OPEN ACCESS

**Scholars International Journal of Chemistry and Material Sciences**

Abbreviated Key Title: Sch Int J Chem Mater Sci
ISSN 2616-8669 (Print) |ISSN 2617-6556 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://saudijournals.com

**Original Research Article**

# Safety and Quality Assessment of Milk Before and After Pasteurization Collected from Different Regions of Punjab

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**DOI:** 10.36348/sijcms.2022.v05i09.001 | **Received:** 25.09.2022 | **Accepted:** 01.11.2022 | **Published:** 05.11.2022

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## Abstract

Milk is an excellent source of proteins, fats and carbohydrates along with minerals and vitamins. It is the balanced diet for all age groups. In Pakistan consumption of milk is increasing day by day. Milk from different animal sources has quality and nutritional differences. Pasteurization found to be increased the milk quality and shelf-life stability by reducing microbial load. The objective of study is to evaluate the differences among the raw and pasteurized milk in terms of safety and microbial distribution. The proximate and quality analysis including moisture, crude protein, crude fat, total soluble solid, pH, acidity, lactose composition, solid-not-fat (SNF) and specific gravity were done for the milk samples. The microbial tests were performed for Total Plate Count and Total Coliform Count before and after pasteurization process. The collected data was analysed statistically to estimate the level of significance. Pasteurized milk of buffalo showed high value for pH 6.65, lactose composition 5.964, crude fat 7.974%, crude protein 6.453%, SNF 6.672%, Total solids 12.646% while pasteurized cow milk showed low value as compare to buffalo milk samples as pH 6.60, lactose composition 4.732, crude fat 4.744%, crude protein 4.353%, SNF 6.128%, Total solids 10.872%. Total Coliform count (TCC) for raw and pasteurized milk of cow was 3.320 CFU/ml and 1.2600 CFU/ml respectively, whereas for buffaloes it was 2.604 CFU/ml and 1.0900 CFU/ml respectively. In case of TPC it was 2.834CFU/ml and 1.132 CFU/ml for raw to pasteurized milk of cow while it was 2.0320 CFU/ml and 1.0720 CFU/ml in buffaloes. Result revealed that pasteurized milk is safer to use and pasteurized milk has low microbial count as well as authenticity in safety and quality when compared with unpasteurized milk.

**Keywords:** Pasteurization, Milk quality, Punjab region, Safety, Raw milk.



## 1. INTRODUCTION

Milk is a widely consumed food due to its great nutritional value and inexpensive cost. Consumers who want fresh taste meals choose pasteurised milk over UHT milk because it has a longer shelf life (Limbo *et al*., 2020). Cow milk is used worldwide not only for calcium need but it also has numerous health benefits like it improves bone health (Aslam *et al.,* 2014). Lactose which forms a major portion of the milk and proteins the most important of which is casein, lipids of medium-chain fatty acids (Mosca and Giannì, 2018).

Milk is an excellent substrate for microorganism proliferation and development. Hands of workers, polluted cleaning water, dirty equipment and farm storage conditions may all contribute to an increase in the number of coliform bacteria in raw milk (Sarkar, 2016). The hygienic quality of raw milk is influenced by coliform bacteria and *E. coli*. A high number of harmful bacteria affect the shelf life and quality of milk while also posing major health risks to humans (Yuen and Alam, 2016).

During pasteurization process, milk is subjected to a specific heat treatment for a certain period of milk can be affected by its time or temperature combination and storage time (Khan *et al*., 2017). Pasteurization of milk is very necessary because raw milk causes very serious pathogenic diseases. Pathogenic and spoilage microbes are killed or

controlled by pasteurization without affecting the organoleptic and nutritional qualities of milk (Disassa *et al.*, 2017).

Udder cleaning, safety and cleaning of milking equipment's, replacement of conventional milking system with machine milking and cattle protection from manure contamination (Sarkar, 2016). The hygienic quality of raw milk is influenced by coliform bacteria and *E. coli*. A high number of harmful bacteria affect the shelf life and quality of milk while also posing major health risks to humans (Yuen and Alam, 2016).

Current pasteurisation procedures employ combined standard conduction-based heating and alternative heating technologies. Industrial pasteurisation technique is built on heat exchangers. Heat transfer overheating is the primary flaw in current technologies It leaves behind chemically complex residues after extended use (or fouling) (Abdullah *et al*., 2022).

Pasteurisation has been the main technique but the adverse effects of heat on quality of milk, such as changes in colour, flavour, or vitamin loss, must be limited without compromising safety. Lowered microbial loads in milk and other dairy products, increased shelf life, and preservation of fresh-like attributes like aroma and vitamins have all been proved to be achieved by high hydrostatic pressure processing , a novel non-thermal processing method (Liu *et al*., 2020).

Adulteration of food includes the mixing of unnecessary, harmful and useless substances into food that deteriorate the food quality. The adulterant nature could be biological or physical. It includes addition of water to milk, inessential matter, or substitution of milk solids. Unintentional adulteration includes addition of undesirable stuffs because of carelessness, unawareness, lack of proper facilities and poor hygiene during processing of food. This could be foods contamination of acquired type by fungi or bacteria, food damage by rodents, entry of stones and dust and from packing material harmful residues, etc (Boujenane, 2019).

