Assessment of Microbial Quality, Physiochemical Properties and Adulteration of Raw Milk in Pokhara

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ABSTRACT

Objectives: This study was conducted to assess the microbial quality, physiochemical properties and adulteration status of raw milk collected from different farms of Lekhanath, Pokhara, Nepal.

Methods: Total of 30 milk samples (15 Cow and 15 Buffalo) were collected from five different farms of Lekhanath, Pokhara. Total coliform count, isolation and identification of *Escherichia coli* and *Staphylococcus aureus* were determined by pour plated and spread plate technique and different biochemical and enzymatic tests, respectively. Additionally, physiochemical properties and adulteration parameters were also tested.

Result: The laboratory analysis revealed that, the total coliform count of raw cow and buffalo milk samples were found as 0.55×10^4 to 2.13×10^6 CFU/ml and 0.34×10^4 to 1.76×10^6 CFU/ml respectively which is higher than the DFTQC and BIS guidelines. *E. coli* and *S. aureus* were found as 40%, 13.33% and 46.67%, 33.33% for cow and buffalo milk respectively. 20% of the raw buffalo milk sample were adulterated with Neutralizer. However, no adulteration was detected for table sugar, formalin, and starch. The average fat content of cow and buffalo milk were found to be 4.13% and 5.47% respectively meeting the minimum requirement set by guidelines. The solid-not-fat and Total solid of the samples were within the acceptable limit and acidity level were within the expected range indicating proper handling and storage of the milk sample.

Conclusion: Thus, the findings suggest that the raw milk samples analysed in this study may not meet the microbiological safety standards for consumption. Proper hygiene practice and quality control measures should be implemented to ensure the safety and quality of milk.

Keywords: Total coliform count, *Escherichia coli, Staphylococcus aureus,* milk adulteration, physiochemical properties.

INTRODUCTION

Milk, a vital source of nutrition, boasts a well-rounded composition encompassing essential nutrients (Baharullah, 2013). Cow and buffalo milk differs slightly in their proportion, as cow milk contains about 3.8% protein, 4.5%fat, 4.9%lactose, 13.9%total solids, and 0.72%ash. On the other hands buffalo milk contains 3.8%protein, 4.9% lactose 7.6% fat, 17% total solids and 0.78% ash, (Burlingame, 2016). However, both are crucial for balance diet (Dhamala, 2018). Despites its nutritional value, milk is susceptible to microbial contamination during production and processing stages, (Sharma, 2018). Potential sources include infected animal, unclean milking,

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equipment contact between milk and faecal matter and environmental sources (Glession, 2013).

Diseases like Tuberculosis and Brucellosis were historically Transmitted through milk but improved sanitary practices and pasteurization have reduce such risks. (Hameed, 2007). Nonetheless, concerns persists about pathogens like *Listeria, Campylobacter, Salmonella Staphylococcus and Escherichia coli,* especially in raw milk *E. coli* in particular poses severe health hazard causing gastrointestinal infection with symptoms ranging from abdominal pain to kidney damage (Abdulsamad, 2007) *Staphylococcus aureus* contamination stemming from poor hygiene during milking or processing can lead to Staphylococcal food poisoning (Jamali, 2015).

Physiochemical properties of milk like fat, solid not fat (S.N.F.), total solid, density of milk vary based on factors like breed, diet, and stage of lactation (Bansal, 2013). Food adulteration is the deliberate act of lowering quality of food available for sale, achieved either by mixing or substituting it with inferior or by the removal of valuable ingredients (Sharma, 2018). To keep milk temporarily fresh some unethical methods were adapted to avoid the financial loses during transportation and sale. The methods involve adding water to increase the volume of milk, thickening agents like starch flour or skimmed milk powder and introducing other ingredients to prevents dilution and extends milks solid contents (Aparnathi, 2020). The safety of milk and its products is a significance global concern particularly in underdeveloped and developing nations where unsanitary conditions prevail (Dhungel, 2019). Addressing these risks requires comprehensive studies to assess microbial quality, identify adulterants and understand their impact on public health.

