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Analysis of Milk Collected From Milk Points for Composition, Adulterants and Microbial Quality in District Swat

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Abstract

Milk contains nutrients which are building block for growth and cannot be fulfill by any other food. In Pakistan Approximately 50 %of the milk produced is consumed as fresh or boiled, one sixth as yoghurt or curd and remaining is utilized for manufacturing of indigenous varieties of milk products such as ice cream, butter, khoa, paneerrabri, kheer, barfi and gulabjamin. A total of 150 samples were collected 50 each from tehsil Babozai, kabal and khwazakhela. The composition was determined through lactoscane milk analyzer, microbial quality through MBRT and adulterant analysis through various procedures. High milk Fat was recorded in tehsil Kabal 5.21% followed by 4.48% in tehsil Khwazakhela and 4.08%in tehsil Babozai. High milk SNF% was 6.92% recorded in tehsil Kabal followed by 6.72% in tehsil Khwazakhela and 6.11% in tehsil Babozai. High milk protein was recorded in tehsil Kabal 3.15% followed by 3.1% in tehsil Khwazakhela and 2.79% in tehsil Babozai.

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High quantity of lactose in milk was recorded as 3.04% in tehsil Kabal followed by 2.96% in tehsail Khwazakhela and 2.69% in tehsil Babozai. High amount of added water was observed as 36.22% in tehsil Babozai followed by 34.07% in tehsail Khwazakhela and 31.69% in tehsil Kabal. Boric acid and starch were not detected in all the samples processed. Formalin was detected in all tehsils as 26%, 46% and 36% in tehsil Babozai, kabal and khwazakhela, respectively. On MBRT no sample (0%) was found to be of excellent quality. The good quality was found to be one sample in tehsil khwazakhela (0.6%), 41.33% were of fair quality, 32% were of poor quality and 26% samples were of very poor quality. In tehsil Babozai 20 samples were of very poor quality which was high value among all the three tehsils. The maximum no for fair quality was observed in tehsil Kabal followed by tehsil Khwazakhela. The colony forming unit per milliliter (cfu/ml) for salmonella on salmonella shagilla agar was found 538 in tehsil Babozai followed by 510 in tehsil Kabal and 370 cfu/ml was observed in tehsil Khwazakhela. The data regarding e.coli count (cfu/ml) shows that high number (595) of colonies of e.coli were observed in milk collected from tehsil Kabal followed by 576 in tehsil Babozai and 480 in tehsil Khwazakhela. Colony forming unit on nutrient agar for various bacterial species showed that maximum number of microorganism were observed in milk samples for tehsil Babozai (590) followed by tehsil Khwazakhela 530 and 475 in tehsail Kabal.

Key Words: Milk; Microbial Quality; Adulterants; Salmonella; Colony forming unit, Agar.

1. Introduction

Raw milk as it leaves the udders of healthy animals normally contains very low number of microorganism. Total bacterial count is usually less than 10^3cfu/ml and many different bacteria are usually present [9,13]. Microorganisms associated with food borne illness may enter the raw milk supply through infected animals, milking personal, or the environment. Tuberculosis caused by *Mycobacterium bovis* and brucellosis caused by Brucella *spp*s are some of the examples. In some parts of the world, milk is still a significant source of these infections [1]. Bacteria related to food borne illness are destroyed by proper pasteurization [6,12]. Post-pasteurization contamination has been found to contribute most of the bacteria in milk that are capable of growth and subsequent spoilage. The bacteria found in raw milk are mostly Gram negative belonging to the genera *Pseudomonas Flavobacterium, hromobacterium, Akaligenes* coliforms [19, 20].

