on milk constituents.

Table I. Physiochemical characterization of fresh raw milk samples

Parameters	Mean±SD	*p* value
Fat	2.85±1.21	< 0.001
SNF	6.27±2.41	< 0.05
Density	20.37±5.65	< 0.001
Lactose	3.43±0.96	< 0.001
Salts	0.50±0.14	< 0.001
Protein	2.32±0.62	< 0.001
Added water	31.65±19.74	< 0.001
Freezing Point	-0.39±0.12	< 0.001
Conductivity	3.67±0.55	< 0.001
pH	6.65±0.19	< 0.001

Milk, due to its unique nutritional profile, is vital for human health across all age groups (16). Physicochemical characterization of fresh raw milk samples revealed significant (P<0.05) inter-sample variation for most parameters analyzed. The observed solids-not-fat (SNF) content (6.27 ± 2.41%) was significantly lower than values (8.39–8.97%) reported by Yoganandi *et al.,* (2014) for Indian cattle. This reduction is potentially attributable to water adulteration, detected at 26.99 ± 19.53% in our samples. Similarly, protein (2.30 ± 0.89%) and lactose (3.33 ± 1.26%) contents were substantially lower than literature values (protein: 3.32–3.87%; lactose: 4.42–4.70%) reported by Yoganandi *et al.,* (2014), further supporting possible adulteration (17). The freezing point (-0.42 ± 0.1°C) was partially aligned with the findings reported by Soomro *et al.,* (2014), approached 0°C (18). This elevation from the typical milk freezing point (approximately -0.54°C) is might be due to a reduced solute concentration resulting from adulteration. pH values (6.69 ± 0.15) were consistent with findings from Ahmad *et al.,* (2013) and Kausar *et al.,* (2023). In contrast, electrical conductivity (3.88 ± 0.86 mS/cm) was lower than typical literature values (3, 19), likely reflecting dilution of ionic constituents. Fat content (2.94 ± 0.94%) showed no significant variation across samples from different sale outlets in this study. However, it was markedly lower than values (4.56–5.25%) reported by Kanwal *et al.,* (2004) for buffalo milk in the Rawalpindi region (20). This discrepancy could indicate breed-specific differences (e.g., comparing cattle to buffalo milk), regional variations, or potential skimming practices.

ADULTERANT SCREENING

Potential adulterants were screened using a commercially available milk adulteration test kit (PCSIR, Lahore). The test panel included detection of: detergents, hydrogen peroxide (H_2O_2), hypochlorite, boric acid (H_3BO_3), formalin (CH_2O), starch, urea, carbonates, sodium chloride (NaCl), sugars, skim-milk powder, pulverized soap, and starch. All tests were performed following the manufacturer's standardized procedures with appropriate quality controls.

Table II. Detection frequency (%) of adulterants in fresh raw milk samples

S. No.	Adulterant	Positivity percentage	Negativity percentage
01	Hydrogen peroxide	0	100
02	Detergent	**10**	90
03	Boric Acid	0	100
04	Starch	0	100
05	Urea	0	100
06	Carbonate	0	100
07	Formalin	0	100
08	Hypochlorite	0	100
9	Sodium Chloride	0	100
10	Sugar	**30**	70
11	Skim Milk	**25**	75
12	Pulverized soap	0	100

The results of present study demonstrate a distinct pattern of milk adulteration in the studied region. The complete absence of certain adulterants (particularly chemical preservatives like formalin and hydrogen peroxide) suggests either effective regulation of these substances or changing adulteration practices. However, the presence of detergent, sugar and skim milk powder at notable frequencies indicates ongoing quality issues, likely motivated by economic gain through volume manipulation.

The findings partially align with a previous study conducted by Chugh and Kaur (2022) in the Delhi-NCR region, which detected adulteration with water, skim milk powder, sugar, and detergent (21). The detection of detergent (in 10% of our samples) is particularly concerning, as it may indicate either poor hygiene practices during milk handling or intentional addition to enhance foaming properties. Furthermore, our results showing the relatively high prevalence of sugar (30%) and skim milk powder (25%) suggest economically motivated adulteration to increase volume and perceived richness. Such practices not only compromise nutritional quality but may also pose health risks, particularly for vulnerable populations. However, our results differ from those of Memon *et al.*, (2018), who reported additional adulterants — including starch, formalin, and sodium chloride — in marketed raw fresh milk in Hyderabad's milk supply (22); these adulterants were not detected in our study. These regional variations may reflect differences in: local adulteration practices, enforcement of food safety regulations, or the availability and cost of adulterants.