This study aims to identify bacterial contamination, assess physiochemical properties, and detects adulterants in raw milk from farms in Lekhanath, Pokhara, Nepal.

METHODS

Study duration and sampling

During the period of December 2022 to February 2023, A total of 30 raw milk samples were collected. The milk sample comprising 15 raw cow milk and 15 raw buffalo milk were collected in a screw capped jar from five different farms of Lekhanath, Pokhara. The collected samples were kept in ice box and transported to laboratory. Samples were processed promptly with in a time frame of 2 hours. Each sample were labelled well.

Microbiological analysis

Total Coliform Count (TCC)

Total coliform test was carried out according to Laboratory Handbook of Dairy Industry, National Dairy Development Board (NDDB, 2001)In which 10^{-6} dilution of milk sample were done, and total coliform count was determined by pour plate method on Violet Red Bile Agar and incubated at 37°C for 24 hours. Following the incubation, the bacteria count was assessed as colony forming unit per millilitre (CFU/ml).

Identification of *Escherichia coli* from isolated plates

For the identification of *E. coli* from isolated plates, colonies were subculture on Nutrient Agar and incubated at 37°C for 24 hours. After incubation Enzymatic test such as catalase and oxidase test and biochemical test such as TSI, MIU, Citrate Utilization test, MR-VP and O/F test were performed.

Isolation and Identification of *S. aureus*

Isolation of *S. aureus* was carried out by spread plate technique. 0.1ml of milk sample from different dilution number was poured into Mannitol Salt Agar (MSA) and spread with the help of bent glass rod. Then the plates were incubated at 37°C for 24 hours. After incubation, isolated golden yellow colony were sub-culture on Nutrient Agar and incubated at 37°C for 24 hours. After incubation, colonies were tested for Catalase test, Oxidase test and Coagulase test.

Physiochemical analysis

Collected samples were tested for physiochemical analysis such as fat, solid not fat, total solid, and acidity according to laboratory manual of method of analysis of food, feed, and water published by National Food and Feed laboratory, Ministry of Agriculture and Livestock Development (MOALD, 2019). Briefly the test was done as follows.

Fat: Fat content of milk was determined by the Gerber's method, with the help of butyrometer.

Solid Non-Fat (SNF): Solid not fat content of milk was determined by Fisherman's formula with the help of fat percentage and corrected lactometer reading (CRL) along with the temperature of milk.

Total Solid: Total solid content of milk was determined by Corrected Lactometer reading by Fisherman's formula with the help of fat percentage and corrected lactometer reading**.**

Titratable Acidity: The acidity of milk was determined by titrating the milk sample against 0.1N sodium hydroxide using phenolphthalein indicator. The acidity is then calculated by using the formula.

Adulteration in milk

Collected samples were tested for adulterant such as Starch, Formalin, Neutralizer, and Table sugar test as per

the laboratory manual of method of analysis of food, feed, and water published by National Food and Feed laboratory, Ministry of Agriculture and Livestock Development, Government of Nepal,2019. Briefly the test was done as follows.

Starch Test: Starch in milk was detected by adding iodine solution to it. The presence of blue colour indicates positive test.

Neutralizer Test: Neutralizer in milk was detected by Rosalic acid method. The presence of rosy-red colour indicates positive test.

Formalin Test: Formalin in milk was detected by adding 90%H_2SO_4 containing traces of $FeCl_3$. Formation of Purple ring at the junction indicate the positive test.

Table Sugar Test: Table sugar in milk was detected by adding 0.5% resorcinol solution. The presence of red colour indicates positive test.

RESULTS

In this study, the microbial quality, physiochemical analysis, and adulteration of the total 30 milk sample (Cow and buffalo) were evaluated by determining the bacterial load, physiochemical properties and adulterants of milk consumed by the population.

