

ANALYSIS OF PHYSICOCHEMICAL AND NUTRITIONAL PARAMETERS OF RAW MILK FROM COMMERCIAL DAIRY FARMS-IMPLICATIONS OF ADULTERATION FOR PUBLIC HEALTH

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ABSTRACT

Objective: Globally, milk adulteration is an important public health issue but more so in developing countries, for small financial gains. This study was designed to assess the physicochemical and nutritional parameters of raw milk from commercial dairy farms from a public health perspective.

Material and Methods: In this study 29 bulk samples of raw cattle milk were selected, one from each dairy farm in Peshawar, using simple random sampling and analyzed using by ultrasonic milk analyser. The main parameters tested for adulteration of milk were urea, starch, hydrogen peroxide, detergent, sorbitol, quaternary ammonium compounds, boric acid, cane sugar, sodium chloride, carbonate, formalin, hypochlorite, freezing point, added water, salt percentages and electrical conductivity (EC). Additionally, nutritional quality was assessed by fats, solids-non-fats (SNF), lactose, and protein. The results were statistically analyzed by t-test using SPSS software.

Results: Results showed that more than 90% of the samples had water adulteration of more than 5% due to which 55% had significantly reduced nutritional parameters but still overall nutritional quality was acceptable in 24% of the samples. 21% of samples had high electrical conductivity reflecting mastitis in animals and consequently considered unsuitable for consumption due to the resultant transmission of either antibiotics or microorganisms into the human body. Furthermore, chemical adulterants were not detected in any sample. t-test showed that most of the parameters were significantly different ($p=0.05$) than the population standard means.

Conclusion: Milk samples had compromised quality due to water addition in half of them and raised EC values in about a quarter of the samples. Despite water adulteration, nutrients like fat and SNF remained within ranges, indicating a cow-buffalo milk blend. Most tested parameters were lower than national averages but above minimum values. No chemical adulterants were detected. There's a need for continuous milk testing to maintain public health standards.

key words: mild adulteration, commercial dairy farms

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INTRODUCTION

Milk is not only an ideal natural collection of carbohydrates, proteins, and fats but also has rich concentrations of vitamins, minerals, and hormones. It plays a vital role in strengthening bones, teeth, and muscles, especially in children. ¹ But, the issue at hand is that we are not consuming nutritious and pure milk in the first place to get

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its above-mentioned benefits, thus rapid screening of milk to detect adulteration is the need of today. Pakistan being the 4th largest milk producing country in the world, has its economy instrumentally dependent on the milk industry. Annual milk production was estimated to be 65.7 million tons in 2022-23.² In developing countries where food safety authorities are rather lenient, free from financial gain, problems of shortage of supply and several other factors take charge and milk falls victim to economically motivated adulteration. ³ The most common adulterant is water, which reduces the nutritional value of milk by many folds and the milk tastes watered down. ³ Dilution with water increases the volume but decreases the number of calories and nutrients like proteins, fats, etc the consumer gets per ml of milk. Furthermore, the water used in the process isn't distilled and is often contaminated. ³

Khyber Pakhtunkhwa (KPK), the second most populous province of Pakistan, produces 4.88 Million liters of milk per year.² According to research done in Peshawar, the average consumption of milk is 430gm per capita daily, or just under one million liters of milk consumed per day cumulatively.⁴ Hence, a public health survey is needed to find out whether milk in the capital city of KPK, Peshawar, is safe for consumption at large or not. A large proportion of the population consumes milk supplied by dairy farms and not packaged milk as the latter is more expensive for the general population. There has been some research on adulteration of milk purchased from the milk shops and packaged milk but there's little data that tests the raw milk directly from the dairy farms. ¹ This study gave an insight into whether physicochemical and nutritional adulteration of milk in Peshawar was present at the sources i.e. dairy farms or done at a later stage in the food chain e.g. at the milk outlets by the vendors. It should be noted that the study does not cover microbiological contamination of milk as it is dependent on milking hygiene practices and handling of milk.

The primary objective of this research was to analyze the different physicochemical and nutritional parameters of dairy farm milk and to assess its nutritional value in Peshawar to make sure that people are drinking healthy and nutritious milk.

MATERIALS AND METHODS

A descriptive cross-sectional study was conducted in the district of Peshawar among the commercial dairy farms registered with the Livestock and Dairy Development Department KPK. The time frame for the study was from May 2022 to August 2022. The sample size for this study was calculated to be 33 but 29 samples were eventually collected because the remaining four farms had been permanently closed due to unknown reasons. Cochran's formula was used to calculate sample size, based on another study done on milk in Peshawar. ^{1,5} The Institutional Research and Ethical Approval Board (IREB) of Khyber Medical College Peshawar gave ethical clearance for this study vide letter no. 436/DME/KMC dated 27-06-2022. Permission for Milk testing was granted by the Office of the District Director Livestock Peshawar vide letter no. 1419 dated 13-06-2022 upon request from the Associate Dean (Research) Khyber Medical College vide letter no.724 dated 31-05-2022. The confidentiality of the respondents was maintained. Farm owners were informed about the purpose of the research study.