The study is designed with the aim:

- To observe the difference between raw and pasteurized milk for the evaluation of safety and quality
- To evaluate the effectiveness of pasteurization in reducing the microbial load in different regions of Punjab

## 2. REVIEW OF LITERATURE

Milk is a healthy and nutritious food. The global demand for food is increasing. Milk can play a part in meeting this rising demand. Milk demand is increasing as the human population grows. Raw milk and raw milk products are consumed in rural regions. Pasteurized milk products are becoming more popular every day since they are the most effective approach to preserve the nutritional value and quality of a perishable meal (Afzal *et al*., 2011).

Milk is averagely composed of water and solids of 87% and 13%, respectively. The solid portion of milk comprised of fat, proteins, vitamins, carbohydrates and minerals. Whey and casein both are of high-quality proteins found in milk having quantity of 18% and 82%, respectively. Essential amino acids are found in casein. Mineral's constituents of milk are calcium, magnesium, potassium, phosphorus (Vitamin D present in milk helps in the absorption and utilization of calcium in human body. Vitamin B2 present in milk is also a good source for healthy eyes and protection of skin (Nagpal *et al.*, 2012).

### 2.1 Food safety

Nowadays, the market offers a wide range of food items, but food safety remains the top priority. Quality control is critical in the food industry, and the quality control system must be effectively maintained (Wilcock *et al*., 2004).

### 2.2 Milk production

Milk contains high nutritional profile due to its balanced nutrient composition. Milk contains protein, fat, vitamins and minerals like zinc, iron, manganese, copper, calcium and fluoride which are vulnerable for the physiological functions in human's body. Lack of these nutrients causes different pathological disturbance and improper muscles growth. Nutritional composition varies among different breeds and species as well as depends on animal diet. Milk comprises of 88% water, 3.2% fat, 8.13% fat solids and 11.4% solid contents (Guetouache *et al*., 2014).

A study was arranged in Bangladesh. Different physiochemical properties of milk sample were analyzed and compared to standards of WHO and Bangladesh (BDS-1985. Different sixty one samples of milk were collected and analyzed. The results for milk sample showed fat 4.2%±0.12%, protein 3.95%±0.17%, lactose 4.58%±0.13%, solid-not-fat 8.54%±0.01%, total solid 12.43%±0.53%, and acidity 0.14%±0.005% . Statistical analysis shown that there is no significant difference between results of samples collected and Bangladesh standard that indicates good quality raw milk. No adulterants and poor quality raw milk were found during study (Hossain and Dev, 2013).

Milk is a significant source of product with a variety of compositions. In quantitative terms, water, fat, protein, and lactose predominate, while minerals, enzymes, antioxidants, and dissolved gases make up the minor components. It satisfies the consumer's desire for increasingly inventive products of reliable quality. The dairy sector must make full use of this raw material's

value, which is both straightforward in appearance and intricate in composition. Cow milk is typically less heavy in lactose, fat, and protein. However, the nutrient composition is comparable (Shinawy *et al*., 2018).

Milk is averagely composed of water and solids of 87% and 13%, respectively. The solid portion of milk comprised of fat, proteins, vitamins, carbohydrates and minerals. Whey and casein both are of high-quality proteins found in milk having quantity of 18% and 82%, respectively. Essential amino acids are found in casein. Mineral's constituents of milk are calcium, magnesium, potassium, phosphorus (Pereira, 2014).

**2.3 Determination of high microbial counts in raw milk**

Milk was tested for microbiological determination in a variety of surveys. Milk samples were collected from various locations for this purpose, and microbial growth variations were detected. The milk samples were analysed with a standard plate count method. A high *E.coli*, *Staphylococcus aureus*, and gram-negative bacteria were all found to be contaminated. (Sarkar, 2016).

Milk is a complex food with full of essential nutrients which helps to protect from the cardiovascular hypertension and haemorrhage diseases. Hypertension is a main cause of cardiovascular disease. Milk contains several minerals like calcium, potassium and magnesium which has anti-hypertensive properties has been playing important role in cardiovascular disease prevention. These minerals have been proved to reduce 20% hypertension and necessary for blood pressure control. Globally, the milk consumption recommended due to the good bone health, maintenance and protection (Pereira, 2014).

**2.4 Biological and chemical compositional study of milk**

Chemical composition of milk is influence by different factors like as specie of an animal, environmental factors and nutritional status of the animal. Milk is considered as a good source of protein in human diet. It provides approximately 32g protein/L, in which 20% is soluble protein called whey protein whereas 80% is insoluble protein named as caseins protein. On the basis of human requirement and digestibility of amino acids these proteins are considered as high-quality proteins because the proteins have different amino acids fractions. Milk contains nine essential amino acids which are required by humans (Severin and Wenshui, 2005).

On the nutritional and economical aspects milk fat is the important component of the milk. The quality and quantity of fatty acids in milk are depending on the origin of animal, feed and stages of lactation. On the fractional basis, seventy percent of fat is composed of saturated fatty acids which decrease the risk of heart diseases according to the nutritional guidelines and thirty percent is unsaturated fatty acids (Gomez-Cortes *et al*., 2018).