Adulteration

Out of the 15 buffalo milk samples tested, three sample were found to be positive for the presence of neutralizer. Starch, formalin and table sugar were absent in all the buffalo milk samples tested. In contrast, all 15 cow milk samples tested negative for the presence of starch, neutralizer formalin and table sugar (Table 1).

Table 1: Adulteration in raw milk

Test	Cow (n=15)	Buffalo (n=15)
Starch test	ND	ND
Neutralizer test	ND	3 (20%)
Formalin test	ND	ND
Table sugar test	ND	ND

ND = not detected.

Physiochemical properties of milk

Out of 15 buffalo milk sample tested the average value for Fat%, Total solid%, Solid not fat% and acidity was found as 5.47%, 13.59%, 8.17% and 0.16 respectively. Similarly, in 15 cow milk samples, the average value for Fat, total solid, SNF and acidity was found as 4.19%, 12.16%, 7.9% and 0.15, respectively (Figure 1 and 2).

Microbial quality of milk

Total coliform count

In 15 cow milk samples the total coliform count ranged from 0.55×10^4 CFU/ml to 2.63×10^6 CFU/ml. Similarly in 15 buffalo milk samples, the total coliform count ranged from 0.34×10^4 CFU/ml to 1.76×10^6 CFU/ml (Table 2 and 3)).

Table 2: Total coliform count of cow milk

S. N.	Farm no	Symbol	Average CFU/ml of TCC
1		C1	213×10^4
2	F1	C2	43×10^4
3		C3	58×10^4
4		C4	TMTC
5	F2	C5	225×10^2
6		C6	164×10^4
7		C7	89×10^4
8	F3	C8	55×10^2
9		C9	74×10^4
10		C10	77×10^4
11	F4	C11	78×10^4
12		C12	67×10^4
13		C13	102×10^2
14	F5	C14	55×10^4
15		C15	124×10^4

Table 3: Total coliform count of buffalo milk

S. N.	Farm no	Symbol	Average CFU/ml of TCC
1		B1	63×10^4
2	F1	B2	34×10^2
3		B3	42×10^4
4		B4	37×10^4
5	F2	B5	65×10^4
6		B6	125×10^4
7	F3	B7	163×10^4
8		B8	105×10^4
9		B9	67×10^4
10		B10	139×10^4
11	F4	B11	TMTC
12		B12	176×10^4
13		B13	43×10^4
14	F5	B14	159×10^2
15		B15	88×10^4

Distribution of *E. coli* and *S. aureus* in milk samples

In 15 cow milk samples, *E. coli* and *Staphylococcus aureus* were identified from 40% and 13.33% of the samples, similarly in 15 buffalo milk samples, *E. coli* and *Staphylococcus aureus* were identified from 46.67% and 33.33% of the samples (Table 3).

Table 3: Distribution of *E. coli* and *S. aureus* in cow and buffalo milk

Milk type	*E. coli*	*S. aureus*
Cow	6 (40%)	2 (13.33%)
Buffalo	7 (46.67%)	5 (33.33%)
Total	**13 (43.33%)**	**7 (23.33%)**