Adulterated food is dangerous for health as it may contain various toxic chemicals, it may be deprived of nutrients required for proper growth and development of human body [18]. In Pakistan problem is not only adulteration but also of dirty adulteration [22]. Milk used by the people for consumption is adulterated to such an extent that there is very less nutritive value in it and may also be toxic for public health [16]. Milk dealers can maximize their profit margin by three ways dilution, extraction of valuable components like milk fat which is removed as cream, addition of cheap substances like starch to increase the value of total solids up to a level which is acceptable by the consumers. In our country raw milk is distributed by a traditional system which involves middlemen called Gawalas. These middlemen (Gawalas) used to adulterate milk to maximize their profit [14,15]. High amounts of carbonates and bicarbonates can interfere with those hormone signals that regulates reproduction and development [22]. Reference [24] reported that levels of carbonates and bicarbonates must be kept constant otherwise higher alkaline values can cause systemic alkalosis, high blood

pressure, renal failure, edema and cardiac failure.

Formaldehyde is a toxic chemical that can kill bacteria and viruses as well as damage human cells. Manufacturers sometimes add Formaldehyde to milk to extend its shelf-life. Although adding formaldehyde to foods is forbidden in many countries, some manufacturers still add it.

2. Materials and methods

The research study was carried out at the University of Swat and the samples were processed at Veterinary Research & Disease Investigation Center (VR&DIC), Balogram, swat. The sampling layout was following.

Table 1: Sampling layout

Serial No	Tehsil	Sample size
1	Babozai	50
2	Kabal	50
3	Khwazakhela	50

2.1 Sample collection

A total of 150 milk samples (each 100 ml) were collected from milk points at autoclavable bottles and transported to VR&DIC using ice box for process. The milk samples each (100ml) were collected from the milk points at retailer shops.

2.2 Sample processing

The sample was processed on the same day of collection or was stored at 4 ^{0}C in case of delay.

2.3 Milk Composition Analysis

Milk samples from milk points were subjected to Lactoscane machine (auto analyzer machine) according to manufacture' protocol for determination of Milk fats (%), solids not fats (SNF), protein (%) and lactose (%), at VR&DIC,.

2.4 Microbial analysis

A total of 150 milk samples 50 from each tehsail were collected in sterile screw cap tubes and transported in ice box to the laboratory. The samples were diluted to1:1000 in sterilized phosphate buffer solution. Than 1ml of the sample was inoculated onto each of macckonkey agar ,salmonella shigella agar and nutrient agar plates and incubated for 24 hours at 37 ^{o}c. After incubation the colonies were counted by standard plate counting

method. By using colony counter & cfu/ml was determined determination of milk microbial quality methylene blue reduction test (MBRT), was performed. Methylene Blue Dye Reduction Test, commonly known as MBRT test was used as a quick method for assessment the microbiological quality of raw milk. This test is based on blue color of dye solution added to the milk and milk get decolorized when the oxygen present in the milk get exhausted because of microbial activity. The sooner the decolonization, more inferior is the bacteriological quality of milk assumed to be.

Table 2: Microbial assessment through MBRT

S.no	Quality	Time for reduction (hours)
1	Excellent	>8hrs
2	Good	06 to 08 hrs
3	Fair	02 to 6hrs
4	Poor	½ to 02hrs
5	v.poor	½ hrs

2.5 Adulterant Analysis

Chemical or adulterant analysis for detection of milk adulterants was carried out by the methods described by [4].

2.6 Alcohol Precipitation Test for keeping quality of milk

We took 2 ml of milk sample and 2ml of 68% of ethyl alcohol in a test tube with cork and the tubes were inverted many times in order to mix it thoroughly. The milk samples were examined for precipitation of casein present in milk. If precipitation occurs the milk samples were near the souring point and not of good quality and in case of no precipitation milk samples were regarded of good quality.

2.7 Detection of Boric Acid

A 50ml of milk and 0.25mg of boric acid was taken in a test tube, then few drops of Phenopthalein were added to the tube, pink color appeared. Then few drops of NAOH (1/10N) and aqueous glycerin (50%) of equal amount (10 ml milk + glycerin 10ml) were added, mixed and results were recorded. Discoloration was the indicator for presence of Boric acid.