In order to assess the usefulness of these proteins as indicators for the analysis of milk pasteurisation, the activity and rates of inhibition of 4 enzymes in fresh buffalo milk were evaluated with respect to the heating process. Gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactic dehydrogenase (LDH) enzyme activity were assessed in between heated at 60℃, 70℃, 80℃, and 90 ℃ for 1, 5, 7, 15, 25, and 35 min. The activity of the enzymes GGT (714 6 601 IU/liter), Lactate dehydrogenase (367 6 182 IU/liter), ALP (296 6 166 IU/liter), and AST (17 8 5 IU/liter) were in that order. There was no difference in the activity when the milk was heated at 508°C for 3 to 15 minutes of any one of the enzyme. All other enzymes displayed resilience to heat inactivation at 608C, except for ALP, which demonstrated the highest susceptibility. At 708°C, ALP activity was fully eliminated after 1 minute, whereas GGT and LDH lost the majority of their activity after 10 minutes, while AST continued to have 50percent activity even after 30 minutes. LDH and GGT ceased their activities at 808°C, whereas AST still had some activity. The findings indicate that LDH and GGT, but not AST, could also be possible indicators of thermal denaturation in milk samples, with GGT possessing the benefit of having the greatest concentration (Sarkar *et al.*, 2016).

**2.5 Effect of pasteurization on milk**

Liu *et al.*, (2020) conducted that full cream milk was compared to two common thermal treatments: high temperature short time (HTST: 72°C for 15 s) and low temperature long time (LTLT: 63°C for 30 min), high hydrostatic pressure processing is used in both processes. After a week of storage at refrigeration temperature of 5°C, the change in activity of microbes, volatile compounds, protein structure, digestibility, and sensory attributes was all investigated. In the milk treated using high pressure and temperature, total plate counts were reduced to nearly the significant level. High-pressured milk had significantly improved denaturation of lactoglobulin when compared to heat-treated milk, but no significant variations in stomach protein digestion were identified. According to the volatile chemical analysis, the profiles of high temperature short time (HTST) and high-pressure processing (HPP) treated milk were essentially the same, whereas low temperature long time (LTLT) milks included higher level of ketones. Sensory evaluation revealed that milk (treated at low temperature for long time) differed the most from the other three milks.

In another research (Deeth, 2021) evaluated that pasteurization is the most widely used thermal

method in the dairy sector, and it has been found the sign of hygienic production of milk for more than a century. Coxiella burnetii is most heat stable pathogen (non- spore forming) has been killed without effecting the chemical composition of milk.

(Felice *et al.*, 2021) studied that the composition of milk and milk products had affected the factors that they fully understand. Cream protein was determined by blast2go. The milk creams of other species were very similar lipid analysis which showed that saturated fatty acids had very lowest amount in sheep cream. Palmitic acids were lowest in sheep cream but it is highest in cow the fat globules used in sheep and cow milk that had thick patches of protein. In Buffalo and goat milk fat globules were related to have larger proteins and cow had absorbed protein. The difference between Buffalo cow goat and sheep milk cream highlighted that these could be used as a use of functional foods for infants.

(Siregar *et al.*, 2021) studied that in Indonesia Buffalo milk had widely available which was used as a traditional food. Main aim of this study was to investigate the chemical properties of Buffalo milk that had been measured by different variables like titratable acid pH moisture and fats protein. In the laboratory descriptive analysis had been done in which samples of 4 breeders of different types of lactation was taken included 6th 5th 4th and 3rd Period of lactation. Resultantly, the composition of Buffalo milk results showed that the nutrition composition of buffalo milk was: protein content of 1.99% - 6.55%, fat content of 2.40% - 15.29%, moisture content of 73.07% - 91.20%, pH of 5.9 - 6.4 and titratable acid of 0.50% - 0.58%. This study concluded that Buffalo milk had good nutritional quality and composition.

According to the studies, if efforts are made to retain microbiological contamination in order to ensure the safety of pasteurized milk, the storage life of pasteurized milk can be enhanced. Milk quality can be improved by following hygienic measures and preventing microbial Ct6ontamination of the milk by preventing microbial contamination of the milk, preventing post-pasteurization contamination of the milk, and ensuring proper pasteurization. (Srey *et al*., 2013).

The raw milk quality was low due to contamination, according to a literature review. Raw milk contamination was caused by the inside udder, exterior udder, utensils used, water, hands and the status of the milkman, as well as the environment of the milk producing region (Ahmed and Abdellatif, 2013). Inadequate packaging might also lead to milk contamination. Another risk of milk contamination is inadequate temperature regulation, which promotes microbial growth and reduces the milk's shelf life (Moussa *et al*., 2013).

The LTLT and HTST pasteurization methods are used to kill bacteria when their concentration is low, around 102 CFU/mL, but not when it is high, around 10 CFU/mL, and since milk that having undergone heat decomposition at 72.9 °C for 25 seconds rather than 86.53 °C for 25 seconds had a relatively low microbial load within a week of pasteurisation heat at 60 °C. The fatal effect of different back postures is identical between 72.9 °C and 85.2 °C. The possibility for this problem makes it challenging to extend the shelf life of microorganisms. However, the psychologically pleasant bacteria proliferate more successfully when isolated. Antibacterial elements start to break down at this temperature (Ranieri *et al*., 2009).