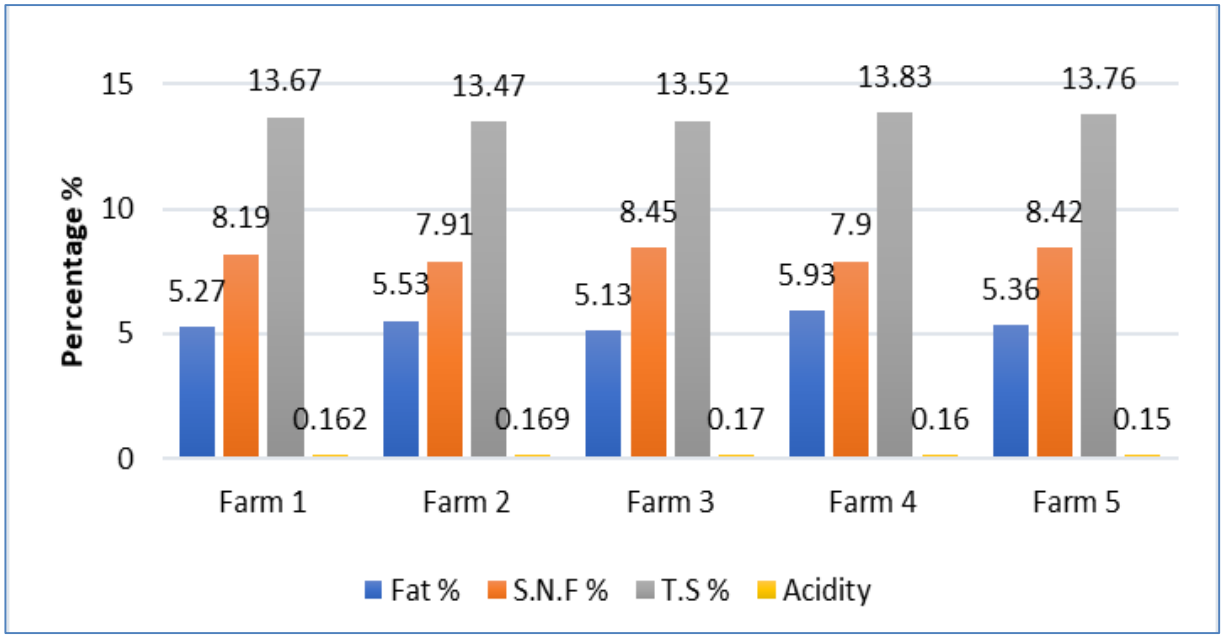

Figure 1: Physiochemical properties of buffalo milk

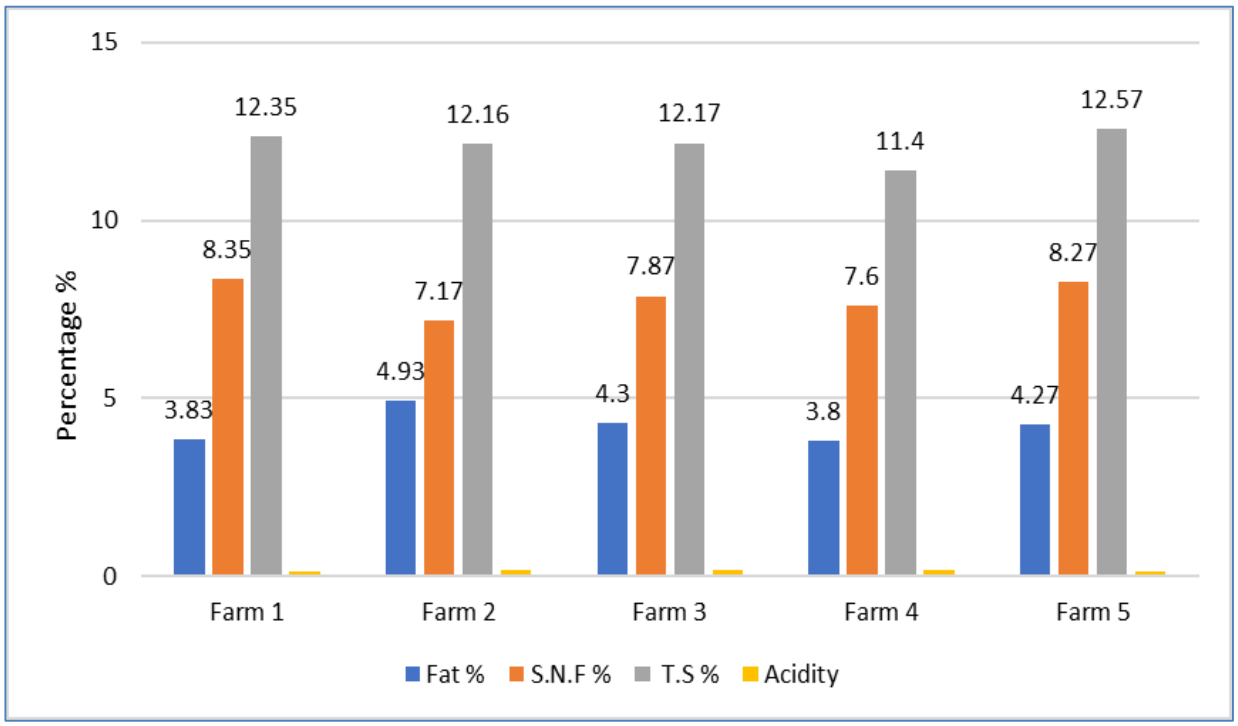

Figure 1: Physiochemical properties of cow milk

DISCUSSION

Food adulteration is the deliberate act of lowering quality of food offered for sale, achieved either by the mixing or substituting it with inferior ingredients or by the removing of some valuable components (Sharma, 2018). The milk samples from farms were tested for adulteration of starch, neutralizer, formalin, and table sugar. During the testing of adulteration of milk, t hree samples (20%) were found to be neutralizer adulterated out of 15 buffalo milk samples. In contrast to buffalo milk samples, all 15 cow milk samples tested negative for the presence of starch, neutralizer formalin and table sugar.

As per the guidelines set by the Department of Food technology and Quality control (DFTQC, 2011), milk and milk products should not contain any adulterants. However, this research showed that 20% of raw milk samples were found to be adulterated with neutralizer. In the similar study conducted by (Parajuli, 2018) and (Chanda, 2012) reported that 40% and 20% of marketed raw milk samples of their study were detected as neutralizer positive. Since, the neutralizer are added to prevent milk from coagulation, which in turn is due to increase microbial activity and acidity of milk (Sharma, 2018). In a similar study conducted by (Parajuli, 2018) and (Limbu, 2020)reported that the extent of adulteration in raw milk of Kathmandu valley and Dharan with table sugar was 10% and 45% respectively. Finding of this shows that raw milk marketed in Lekhanath, Pokhara, was free from table sugar. Table sugars are added to increase the S.N.F content, it can be inferred that the quality of milk sold from farms in Lekhnath, Pokhara is completely better than that of Kathmandu valley and Dharan. Table sugars were used as an adulterant because of its easy availability and non-harmful from health point of view. Starch and formalin were used to increase S.N.F content and self life of milk (Aparnathi, 2020). Starch and formalin were not found as adulterant in the study by (Limbu, 2020) and this study too. It may be because, starch and formalin were expensive, difficult to homogenised and harmful (Parajuli, 2018).