2.8 Detection of Formaldehyde (Henher's Test)

A 5ml of milk sample was taken in test tube and 5ml of distilled water was added. Then about half volume of concentrated sulphuric acid (5ml) was also added to it. A ring was formed in case of presence of formaldehyde or positive sample.

2.9 Detection of Starch

A 5ml of milk sample was taken in a test tube and brought to boiling condition. It was allowed to cool at room temperature. Then 1-2 drops of iodine solution was added to it. Formation of blue color indicated the presence of starch in milk sample.

2.10 Statistical analysis

The data collected were analyzed through descriptive statistics by using Microsoft excel and Statistix 8.1.

3. Results

3.1 Milk composition of samples collected from milk points at Tehsil Babozai

Data on milk composition collected from tehsil Babozai are given in Table. 3. The mean value for fats content in tehsil Babozai was recorded as 4.08%. The minimum value for fats was observed as 2.40% and maximum was 6.00% in tehsil Babozai. Data collected regarding solids not fats (SNF) showed the mean value of 6.11%, minimum value was 3.91% and maximum was observed as 7.32%. Protein constituted 2.79% of milk composition while the minimum value recorded for protein was 1.77 % and maximum was observed as 3.35%. Lactose content of milk 2.69% was recorded in which the minimum value was 1.71% and maximum was 3.22% among all the samples collected. Data for the added water showed mean value of 36.22% while the lower level of added water was 0.00% and high recorded as 47.88%.The mean value of PH was observed as 5.95 in which the minimum value was 5.75 while maximum value has been recorded 6.20.

Table 3: Milk composition (%) of samples collected from milk points at Tehsil Babozai

Nutrient	Fats %	SNF%	Protein%	Lactose%	Added Water%	PH
Mean	4.08	6.11	2.79	2.69	36.22	5.95
Minimum	2.40	3.91	1.77	1.71	0.00	5.75
Maximum	6.00	7.32	3.35	3.22	47.88	6.20

3.2 Milk composition (%) of samples collected from milk points of Tehsil Kabal

Data collected on milk composition of tehsil Kabal are given in Table 4. Mean value for fats, SNF, protein, lactose, added water and PH was observed as 5.21%, 6.92%, 3.15%, 3.04%, 31.69% and 6.37 respectively .The

minimum value for fats, SNF, protein, lactose, added water and PH was recorded as 2.82% ,5.11%, 2.30%, 2.24%, 14.30% and 6.00 respectively and maximum value for fats, SNF, protein, lactose, added water and PH was recorded as 6.48%, 8.52%, 3.90%, 3.75%, 50.96% and 6.67 respectively.

Table 4: Milk composition (%) of samples collected from milk points of Tehsil Kabal

Nutrient	FAT%	SNF%	PROTEIN%	LACTOSE%	ADDED WATER%	PH
Mean	5.21	6.92	3.15	3.04	31.69	6.37
Minimum	2.82	5.11	2.30	2.24	14.30	6.00
Maximum	6.48	8.52	3.90	3.75	50.96	6.67

3.3 Milk composition of samples collected from milk points of Tehsil Khwazakhela

Data presented on milk composition of tehsil Khwazakhela are showed in Table 5. The mean values for fats, SNF, protein, lactose, added water and PH were 4.48%, 6.72%, 3.10%, 2.96%, 34.07% and 6.17 while the minimum and maximum values for fat, SNF, protein, lactose, added water and PH were recorded as 2.53%, 5.03%, 2.28%, 2.21%, 10.76% and 6.06 and 6.25%, 8.74%, 3.99%, 3.85%, 52.11% and 6.36 respectively.