**2.6 Storage conditions of raw and pasteurized Milk**

The number of factors affecting the microbial status of fresh milk includes storage, handling, and animal health. Various factors that impact milk quality were discussed in this study. The key elements that impact the microbiological quality of milk include storage tanks, pollution from the environment, and animal health. The key elements that impact the microbiological quality of milk include storage tanks, pollution from the environment, and animal health. To enhance milk microbiological quality, changes to farm operations such as payment policies, pre-and post-milking sanitary activities, adequate infrastructure, and improved feeding methods have been suggested (Sarkar, 2016).

The shelf life of pasteurised milk is more influenced by the storage temperature. When kept at 6.1 °C, milk has a storage life of approximately 10 to 20 days. The shelf life of pasteurised full cream milk is 31–11 days, while that of pasteurised skim milk is 31–11 days when stored at a temperature between 4–10 °C. This is because psychotropic bacteria exhibit more lipolytic and proteolytic activity after 2-3 days at 10 °C compare to 4-6 days at 4 °C. At the period of milk storage, activity was found to be 15 times higher at 4–7 °C than at 15 °C. The compositional qualities of milk are also affected by environmental factors and seasonal influences (Nada *et al*., 2012).

Milk sold on the street has a high concentration of microorganisms. The major cause is polluted environment. This type of milk has a lot of *Staphylococcus* and *E. coli* in it. 25 out of 135 samples were found to be heavily infected with *Staphylococcus* and *E. coli*. Greater causes are unsanitary environments. Pasteurization of milk before distribution can help to avoid or reduce microbial contamination (McCarthy *et al.*, 2007).

**2.7 Foodborne diseases caused by microorganisms found in food**

Everyone has the right to eat food that is safe, nutritious, wholesome, and healthful. Foodborne disease is a big problem and health concern all over the

world. By consuming liquid, semiliquid, or solid food, any chemical or biological agent might cause foodborne illness (Scallan *et al.,* 2011).

Food safety is a global issue that requires attention for the benefit of human health. All of the steps, situations, and procedures involved in acquiring safe and nutritious food for human consumption are referred to as food safety. Food contamination and food-borne illness are widespread as a result of all of these activities. Listeriosis is a more serious infection caused by Listeria monocytogenes (Control and Prevention, 2008).

### 2.8 Quality parameters of pasteurized milk

The pasteurization procedures were originally designed to inactivate M. tuberculosis, but because the main heat resistant bacteria in milk is C. burnetii, the pasteurization process was limited to at least a 5-log reduction of this water repellent pathogen. The HTST pasteurization procedure eliminates nearly all pathogenic microorganisms and is believed effective for reducing germs to a 4-5 log reduction (Shannon *et al.,* 2005).

### 2.9 Factors influencing microbiological quality of pasteurized milk

The quality of raw milk has an impact on the storage life of pasteurised milk. Prior to processing, storage time, heat treatment employed, heat resistant microbe concentrations, post-pasteurization confinement extents, packing technology used, and to some extent the impact of light (Rysstad and Kolstad, 2006).

Milk with low bacterial count, normal composition, without toxic substances and adulterants, low titratable acidity, better taste and generous in keeping quality known as quality milk. The quality of fresh milk for public consumption sold at Quetta was examined by chemical composition. 100 samples of milk randomly collected from different milk shops. The Chemical composition determined by different parameters like fat%, protein%, solid not fat%, total Solid%, acidity% and specific gravity. Raw milk samples results showed highest mean% of fat 2.23% ±0.46, protein 3.43% ±0.63, solid not fat 6.82% ±1.60, total solid 9% ±1.92, specific gravity 1.025 ±0.01 and acidity 0.21% ±0.05. The mean percentages of solid-not-fat, protein, total solid, acidity and specific gravity of milk samples were found non-significantly different statistically whereas the mean fat percentages milk samples were found considerably different. The major constituents like fat, protein, solid not fat and total solids of marketed milk in Quetta which were much lower than the pure milk that indicated the milk poor quality (Tohma *et al.,* 2017).

The shelf life of milk is affected by the chemical composition and metabolic activity of the milk. Whole milk has a longer life cycle than skim milk when stored at 4.5°C or 7°C, which may lead to higher protease activity in skim milk than whole milk or protein protection from enzymatic cell lysis or protease inhibition in whole milk (Dosti *et al*., 2005).

Cool-bear *et al.,* (2021) conducted that heat treatment of the milk to increase its storage period by minimizing the risk of enzymes and bacteria making the food hazardous or spoiling has an effect on its sensory parameters. If the heat load is greater, the outcome should be worse; the combined effect of time-temperature used in the making of the dairy industry's variety of liquid milk products shows a range to enhance storage life and to minimize changes in original sensory properties that are detrimental to consumer acceptability.

## 3. MATERIALS AND METHODS

Milk sample all species like cow and buffalo fresh samples were collected from the different areas of Faisalabad. The milk samples were microbiologically analysed in the Dairy Technology laboratory of the National Institute of Food Science and Technology University of Agriculture Faisalabad.

### 3.1. Procurement of sample:

In order to collect the representative and random samples total 40 samples were collected (20 from each. The samples were collected in sterilized laboratory bottles available in the Food Microbiology and Biotechnology laboratory and in the dairy Technology laboratory. The milk sample was immediately transported to the laboratory in the icebox.