Milk adulteration remains a persistent challenge in developing countries, significantly altering the physicochemical properties of milk and compromising its nutritional quality (23, 24). This practice has become increasingly prevalent due to several interconnected factors: rapid urbanization, population growth, and the consequent surge in milk demand have created economic incentives for adulteration (22). Adulterators primarily employ this deceptive practice to artificially increase milk volume, thereby maximizing profits while disregarding food safety standards. The most common adulterants - including water, vegetable proteins, and chemical extenders - are deliberately chosen for their ability to mimic genuine milk characteristics while substantially reducing production costs. This economically motivated fraud not only deceives consumers but also poses significant public health risks, particularly when harmful substances are used as adulterants.

AFLATOXIN-M1 CONTAMINATION LEVELS

The analysis of raw dairy milk samples from Nall revealed significant Aflatoxin-M1 (AFM1) contamination, as presented in Table III. Of the tested samples, 65% were contaminated with AFM1, with 20% exceeding the stringent EU regulatory limit of 0.05 μg/kg. The remaining 45% of positive samples contained detectable levels below this threshold, while 35% showed no quantifiable contamination (below detection limit). These findings indicate substantial AFM1 exposure in the local dairy supply, posing potential health risks to consumers.

Table III. Aflatoxin-M1 contamination status

S. No.	Contamination level	Percentage
1.	More than 0.05 ug/kg	20
2.	Less than 0.05 ug/kg	45
3.	Not detected	35

Regional comparisons demonstrate consistently high AFM1 contamination across Pakistan, though with notable geographical variations. While the current study found 65% of Nall's milk samples contaminated, previous research reports even higher prevalence—76.3% in Punjab's milk supply [25] and 96.43% in Sindh, where 70% of samples exceeded US regulatory limits [26]. These findings, corroborated by Yunus et al.'s (2019) risk assessment, confirm that Pakistani consumers face widespread AFM1 exposure. The observed inter-study differences likely arise from multiple factors: geographical disparities in feed storage conditions promoting Aspergillus growth, seasonal fluctuations in fungal contamination, variations in analytical detection limits, and divergent dairy farming practices [27]. Of particular concern is that 20% of Nall's samples surpassed the EU's strict 0.05 μg/kg threshold—a troubling finding given AFM1's documented hepatotoxicity and carcinogenicity with chronic exposure. Even the 45% of samples with detectable but lower-level contamination merit concern, as they indicate persistent background exposure that may cumulatively impact public health, especially in vulnerable populations.

CONCLUSION

Substantial compositional deviations were observed, confirming adulteration with water, detergent, hypochlorite, sugar, and skimmed milk, as well as AFM1 contamination levels exceeding permissible limits. This compromised milk safety profile demands urgent intervention from food regulatory bodies, including systematic surveillance and stringent regional controls.

References:

1. Poonia A, Jha A, Sharma R, Singh HB, Rai AK, Sharma N. Detection of adulteration in milk: A review. International journal of dairy technology. 2017;70(1):23-42.
2. Al-Bedrani DI, ALKaisy QH, Rahi AK. Evaluation of milk source on physicochemical, texture, rheological and sensory properties of yogurts. Journal of Applied and Natural Science. 2023;15(1):128.
3. Ahmad S, Anjum FM, Huma N, Sameen A, Zahoor T. Composition and physico-chemical characteristics of buffalo milk with particular emphasis on lipids, proteins, minerals, enzymes and vitamins. J. Anim. Plant Sci. 2013; (sup I):62-74.
4. Ahmed M, Khaleeq A, Huma R, Qadir MA, Shafiq MI, Israr A, Ali A, Shahzad S. Optimization and validation procedure for elemental composition of fresh and pasteurized milk in Pakistan employing microwave digestion followed by ICP-OES: A contribution to risk assessment. Food Analytical Methods. 2016;9:2933-42.
5. Swathi JK, Kauser N. A study on adulteration of milk and milk products from local vendors. International Journal of Biomedical and Advance Research. 2015;6(09):678-81.
6. Ionescu AD, Cîrîc AI, Begea M. A review of milk frauds and adulterations from a technological perspective. Applied Sciences. 2023 Aug 30;13(17):9821.
7. Rashid N, Bajwa MA, Rafeeq M, Tariq MM, Abbas F, Awan MA, Khan MA, Shahzad I, Rehman A, Ahmad Z. Prevalence of aflatoxicosis in broiler chickens in Quetta, Pakistan. Pakistan Journal of Zoology. 2013;45(4).