Table 5: Milk composition of samples collected from milk points of Tehsil Khwazakhela

Nutrient	FAT%	SNF%	PROTEIN%	LACTOSE%	ADDED WATER%	PH
Mean	4.48	6.72	3.10	2.96	34.07	6.17
Minimum	2.53	5.03	2.28	2.21	10.76	6.06
Maximum	6.25	8.74	3.99	3.85	52.11	6.36

The percent mean data in Fig. 1 showed that high milk Fat was recorded in tehsil Kabal 5.21% followed by 4.48% in tehsil Khwazakhela and 4.08%in tehsil Babozai. High milk SNF% was 6.92% recorded in tehsil Kabal followed by 6.72% in tehsil Khwazakhela and 6.11% in tehsil Babozai. High milk protein was recorded in tehsil Kabal 3.15% followed by 3.1% in tehsil Khwazakhela and 2.79% in tehsil Babozai. High quantity of lactose in milk was recorded as 3.04% in tehsil Kabal followed by 2.96% in tehsail Khwazakhela and 2.69% in tehsil Babozai. High amount of added water was observed 36.22% in tehsil Babozai followed by 34.07% in tehsil Khwazakhela and 31.69% in tehsil Kabal.

3.4 Adulterant analysis for formalin, boric acid and starch determination of keeping quality by alcohol precipitation test of milk samples in tehsil Babozai

A total of 50 samples were collected from tehsil Babozai and processed for the determination (presence) of milk adulterants through different procedure. Milk keeping quality for these samples was also determined through alcohol precipitation test (APT). Among all these 50 samples 16 samples (32%) were positive on alcohol precipitation test, It means that their keeping quality is poor. For formalin 13 samples (26%) were found to be positive. The starch and boric acid test was found to be negative for all the samples processed.

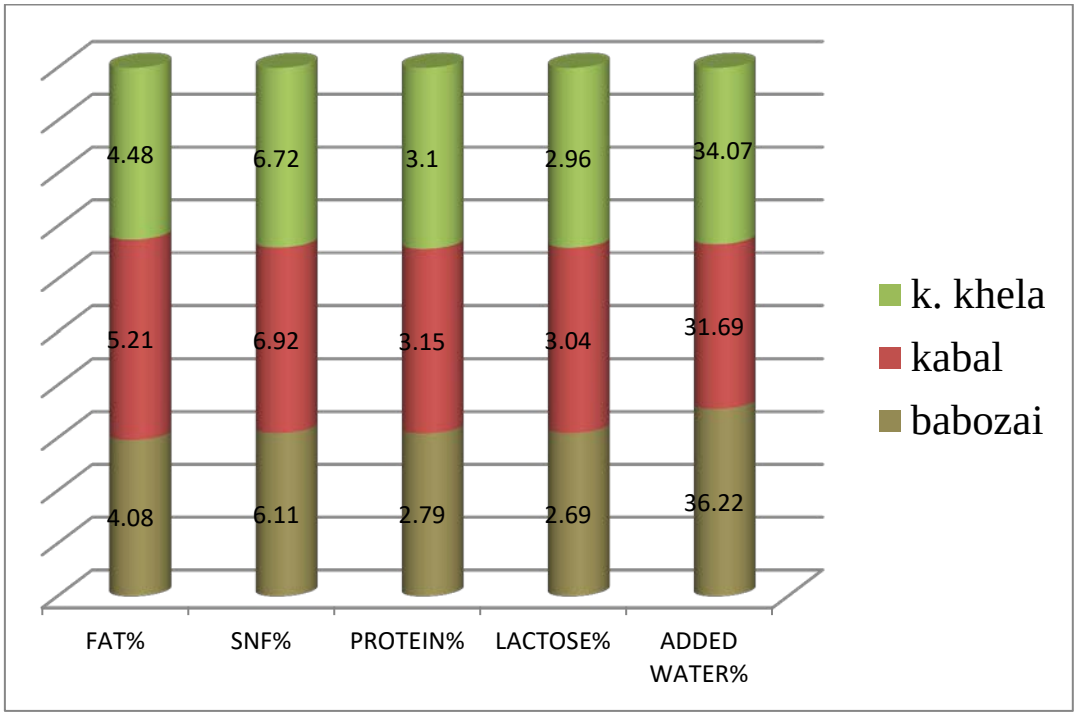

Figure 1: Mean data on milk composition of tehsil babozai, kabal and khwaza khela