**Table 3.1: Buffalo Milk Collection**

| Sr. No | Specie | Milk Collection farms |
|---|---|---|
| 1 | Buff | farm-A |
| 2 | Buff | farm-B |
| 3 | Buff | farm-C |
| 4 | Buff | farm-D |
| 5 | Buff | farm-E |

**Table 3.2: Cow Milk Collection**

| Sr. No | Specie | Milk Collection farms |
|---|---|---|
| 1 | Cow | farm-A |
| 2 | Cow | farm-B |
| 3 | Cow | farm-C |
| 4 | Cow | farm-D |
| 5 | Cow | farm-E |

### 3.2. Analysis for raw and pasteurised milk

Physicochemical characteristics i.e. Specific gravity, pH, titrate able acidity, ash, protein and lactose content was measured according to the method of AOAC (2016).

### 3.3. Physico-chemical analysis of raw and pasteurised milk

#### 3.3.1. PH

The pH of the milk of cow and buffalo was measured with the help of digital pH meter. First of all calibration of the pH meter was done by pH buffers of 4 and 7. Milk sample of 20ml was taken in a beaker. Then the milk electrode was immersed in the milk sample and constant reading was taken. Then the correction factor was applied to calculate the final correct reading (AOAC, 2016).

#### 3.3.2. Titratable Acidity

Milk acidity was determined according to AOAC (2016) with reference number of 947.05 by titration method. 10 ml of the milk sample was taken in the Erelnmeyer flask and 2-3 drops of the phenolphthalein indicator was added in this milk sample. Against 0.1N NaOH the milk titration was done till the light pink colour. The acidity % age was measured according to the formula:

$$\text{Acidity (\%)} = \frac{\text{0.1 N NaOH used (mL)} \times 0.009}{\text{Sample Vol. (mL)}} \times 100$$

#### 3.3.3. Total Solids

The solid content was measured according to AOAC (2016) by the following method no. 952.23. Oven drying method was used to calculate the total solid content of the milk samples 5 mL of the milk sample was taken in a dry, clean and weighed china dish. Then this china dish was placed in hot air oven for 3 hours at 100℃. After that the china dish was placed in desiccator for half hour to cool the sample. By the use of the following formula total solid percentage was measured.

$$\text{Total solids \%} = \frac{\text{Residue Wt. after drying}}{\text{Sample Vol. (mL)}} \times 100$$

#### 3.3.4. Fat

Gerber method was used for fat determination in the milk of all milk samples according to the method described by AOAC (2016). In the butyrometer 10.94 mL of the milk sample was taken. 1 mL isoamyl alcohol and 10 mL $H_2SO_4$ was added in the milk and 65℃ centrifugation was done for 5 minutes at 1100 rpm.

#### 3.3.5. Crude Proteins

In the milk of all species total protein content was measured according to the international dairy federation method, IDF 20-1 (2001). From the protein and the other nitrogenous sources protein was converted into the ammonium sulphate and is then this ammonium sulphate is distilled in the boric acid and solution and then was titrated against the acid of the known normality.

**Procedure**

**1. Digestion**

In the digestion tube 3 g of the milk sample was taken in the digestion tube with the digestion tablet with adding 20 mL of conc. $H_2SO_4$. To avoid the frothing, the process of the digestion was initially by slow heating for 45 minutes and then at the temperature of 80 ℃ till the appearance of the clear or pale green colour. Digestion sample was cooled for about 30 minutes. 100 mL of the distilled water was transferred to the volumetric flask of 250 mL.

**2. Distillation**

In the micro Kjeldahal apparatus 10 mL NaOH and 10 ml of the digestion sample was distilled. In the 4% of the boric acid solution the ammonia procedure was tapped containing few drops of the methyl red indicator. Boric acid colour changed from red to yellow colour after the addition of ammonia. After the first appearance of the yellow colour the distillation was continued to catch the maximum ammonia.

**3. Titration**

All of the content was titrated against the 0.1 N $H_2SO_4$ Solutions till the light pink colour was obtained. $H_2SO_4$ volume used was noted.

**Calculation**

With the formula given below, total nitrogen content and that value multiplied to get the total protein.

$$\text{\% N} = \frac{\text{H2SO4 vol. used (mL) x 250 x0.0014}}{\text{Digestion vol. (mL) x Didested sample vol.}} \times 100$$

#### 3.3.6. Moisture content

Determination of moisture content of milk was done with respect to the method described by AOAC (2016) method number 934.06. At first, equal sized china dishes were taken, washed, cleaned and dried. After that they were weighed. 10 g sample milk was taken in each china dish. China dishes with milk sample were weighed again and then the readings were noted.

Milk sample containing china dishes were placed for 24 hours at 105 °C in hot air oven. The samples were taken out and weighed. The procedure was repeated again until a constant weight of samples attained. To cool down the china dishes, they were placed in a desiccator. Following formula was used to check moisture content:

$$\text{Moisture (\%)} = \frac{\text{Final weight(g)} - \text{Initial weight(g)}}{\text{Weight of sample}} \times 100$$

#### 3.3.7. Solid Not Fat

Solid not fat (SNF) content of milk samples would be measured by LR reading as described in AOAC (2016). A total solid other than butter fat constitutes the milk SNF. The sugar, proteins and minerals were other solids of milk constituting major portion of total solids of milk constituting major portion

of total solids. Following formula was used for determination of SNF contents in samples.