A master list of 417 registered dairy farms in Peshawar was procured from the Livestock and Dairy Development Department KPK. 33 Farms were selected using a simple random sampling technique (computer-generated sequence) from the master list using an online randomizer tool from the website: <https://www.randomizer.org>.

All the collected samples were bulk samples, as they are dispensed to the public in the same way, and not from an individual animal. Obtained samples were of fresh milk that hadn't gone through any intervention i.e. pasteurization, boiling, ultra-heat treatment (UHT), freezing, or packaging. In case any farm wasn't able to provide the milk samples, a substitute nearest farm was selected from the same location, or a raw milk sample was taken from the nearest sourced milk shop that had been supplied by the designated farm. This sampling strategy was chosen to improve the external validity of the study. The collection of samples was done in the morning hours over a couple of consecutive days.

The variables of the study were the following parameters: The chemical adulterants; urea, starch, hydrogen peroxide, detergent, sorbitol, quaternary ammonium compounds, boric acid, cane sugar, sodium chloride, carbonate, formalin, and hypochlorite. The nutritional parameters tested were fats, solids-non-fats (SNF), lactose, protein, and salts. Apart from these, electrical conductivity, freezing point, added water, pH, and density of milk were also tested.

Inclusion criteria were raw milk from the commercial dairy farms of Peshawar. Exclusion criteria were any dairy farm that had been permanently closed.

Approximately 500 ml of raw milk was collected in line with the standard protocol of milk collection and transported aseptically to the laboratory for analysis. Samples were given identification numbers and addresses of the farms to ensure systematic collection. The samples were stored in a chilled container but not frozen and delivered to the testing facility within 48 hours.

Milk analysis for all the above-mentioned variables was done using Milkotronic Ltd. Lactoscan SA50 Ultrasound milk analyzer. The ultrasonic working principle is based on measuring the speed of the ultrasound in milk and dairy products. All results from the Lactoscan analysis are based on a direct measurement of the parameters, which means confidence in the results. Analysis was done at the Milk Testing Laboratory, Civil Veterinary Hospital, Peshawar. The data was presented in the form of tables with standard deviation values and t-test results.

RESULTS

A total of 29 samples were analyzed. The raw data for all the parameters of adulteration are listed in Appendix A. The results showed that all the samples were negative for chemical adulterants. More than 90% of the samples showed water adulteration to some extent (Figure 1). After combining all factors of adulteration, the following results were calculated: 55% of samples had reduced nutritional status due to the addition of more than 5% water. 21% of samples had high electrical conductivity (6.5-12 mS/cm) reflecting mastitis in animals. However, 24% of samples

were largely unaltered as all parameters were within acceptable range including water addition of less than 5%.

A two-tailed t-test was performed on the major parameters of all the samples. The results of this study showed that the means of most of the physicochemical and nutritional parameters were significantly different from the standard means ($p=0.05$). The standard milk parameters used for comparison and analysis are given in Table 1. The mean values of the tested parameters, along with statistical analysis are listed in Table 2.

All values quoted in the table have been numbered with a reference number of the sources. Both mean values and minimum values have been mentioned. The sources include mean values taken from studies done on Pakistani cow and buffalo milk. The minimum values have been taken from international food regulation bodies. The letter 'a' denotes the values used for the t-test 'standard mean.'

The bar chart shows the percentage of water that has been added to the milk samples. A total of 2 samples had no water added to them, while 13 samples had water addition between 1-10%, similarly 6 samples had 10-20%, 3 samples had 20-30%, 3 samples had 30-40% and only 2 samples had greater than 40% water added in them.

DISCUSSION

Multiple factors can change the composition of milk naturally e.g. nutrition of the animal, breed of animal, seasons, emotional status of the animal, milking intervals, etc. The fat content of milk is the most variable component as compared to other variables.⁷ In this study, fat values were significantly higher than the mean fat value of Pakistani cattle milk samples from another study ($p=0.05$).⁸ The distribution showed that none of the samples had a fat percentage less than 3.2% (minimum standard for cow milk), while 5(17%) samples had 3.2-4.5% fat, 16(55%) samples had 4.5-6% fat (minimum standard for mixed milk) and 8(28%) samples had a fat percentage greater than 6% (minimum standard for buffalo milk).⁹

Solid-non-fat (SNF) is another major component of milk that includes all other solids like casein, albumin, globulin, and lactose. Like fat, SNF can vary too, and does not provide accurate evidence for the presence of extraneous water in milk. SNF mean value was higher than the mean of Pakistani samples but not significant at $p=0.05$.¹⁰ It had the following distribution: 14 (48.3%) samples had less than 8.3% SNF present in them (minimum cow