Table 6: Detection of formalin, boric acid, starch and determination of keeping quality (APT) of milk samples in tehsil Babozai

Test	Total sample size	Alcohol precipitation test	Starch test	Formaldehyde test	Boric acid test
Positive	50	16	0	13	0
Negative		34	50	37	50
Percent positive		32	0	26	0

3.5 Adulterant analysis for formalin, boric acid and starch and determination of keeping quality through alcohol precipitation test of milk Samples in tehsil Kabal

In tehsil Kabal (Table. 7) 12 samples (24%) were found to be positive on APT that is their keeping quality was

poor. Out of 50 samples tested for formalin detection 23 (46%) samples were found to be positive. All the samples were negative for boric acid and starch.

Table 7: Detection of formalin, boric acid, starch and determination of keeping quality (APT) of milk samples in tehsil Kabal

Test	Total sample size	Alcohol Precipitation Test	Starch test	Formaldehyde test	Boric acid test
Positive	50	12	0	23	0
Negative		38	50	27	50
Percent positive		24	0	46	0

3.6 Adulterant analysis for formalin, boric acid and starch and determination of keeping quality through alcohol precipitation test of milk samples in tehsil khwazakhela

The data regarding tehsil Khawazakhela (Table. 8) shows that 13 samples (26%) were positive for APT i.e. was of poor keeping quality. Starch and boric acid was not detected in any of samples tested. The formalin was found to be present in 18 (36%) samples.

Table 8: Detection of formalin, boric acid, starch and determination of keeping quality (APT) of milk samples in tehsil Khwazakhela

Test	Total sample size	Alcohol precipitation test	Starch test	Formaldehyde test	Boric acid test
Positive	50	13	0	18	0
Negative		37	50	32	50
Percent positive		26	0	36	0

No.3.7. Microbial quality assessment through MBRT of milk samples collected from tehsil Babozai, Kabal and Khwazakhela.

A total of 150 samples were collected from milk points in tehsil Babozai, kabal and khwazakhela (50 from each) and MBRT (Methyline blue reduction test) test was performed to determine the microbial quality of milk. Among the entire sample tested (150) from all the three tehsils no sample (0%) was found to be of excellent quality.

The good quality was found to be one sample in tehsil khwazakhela (0.6%), 41.33% were of fair quality, 32% were of poor quality and 26% samples were of very poor quality. In tehsil Babozai 20 samples were of very poor quality which was maximum among all the three tehsils.

The maximum number for fair quality of milk sample was observed in tehsail Kabal (27) followed by tehsil Khwazakhela (25).

Table 9: Microbial quality assessment through MBRT of milk samples collected from tehsil babozai, kabal and khwazakhela

S.no	Quality	Time for reduction (hours)	Results			Total percentage
			Babozai	Kabal	Khwazakhela	
1	Excellent	>8hrs	00	00	00	0.00%
2	Good	06 to 08 hrs	00	00	01	0.60%
3	Fair	02 to 6hrs	10	27	25	41.33%
4	Poor	½ to 02hrs	20	15	13	32%
5	v.poor	½ hrs	20	08	11	26%

3.8 Microbial analysis of milk

The colony forming unit per milliliter (cfu/ml) at 1: 1000 dilution for *salmonella* on salmonella shagilla agar was found to be 538 in tehsil Babozai followed by 510 in tehsil Kabal and 370 cfu/ml was observed in tehsil Khwazakhela.

The data regarding e.coli count (cfu/ml) shows that high number 595 of colonies of *e.coli* were observed in milk collected from tehsil Kabal followed by 576 in tehsil Babozai and 480 in tehsil Khwazakhela. Colony forming unit on nutrient agar for various microbial species showed that maximum number of microorganism were for tehsil Babozai 590 followed by tehsil Khwazakhella 530 and 475 in tehsail Kabal.