Calculations:
Solid not fat percentage= CLR + 0.2 ×F
CLR = Corrected Lactometer readings
F=Fat percentage of milk

### 3.3.8. Temperature

When milk samples were arrived, their temperature was tested with thermometer and the temperature was 15-25℃. After pasteurization the temperature of the milk samples were again tested.

### 3.3.9. Lactose composition

Lactose content of the milk of all milk samples was estimated according to the method described by AOAC (2016). By mixing the equal columns of the Fehling A and Fehling B solutions the Fehling solution was prepared.

**Preparation of the Chemical/Reagents**

- Methylene blue indicator
- $CuSO_4$ Solution (Fehling A) $CuSO_4.5H_2O$ (69.28g) was completely dissolved in the 1L of the distilled water and then filtered with the Whatman filter paper of No.4
- Rochelle salt (potassium sodium tartrate, 346 g), alkaline tartrate solution (Fehling's B) and 100 g NaOH was dissolved in the 1L of the distilled water.

**Method**

Milk sample of the 40 Ml poured in the beaker and then heated in the water bath up to the temperature 65℃. 5-8 drops of the acetic acid were added and then left to proteins precipitate for 5 min. Then the acidified sample was filtered. It was then diluted with distilled water to make the final volume to the 100mL.Then this filtered was added in the burette. Then this was slowly poured to the conical flask which contained 5 mL of the boiling Fehling's A and Fehling's B until the appearance of the blue colour. To get brick red colour as end point, the titration was done completely.

**Calculation**

0.064 factor was multiplied with the total lactose content and the final lactose content was measured by the use of the following formula.

| Microorganism | Media |
|---|---|
| Coliform | Eosine methylene blue (EMB)agar |
| TPC | Nutrient agar |

$$\text{Lactose (\%)} = \frac{\text{Equivalence obtained from lactose} \times \text{Dilution}}{\text{10 Vol. of acidified sample}} \times 100$$

### 3.3.10. Specific Gravity

Specific gravity of all of the samples was determined by the use of lactometer by the method described in AOAC (2016).

**Procedure**

First of all cylinder of 200 mL volume was taken. Milk sample was filled in it up to the mark. The lactometer was used for taking the measurement. Care was taken during taking the reading that it was not touching the wall of the cylinder. After waiting for some time the reading was taken. The practical was performed thrice to take the mean value. The specific was calculated with the help of the following formula;

$$\text{Specific Gravity} = \frac{\text{Lactometer Reading} + 1}{1000}$$

### 3.4. Microbial analysis of milk samples

The microbial analysis of milk sample were done by total plate count (TPC) and Total coliform count (TCC) and for intended purpose sample prepared by following method; 20 ml milk from each representative sample was mixed in 220ml of sterile buffered peptone water in stomacher bag and then the sample was stomached for 2 minutes in Seward stomacher for the homogenization. The homogenization was done at 200 rpm (Khan *et al.*, 2007).

**Selective media used for the Microbial evaluation**

The following microorganism were analysed by using their specific agar media described in the table. The media was prepared according to the method mentioned on the label of the specific selective media.

**Specific microorganisms detected from the different milk samples and their specific selective media**

Microbial evaluation was done in both raw and pasteurised milk.

### 3.5. Pasteurization

Pasteurisation was done in Dairy Science Laboratory of National Institute of Food Science and Technology, University of Agriculture, Faisalabad.

### 3.6. Analysis of pasteurised milk

After pasteurisation, microbial examination was done in all samples HTST pasteurization method was used (Ranieri *et al*., 2009).

Following method was used for microbial evaluation of both raw and pasteurised milk.

**3.6.1. Serial dilutions**

9 ml saline solution (8.9g NaCl /1000ml of distilled water) was filled in the labelled test tubes and sterilization was done in the autoclave 121℃ for 15 minutes at the pressure of 15 psi for dilution purpose. 10 fold serial dilutions were prepared with the help of the micro –pipette and was transferred into the test tube labelled as 1 and thoroughly mixed then 1 ml was taken from the test tube 1 and was transferred to the test tube 2 and so on.

**Sample inoculation and spreading**

Petri plates for the inoculation purpose and spreading was done by ethanol sterilised spreader. Similarly 0.1 ml was spread to the test tube 2 to the petri-plate 2 and in this way spreading was done continued and so on. The process of inoculation was done under the sterilised conditions. Bunsen burner was used to sterilize the spreader to avoid the contamination process.

**Incubation**

37℃ temperature was used in the incubator to incubate the microbes keeping at 24-36 hours to get the microbial growth.

**Counting the colonies**

With the help of colony counter, counting of colonies was done from the petri plates in order to get the final results the following formulae was used according to the method described by (Robinson *et al*., 2005).

$$\text{CFU/mL} = \frac{\text{Average No of colonies from duplicate plates}}{\text{Dilution Factor x Vol. plated}}$$

$$\text{Dilution Factor} = \frac{\text{Sample Vol}}{\text{Sample Vol.} + \text{Diluent Vol.}}$$

**3.7. Statistical Analysis**

Experimental units were randomly allocated, and the obtained results was subjected to t1o way ANOVA to achieve the maximal information about dependent variables from a minimal number of possible experiments for calculating level of significance ($\alpha \leq 0.05$). Whereas, Tukey's HSD test was applied to test means difference (Montgomery, 2017).