Table 1: standard means and ranges of milk parameters

Source of Milk	Fat	SNF	Densit @ 18°C	Lactose	Salts	Protein	Freezing Point	pH	Conductivity (mS/cm) ¹⁸
Cow	4.95%±1.9010 a Min 3.2% ⁹	8.24%±0.7310 a Min 8.39	1030.711 lbs./ft ³ a	4.5-4.8% ¹³ a	0.8-0.9% ¹⁸ a	3.56%±0.0510 a Min 3% ¹²	-0.522°C ±0.00215 a Min ≤ -0.525°C ⁶	6.62±0.1010 a	Normal 3-5.5 a
Buffalo	7.45%±0.4210 Min 6.0% ⁹	10.36%±1.710 Min 9.0% ⁹	1034.111 lbs./ft ³	4.7-5.0% ¹³		4.17%±0.5110	-0.545°C ±0.01016	6.71±0.1110	Sub-Clinical Mastitis 5.5-6.
Mixed ⁹	Min 4.5% ⁹	Min 8.5% ⁹							Clinical Mastitis 6.5+

i. All values quoted in the table have been numbered with a reference number of the sources. Both mean values and minimum values have been mentioned. The sources include mean values taken from studies done on Pakistani cow and buffalo milk. The minimum values have been taken from international food regulation bodies.

ii. The letter 'a' denotes the values used for the t-test 'standard mean.'

Table 2: T-Test Analysis Of Tested Parameters Of Milk

	Fat	SNF	Density	Lactose	Salts	Protein	Added water	Freezing point oC	pH	Electrical Conductivity
Sample Means	5.45%	8.12%	1030.09	3.69%	0.56%	3.81%	15.37%	-0.450	6.05	5.21
Std. Deviation	±1.15	±1.23	±4.44	±0.53	±0.08	±0.57	±14.27	±0.08	±0.67	±2.22
t- test* t(29), p=0.05 tc =2.048	2.341	0.525w	0.740	9.790	19.33	2.362	-	4.80	4.581	2.329

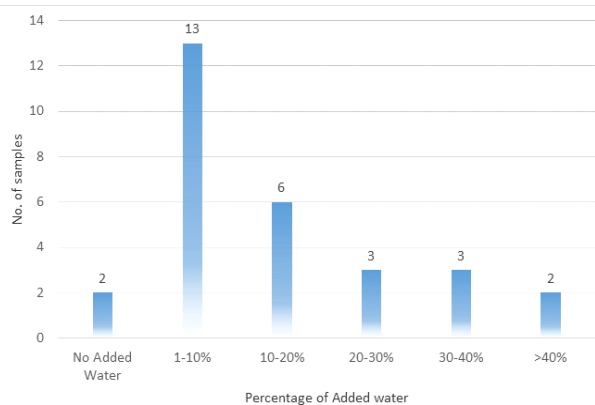


Fig 1: Percentage of added water in milk samples

standards), while another 7 (24.1%) samples had 8.3-8.9% (minimum standard for mixed milk) whereas 8(28%) samples had values greater than 9% (minimum for buffalo milk) of SNF.⁹ The above-mentioned minimum cut-off percentages of fat and SNF in cow, buffalo, and mixed milk were taken from 'Food Safety and Standards Regulations, India', to interpret the results, as the cattle breeds were found to be similar in the two countries.⁹

The mean density of the tested samples was 1030.09 ± 4.44 while the density of standard whole milk at 18°C is around $1032 \text{ kg/m}^3 \pm 2$.¹¹ Milk densities were not significantly different ($p=0.05$) from the standard means.

The fat, SNF, and density values were high despite adulteration with water; this could be the consequence of the admixture of cow milk with buffalo milk where the latter is naturally higher in these values. Many dairy farm owners themselves stated that they mixed the different types of milk. Mixing milk from different types of animals, especially cows and buffaloes is a familiar practice and comes under the definition of adulteration.¹⁰ This may be done to reach regulation standards. Cow and buffalo milk differ in regards to their fat, SNF, and lactose content which are all higher in buffalo milk. Although there are methods to detect this admixture of milk, they are complex and not usually performed generally, as countries like Pakistan and India don't prohibit the mixing of milk from more than one species of animal.

The protein content was within permissible limits in a dominant chunk of the samples and only 4 (13.8%) samples had protein content $<3\%$, which is the minimum standard according to the 'Australia New Zealand Food Standards Code'.¹² However, the mean protein content of the samples in this study was 3.81% which is more than the mean protein content of Pakistani cow milk (3.56 %) but less than Pakistani buffalo milk (4.17%).¹⁰ This further

endorses the phenomenon of mixing the milk of different cattle.