8. Goncalves L, Dalla Rosa A, Gonzales SL, FELTES MM, Badiale-Furlong E, Dors GC. Incidence of aflatoxin M1 in fresh milk from small farms. Food science and technology. 2017;37(spe):11-5.
9. Luqman M, Arif A, Zahra N, Wajid U, Abid S. Detection And Removal Of Seasonal Afm1 Occurrence In Various Milk Samples Collected From Different Shops Of Lahore. Chelonian Research Foundation. 2024;19(01):422-33.
10. Unusan N. Occurrence of aflatoxin M1 in UHT milk in Turkey. Food and Chemical Toxicology. 2006;44(11):1897-900.
11. Jafari K, Fathabad AE, Fakhri Y, Shamsaei M, Miri M, Farahmandfar R, Khaneghah AM. Aflatoxin M1 in traditional and industrial pasteurized milk samples from Tiran County, Isfahan Province: a probabilistic health risk assessment. Italian Journal of Food Science. 2021;33(SP1):103.
12. Ferrari L, Fumagalli F, Rizzi N, Grandi E, Vailati S, Manoni M, Ottoboni M, Cheli F, Pinotti L. An eight-year survey on aflatoxin B1 indicates high feed safety in animal feed and forages in northern Italy. Toxins. 2022;14(11):763.
13. Tadesse S, Berhanu T, Woldegiorgis AZ. Aflatoxin M1 in milk and milk products marketed by local and industrial producers in Bishoftu town of Ethiopia. Food control. 2020;118:107386.
14. Ferrari L, Rizzi N, Grandi E, Clerici E, Tirloni E, Stella S, Bernardi CE, Pinotti L. Compliance between food and feed safety: Eight-year survey (2013–2021) of aflatoxin M1 in raw milk and aflatoxin B1 in feed in northern Italy. Toxins. 2023;15(3):168.
15. Rashid N, Bajwa MA, Rafeeq M, Khan MA, Ahmad Z, Tariq MM, Wadood A, Abbas F. Prevalence of aflatoxin B1 in finished commercial broiler feed from west central Pakistan. J. Anim. Plant Sci. 2012;22(1):6-10.
16. Górska-Warsewicz H, Rejman K, Laskowski W, Czeczotko M. Milk and dairy products and their nutritional contribution to the average polish diet. Nutrients. 2019;11(8):1771.
17. Yoganandi J, Mehta BM, Wadhwani KN, Darji VB, Aparnathi DK. Comparison of physico-chemical properties of camel milk with cow milk and buffalo milk. Journal of Camel Practice and Research. 2014;21(2):253-8.
18. Soomro AA, Khaskheli M, Memon MA, Barham GS, Haq IU, Fazlani SN, Khan IA, Lochi GM, Soomro RN. Study on adulteration and composition of milk sold at Badin. Intl J Res Appl Nat Social Sci. 2014;2(9):57-70.
19. Kausar R, Hameed A, Jamil H, Iqbal Z, Bahadur SU, Jabbar J, Sabir A. Comparative analysis of buffalo and cow milk for quality characteristics and β-N-acetyl-glucosaminidase activity in non-infected animals. Emirates Journal of Food and Agriculture. 2023;35(3):203-9.
20. Kanwal R, Ahmed T, Mirza B. Comparative analysis of quality of milk collected from buffalo, cow, goat and sheep of Rawalpindi/Islamabad region in Pakistan. Asian Journal of Plant Sciences. 2004;3(3):300-5.
21. Chugh R, Kaur G. A Study on Milk Adulteration and methods of detection of various Chemical Adulterants qualitatively. InIOP Conference Series: Materials Science and Engineering 2022 (Vol. 1225, No. 1, p. 012046). IOP Publishing.
22. Memon MA, Khaskheli M, Kamboh AA, Soomro NA, Mangsi AS, Barham GS, Korejo NA. Surveillance of milk adulteration and its influence on physico-chemical characteristics of milk in Hyderabad, Pakistan. A Review. J. Anim. Health Prod. 2018;6(1):5-12.
23. Barham GS, Khaskheli M, Soomro AH, Nizamani ZA. Adulterated milk used for consumption in Thatta District of Sindh, Pakistan. American Journal of Agricultural Science. 2015;2(3):91-6.
24. Haasnoot W, Smits NG, Kemmers-Voncken AE, Bremer MG. Fast biosensor immunoassays for the detection of cows' milk in the milk of ewes and goats. Journal of Dairy Research. 2004;71(3):322-9.
25. Sadia A, Jabbar MA, Deng Y, Hussain EA, Riffat S, Naveed S, Arif M. A survey of aflatoxin M1 in milk and sweets of Punjab, Pakistan. Food control. 2012;26(2):235-40.
26. Jawaid S, Talpur FN, Nizamani SM, Afridi HI. Contamination profile of aflatoxin M1 residues in milk supply chain of Sindh, Pakistan. Toxicology reports. 2015;2:1418-22.
27. Yunus AW, Imtiaz N, Khan H, Ibrahim MN, Zafar Y. Aflatoxin contamination of milk marketed in Pakistan: A longitudinal study. Toxins. 2019;11(2):110.