## 4. RESULTS AND DISCUSSIONS

Results of all these parameters are discussed under the respective headings. Total 10 milk samples of cow and buffalo were collected from different farms the following analyses described below:

**4.1 Physiochemical characteristics of milk**

**4.1.1 PH**

Statistical results and mean values for pH contents in milk from cow and buffalo is shown in Table 4.1a and Table 4.1b. This ANOVA table shows that raw and pasteurize milk of buffalo and cow have significant difference in pH content among each other this permits further study of this experiment. The mean values (Table 4.1b) of pH contents in milk samples showed a significant variation with maximum pH content in 6.74 in buffalo raw milk followed by raw cow milk as 6.64, respectively. Whereas the table shows mean values of pH content in pasteurize milk of buffalo was maximum 6.65 and in cow 6.60 The findings of current/recent study are found relatively closed by the results of the Mahmood *et al.,* (2010) who analyzed the pH value in the raw and pasteurize milk of buffalo was relatively high as compared to the cow milk.

**Table 4.1a: Analysis of variance for raw and pasteurize milk for pH:**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Treatment** | 1 | 0.000084 | 0.000084 | $0.06^{NS}$ |
| **Specie** | 1 | 0.083076 | 0.083076 | 55.83** |
| **Treatment*Specie** | 1 | 0.035196 | 0.035196 | 23.65** |
| **Error** | 16 | 0.023807 | 0.001488 | |
| **Total** | 19 | 0.142163 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), $^{NS}$ = Non-Significant (P>0.05)

**Table 4.1b: Mean value of raw and pasteurize milk for pH**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 6.5 | 6.73 | $6.63^{a}$ |
| **Pasteurize** | 6.60 | 6.65 | $6.62^{a}$ |
| **G.M** | $6.56^{b}$ | $6.695^{a}$ | |

**4.1.2. Specific gravity**

Statistical results and mean values for specific gravity contents in milk from cow and buffalo is shown in Table 4.2a and Table 4.2b. This ANOVA table shows that raw and pasteurize milk of buffalo and cow have significant difference in specific gravity among each other this permits further study of this experiment. The mean values (Table 4.2b) of specific gravity in milk samples showed a significant variation with maximum value of specific gravity is 1.0299 in buffalo raw milk followed by raw cow milk as 1.0320, respectively. Whereas the table shows mean values of specific gravity content in pasteurize milk of buffalo was 1.0302 and in cow 1.0292. The findings of current/recent study are found relatively closed by the results of the Mahmood *et al.,* (2010) who analyzed the value of

specific gravity in the raw and pasteurize milk of buffalo was relatively high as compared to the cow milk. However, my findings are in contradiction with the Abbas *et al*., (2013).

**Table 4.2a: Analysis of variance for raw and pasteurize milk for Specific Gravity**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Treatment** | 1 | 0.000008 | 0.000008 | $1.74^{NS}$ |
| **Specie** | 1 | 0.000013 | 0.000013 | $2.78^{NS}$ |
| **Treatment*Specie** | 1 | 0.000002 | 0.000002 | $0.38^{NS}$ |
| **Error** | 16 | 0.000073 | 0.000005 | |
| **Total** | 19 | 0.000095 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), NS = Non-Significant (P>0.05)

**Table 4.2b: Mean Value of raw and pasteurize milk for Specific gravity**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 1.0320 | 1.0299 | $1.3096^{a}$ |
| **Pasteurize** | 1.0292 | 1.0302 | $1.02970^{a}$ |
| **G.M** | $1.03112^{a}$ | $1.02954^{a}$ | |

### 4.1.3. Total Solids % age

Milk minus water and the remaining constituents are called as total solids. Total solid of milk contains the protein, fat, mineral and lactose. The low content of total solids may be indicated adulteration of milk with water and it also skimming of milk was done. It is known that milk total solids content is important in terms of its whole quality characteristics and its normal range in milk is 9-16% that is derivative of quality features of final products and some time the structural/textural parameters.

Statistical results and mean values for Total solid contents in milk from cow and buffalo is shown in Table 4.3a and Table 4.3b. This ANOVA table shows that raw and pasteurize milk of buffalo and cow have significant difference in total solids among each other this permits further study of this experiment. The mean values (Table 4.3b) of total solids in milk samples showed a significant variation with maximum value of total solid content is 13.876 in buffalo raw milk followed by raw cow milk as 12.102, respectively. Whereas the table shows mean values of total solids content in pasteurize milk of buffalo was 12.646 and in cow 10.872. The findings of current/recent study are found relatively closed by the results of the Mahmood *et al.*, (2010) who analyzed the value of total solids content in the raw and pasteurize milk of buffalo was relatively high as compared to the cow milk.