As per the Department of Food technology and Quality control guidelines for milk and milk products, it is advised to have a minimum of 3.5% and 5% of milk fat for cow and buffalo milk. The present study showed 4.13% and 5.47% as an average fat content of cow and buffalo milk samples of different farms. These findings indicate that both type of milk meet the minimum fat content requirements as recommended by DFTQC guidelines for milk and milk products. The fat content of cow milk varied among the farms. (Parajuli, 2018) and (Teklemichael, 2012) reported 3.86% and 3.79% of fat content respectively. The average S.N.F. content in the tested milk was measured at 7.91% and 8.17% respectively. These S.N.F. values are higher than those reported by (Parajuli, 2018) and lower than the finding of (Debebe, 2010), who reported 7% and a range of minimum (8.3±0.30) to maximum (8.7±0.36). According to NDDB, the recommended S.N.F content in milk should be 8%. In most of the milk samples from different farms the average SNF content was found to be near to or equal to or higher than to that, which is acceptable as per the standard.

The average Total solid of cow and buffalo milk was found as 12.17% and 13.57% respectively. This value is higher than the (Parajuli, 2018) and (Teklemichael, 2012)who reported 10% and 12.58% respectively. As per the European Union standards, cow milk should have a total solid content of at least 12.5%, which is considered a recognized quality standard. The variation of fat, S.N.F and T.S content of milk can be attribute to various factors, such as genetics, stage of her lactation, feeding habits, animal health and seasonal changes (Sharma, 2018)

When analysing milk (cow and buffalo) samples, the average titratable acidity was measured as 0.15% and 0.16% respectively. The results indicate slight variation in acidity among the samples. According to Department of food technology and quality control, the average titratable acidity of milk is 0.16. (Gemechu, 2015) reported that the acidity of raw milk varies between 0.14% to 0.16%, expressed as lactic acid. In the similar study conducted by (Maskey, 2019),the average titratable acidity of cow milk was reported as 0.20% which is higher than present study.