Table 9: Colony forming unit per milliliter at 1:1000 dilution factor for bacteria on SS, Macconkey and nutrient agar

s.no	Tehsails	Salmonilla cfu/ml on ss agar	e.coli cfu/ml on macconkey	Cfu/ml on ntrient agar
1	Babozai	538	576	590
2	Khwazakhella	370	480	530
3	Kabal	510	595	475

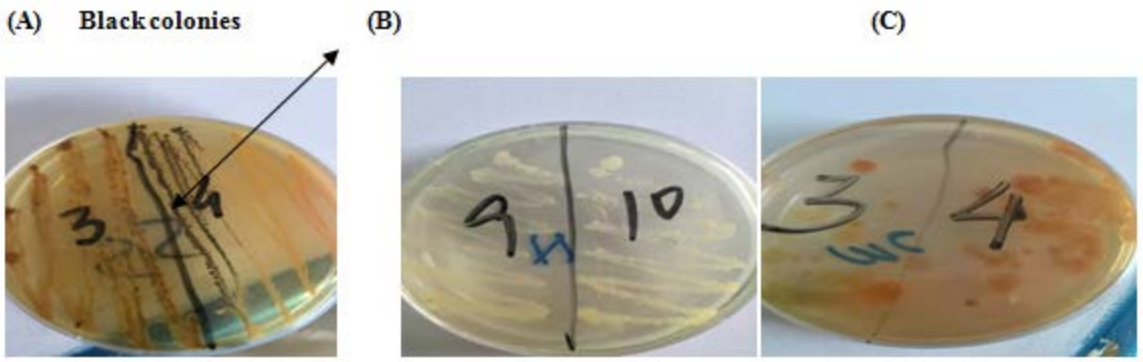

Figure 2: (A) *Salmonella* colonies on SS agar (B) bacterial colonies on nutrient agar (C) e.coli on macconkey agar

(D) **(E)**

Figure 3: Formalin positive samples (D and E)

4. Discussion

The results regarding the study on compositional, adulterant and microbial analysis of milk samples collected from three tehsils of district swat are discussed as below.

Reference [10] reported the nutritional composition of milk samples collected from various educational canteens and public places which shows the mean value of protein 1.12% and 1.33%, fat 2.06% and 1.40% and solid not fats 5.10 % and 4.77%, respectively. Reference [32] reported the fat composition of raw milk collected from different dairy owners was between 2.5% and 4.5%. [5] reported the nutritional composition of milk collected from cattle's of various regions in which the minimum protein was 2.54% and maximum was 4.19% while the mean value was 3.42%. The minimum fat content was recorded as 3.23% and maximum 5.34% while the mean value was 4.09%. The lactose composition was recorded as, minimum 4.40%, maximum 5.33% while the mean value was recorded as 4.82%. Reference [12] reported the milk composition of different samples collected from milk centers in which the mean value of protein content was 3.96% to 3.23%. For fat 5.41% to 4.60%, lactose content as 4.03% to 3.27% and solid not fat content was 8.81% to 7.49%.

Nutrients or feed effect milk fat and protein content. Fat is more sensitive to change in diet and protein to lesser extent. Lactose, minerals and other solid content of milk do not change well to dietary changes. Composition of milk is also affected by bread of animals and species of animals, by season, age of animal, environment, and stage of lactation, level of milk production, disease and genetics. The fat and protein content are high in early and late lactation while lower in peak and mid lactation. Disease such as mastitis (inflammation of udder) changes the milk content such as reduction of fat and casein content of milk. Season also affect milk content. During fall and winter milk fat and protein content increase and decrease during spring and summer. This is indirectly related to feed changes according to season. Age effect protein content but not fat. Protein contents decrease with age advancing.

The difference in milk composition in our research among different tehsils and among previous researchers may be due to many factors including species, breed of animals, stage of lactation, type of feed, season and level of water adulteration. Some retailers also add some ice in milk to keep it cool and prevent spoilage. This factor may change the effect of the composition of milk. In swat milk comes to retailer shops mostly from buffalos and cows.