The percentage of salts and lactose was considerably low as compared to the sample means of other studies. 5(17%) samples had lactose percentages between 2-3%, while 16(55%) samples had lactose concentrations between 3-4%, and 8(28%) samples showed lactose percentages greater than 4%. The average lactose level in cow and buffalo milk in regional research was 4.85% and 5.48% respectively whereas in this study the mean lactose was 3.69% which was significantly low ($p=0.05$).^{2,13} This can also probably be attributed to the addition of water.

It is important to note that water was added to most of the samples; up to 40% in some milk samples (Figure 1). This was deduced by the milk analyzer from the freezing point which decreases as water is added to milk, using dissolved solids. The freezing point of milk is the most constant and precise value for determining the presence of added water, due to biophysical reasons.¹⁴ Evidence shows the average freezing point of cow and buffalo milk is $-0.522^\circ\text{C} \pm 0.002$ and $-0.545^\circ\text{C} \pm 0.010$ respectively^{15,16}, whereas in this study, the sample mean freezing point was $-0.450^\circ\text{C} \pm 0.08$. This adulteration causes milk's nutritional value to be largely reduced, including the consumed calories/ml. This is a significant risk to public health nutrition.

The electrical conductivity (EC) of milk is measured to test for mastitis in animals. It is affected by the electrolytes present in milk, can be increased by adding salts and decreased by adding water in milk, but is always higher than the normal average in animals with mastitis.¹⁷ It is one of the major parameters to judge milk quality and the presence of mastitis quickly and reliably in animals. In this study 6 (21 %) samples showed raised EC (6.5-12 mS/cm) and indicated clinical mastitis, 2 samples had 5.5-6.5% (subclinical mastitis) and 14 samples had 3.5-5.5% (healthy). These cut-off values and categories were provided by another research.¹⁸ Animals receiving antibiotics for these infections can pass them onto humans through their milk, which is detrimental to health. Alternatively, untreated animals can pass on microorganisms such as *S. aureus*, *Str. Agalactiae*, *Str. Dysgalactiae* and *E. coli* etc in milk, causing diseases in humans.¹⁹

Another parameter of milk analysis was pH. The normal pH of fresh cow milk is 6.6 -6.9. The addition of preservatives and other adulterants can raise the pH of milk above the standard.²⁰ It was observed that the pH of milk was not a reliable indicator for detecting subclinical mastitis in lactating cattle.²¹ The pH value of milk at best

shows spoilage caused by bacterial fermentation which is based on the appropriate storage and timely usage of the milk by the end users. Hence this parameter was insignificant to our findings.

This study showed that none of the samples had any chemical toxins present, though previous studies reported the presence of chemical adulterants in milk consumed in Peshawar ^{4,22}. They included both packaged and fresh milk whereas our study was conducted on raw milk from dairy farms only. Furthermore, the lack of chemical adulterants might be due to the strict implementation of a ban by the provincial government. According to the Punjab Food Authority in Pakistan, only half of the milk samples were found to be adequate for drinking. Another report revealed that three-quarters of the samples of milk collected from Peshawar were found unhealthy and unhygienic. ²²

Despite best efforts to conduct the study, it had certain limitations. Only those farms that were registered with 'The Livestock and Dairy Development KPK Authority' had the farm details mentioned, allowing for a scientific sampling strategy; and were easily accessible. This may have omitted a few private small-scale farms from the study but not significant enough to affect the outcome of the study as they are not the major suppliers. Moreover, on-site testing at the farm could've reduced any systematic errors.

CONCLUSION

Water was the main adulterant resulting in significantly decreased nutritional quality in half of the milk samples. About a quarter of milk samples had high electrical conductivity indicating mastitis. Most nutritional parameters were more than the minimum values but lower than the national average milk values. An admixture of buffalo and cow milk was done. There were no chemical adulterants/preservatives detected in the milk. Continuous monitoring of milk should be made mandatory, with regular testing of dairy farms to uphold public health safety standards.

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Authors Contribution:

Following authors have made substantial contributions to the manuscript as under

Authors	Conceived & designed the analysis	Collected the data	Contributed data or analysis tools	Performed the analysis	Wrote the paper	Other contribution
Azhar S	✓	×	✓	×	✓	×
Jamil W	✓	✓	×	✓	✓	×
Ali AH	✓	×	×	✓	×	×
Iftikhar B	✓	×	✓	×	✓	×
Orakzai M	×	✓	✓	×	×	✓
Afaq Y	✓	×	×	✓	×	×
Arshad A	×	×	✓	×	✓	×
Azhar A	✓	×	✓	×	✓	×
Ali W	✓	×	✓	×	✓	×

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical Approval:

This Manuscript was approved by the Ethical Review Board of
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