The coliform count for cow milk sample ranges from 0.55×10^4 CFU/ml to 2.13×10^6 CFU/ml. Similarly in 15 buffalo milk samples, the total coliform count ranged from 0.34×10^4 CFU/ml to 1.76×10^6 CFU/ml. Higher coliform count observed in this study can be attribute to inadequate hygiene practice on the farm, the use of contaminated water during milking and the use of contaminated utensils

by farmers. While it is impractical to eliminate coliforms from milk, their presence does not necessarily indicate faecal contamination. However, it does serve as a reliable indicator of poor hygiene and sanitation during milking and subsequent handling process (Hassan, 2015). The raw milk samples from different farms were found to be unsafe from microbiological quality standard for human consumption. The BIS (Bureau of Indian Standards) Standard for coliform count in milk is 1 coliform per 10 millilitres, which can be expressed as 1:10 coliform per ml. Both cow and buffalo milk sample have coliform count significantly higher than the BIS standard. This finding was higher than some previous studies, (Limbu, 2020), (Koirala, 2016) (Shrestha, 2012).However, this finding was lower when compared to (Rizal, 2023), who reported that total coliform count from farm milk was 39.83×106±77.39. Coliforms serve as indicator organism in food because their presence indicates potential contamination. Elevated coliform counts of raw milk can result from factors like poor hygiene, contaminated water, unsanitary milking practice and unclean equipment (Arjyal, 2004).

E. coli and *S. aureus* were isolated from 40% and 13.33% cow milk sample and 46.67% and 33.33% from buffalo milk sample. *E. coli* can be confirmed by a positive indole test and their inability to utilize citrate as a sole carbon source (Sharma, 2018).The reason for the presence of *S. aureus* bacteria in raw milk could be attribute to inadequate personal hygiene among milkers, milk handlers, and insufficient cleaning procedure (Sankhar, 2015).The prevalence of *Escherichia coli* and *Staphylococcus aureus* in milk sample has been studied by multiple researchers, namely (Rizal, 2023), (Bohora, 2022), (Chaulagain, 2022), (Limbu, 2020), (Acharya, 2017) and (Koirala, 2016). (Rizal, 2023) reported that the 23.1% of the raw milk sample from farms were contaminated with *E. coli*, while 50% showed presence of *S. aureus*. These percentage are higher than the findings of (Acharya, 2017),who reported 20% and 24% of marketed raw milk samples were contamination with *E. coli* and *S. aureus* respectively. However, the prevalence rates reported by Rizal, et al., (2023) are similar to this study.

When proper hygiene and sanitation practice are neglected on farms, spoilage organism can originate from animal's excreta can multiply significantly, particularly in warm temperature.

The variation in contamination rates between studies could be the difference in the time, sampling size and sampling locations, Farming practices, sanitation, and quality control measure (Shrestha, 2012).

The result obtains from the study on microbial quality, physiochemical analysis, and adulteration of raw milk from different farms of Lekhanath, Pokhara indicated that the current situation needs improvements from microbial and adulteration point of view as some of the samples were adulterated with neutralizer, while none of the samples were free from coliform contamination.

Conclusion

Raw milk samples from different farms were found to be free from adulteration with starch, formalin, and table sugar but 3(20%) of the buffalo milk sample were adulterated with neutralizer. The physiochemical properties of both cow and buffalo milk were within the contamination and poor hygiene. Overall, these findings highlight the need for improved quality control measures and hygiene practices in the production and handling of raw milk to ensure its safety for consumption.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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