In Swat three breeds of buffalos are present. Fat content in milk of Azakheli buffalo is very high among three breeds. But in retailer shops mostly mix milk are sold and consume. i.e. They come from different species and breeds of animals. The different in fat% may be due to: 1). Different species and breed of animals 2). May be due to level of water addition 3). May be due to different feeding practices. In our research we found that some samples were below the standard .i.e. fat and other contents were not present at normal level. They are needed for human health and nutrition.

Reference [11] reported the adulteration results in samples collected from different dairy owners in which the starch was 0% and formalin positive samples were 32%. Somewhat contrary to our findings [11] also reported milk adulteration in samples that were collected from different dairy owners in which the formalin, boric acid and starch are 0% reported. With accordance to our study [14] and [17] did not reported starch and boric acid in milk samples.

Reference [7] reported the adulteration results in raw milk samples collected from different milk points in which the adulteration of water was reported as 100%. Two types of preservatives are commonly used that is formalin and starch. The starch was detected in 12% and formalin in 10% positive of the collected samples. [10] also reported the adulteration in milk samples collected from various school canteens and public places in which the addition of water was 97% and 93%, respectively and their mean value was 95% while the addition of formalin was 23% and 27%, respectively and their mean value was 25%. Starch was not detected in any of the samples processed.

Reference [23] reported 15% very poor , 73% fair quality, 10% good quality and only 02% very good quality of milk samples. Reference [10] collected milk samples from educational canteens and public places and subject them to MRBT. They found that 3 and 17% samples were of poor quality, 27 and 10% good quality and 47% & 56%of excellent quality in canteens & public places respectively. Reference [3] collected samples from different

rural and urban areas in which 11 and 50% were of poor quality on MRBT respectively. The fair quality and good quality milk from only rural areas were 3% and 50% respectively. Reference [2] also reported the MBRT result of three samples collected from different dairy owners in which raw milk one sample was of fair quality and 2 3 were both of poor quality. Reference [8] reported that in raw milk samples out of 10 samples, 6 samples were found to be having high number of microbial colonies. They determine CFU at 1:1000dilution and recorded 627 to be high number of colonies. In raw milk the bacteria isolated were mostly e.coli. Reference [9] studied 10 raw and 10 pasteurized milk samples. The coliforms colonies were less in number in pasteurized milk sample as compared to raw milk samples. They obtain maximum number of 300 colonies at 1:10000 cfu in raw milk sample and 74 cfu/ml in pasteurized milk samples [3] isolated e.coli, shagilla, salmonella and pseudomonas species from milk sample collected from rural and urban areas. The bacteria isolated were mostly e.coli. Contamination of milk may be due to unhygienic milking practices and contaminated water. In our experiment the reduced number of cfu/ml may be due to reason that the milk collected were boiled. Contamination of milk may be from udder of animals and through environment. It is also hypothesized that feeding and habitat of animals may affect the microbial quality of milk. Fresh milk which was collected from healthy cows contain low bacterial load which is less than 10^3 CFU/ml [14]. It can be increase it up to 100 folds or more if the milk is stored for sometime at ambient (30 to 35ºc) temperature [8]. Bacterial load in raw milk are due to many factor such as health of the animals. Unhygienic condition cleanliness of housing areas, type of feeding, water and utensils used for storage and hygienic condition for milk handling. Reference [8] reporeted that load of pathogenic bacteria may be a high threat to the public health and especially to those who are consuming raw milk .Presence of bacteria can minimize the keeping quality and their toxins and enzymes can survive in pasteurized milk.

5. Conclusion

Composition of milk was not uniform in all tehsils and some were of below standard. Formalin was present in some of the milk samples collected from three tehsils. Microbial and keeping quality of milk was poor in the selected tehsils.

6. Recommendation

Further studies on milk composition in district swat on branded and unbranded milk are recommended Some more adulterant like urea, detergents, and soaps may be investigated.

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