**Table 4.3a: Analysis of variance for raw and pasteurize milk for Total Solids**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Treatment** | 1 | 7.5645 | 7.5645 | 13.68* |
| **Specie** | 1 | 15.7283 | 15.7283 | 28.45** |
| **Treatment*Specie** | 1 | 0.0000 | 0.0000 | $0.00^{NS}$ |
| **Error** | 16 | 8.8468 | 0.5529 | |
| **Total** | 19 | 32.1395 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), NS = Non-Significant (P>0.05)

**Table 4.3b: Mean Value of raw and pasteurize milk for Total Solids**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 12.102 | 13.876 | $12.9888^{a}$ |
| **Pasteurize** | 10.872 | 12.646 | $11.7588^{b}$ |
| **G.M** | $11.4870^{b}$ | $13.2606^{a}$ | |

### 4.1.4. Lactose composition

Lactose is the unique sugar present in milk and is hydrolyzed producing glucose and galactose by lactase action in the intestine. Concentration of lactose can change a lot in dairy products.

Statistical results and mean values for Total solid contents in milk from cow and buffalo is shown in Table 4.4a and Table 4.4b. This ANOVA table shows that raw and pasteurize milk of buffalo and cow have significant difference in lactose composition among each other this permits further study of this experiment. The mean values (Table 4.4b) of lactose composition in milk samples showed a significant variation with maximum value of lactose composition is 5.842 in buffalo raw milk followed by raw cow milk as 3.521 respectively. Whereas the table shows mean values of lactose composition in pasteurize milk of buffalo was 5.964 and in cow 4.732. The findings of current/recent study are found relatively closed by the results of the

Mahmood *et al.*, (2010) who analyzed the value of lactose composition in the raw and pasteurize milk of buffalo was relatively high as compared to the cow milk.

**Table 4.4a: Analysis of variance for raw and pasteurize milk for Lactose composition**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Treatment** | 1 | 7.429 | 1.458 | 16.42** |
| **Specie** | 1 | 5.993 | 2.879 | 33.10** |
| **Treatment*Specie** | 1 | 16.36 | 1.532 | 18.59** |
| **Error** | 10 | 0.002 | 0.000 | |
| **Total** | 18 | 28.48 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), NS = Non-Significant (P>0.05)

**Table 4.4b: Mean Value of raw and pasteurize milk for Lactose composition**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 3.521 | 5.842 | $0.13872^{b}$ |
| **Pasteurize** | 4.732 | 5.964 | $0.15330^{a}$ |
| **G.M** | $0.1327^{a}$ | $0.52638^{a}$ | |

### 4.1.5. Fat

Fat of any food item is highly significant in relations to the nutritive value. It is also responsible for different functional properties such as flavor/taste, mouth feel, structure/texture and palatability rather than providing energy. Fat contents also source of essential fatty acids which do not produced by body, collectively in our daily diet it may also be consider as one of the main ingredients.

Statistical results and mean values for fat content in milk from cow and buffalo is shown in Table 4.5a and Table 4.5b. This ANOVA table shows that raw and pasteurize milk of buffalo and cow have significant difference in fat content among each other this permits further study of this experiment. The mean values (Table 4.5b) of fat content in milk samples showed a significant variation with maximum value of fat content is 5.832 in buffalo raw milk followed by raw cow milk as 3.588 respectively. Whereas the table shows mean values of fat content in pasteurize milk of buffalo was 5.974 and in cow 4.744. The findings of current/recent study are found relatively closed by the results of the Mahmood *et al.*, (2010) who analyzed the value of fat content in the raw and pasteurize milk of buffalo was relatively high as compared to the cow milk.

**Table 4.5a: Analysis of variance for raw and pasteurize milk for Fat %**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Specie** | 1 | 2.106 | 2.1060 | 14.15* |
| **Treatment*Specie** | 1 | 15.086 | 15.0858 | 101.33** |
| **Error** | 1 | 1.285 | 1.2852 | $8.63^{NS}$ |
| **Total** | 16 | 2.382 | 0.1489 | |
| **Specie** | 19 | 20.859 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), NS = Non-Significant (P>0.05)

**Table 4.5b: Mean value of raw and pasteurize milk for fat %**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 3.588 | 5.832 | $0.13882^{b}$ |
| **Pasteurize** | 4.744 | 5.974 | $0.15360^{a}$ |
| **G.M** | $0.14513^{a}$ | $0.54729^{a}$ | |

### 4.1.6. Protein

Generally, Milk is considered to be an important source of protein in diet of humans, by supplying nearly 32g/l protein. Protein in milk is rich of amino acids that plays role in muscle synthesis. It is another important concern for assessing the nutritive quality of food samples. Dairy proteins (casein and whey) are only supplied by milk and couldn't be substituted by any of other sources so their profile in milk is important in terms of nutritive value.

Statistical results and mean values for protein content in milk from cow and buffalo is shown in Table 4.6a and Table 4.6b. This ANOVA table shows that raw and pasteurize milk of buffalo and cow have significant difference in protein among each other this permits further study of this experiment. The mean values (Table 4.6b) of protein content in milk samples showed a significant variation with maximum value of protein is 6.508 in buffalo raw milk followed by raw cow milk as 4.2180 respectively. Whereas the table shows mean values of protein content in pasteurize milk of buffalo was 6.453 and in cow 4.353. These results were cross

matched with the earlier research of Khan *et al.*, (2007) who worked on effects of milking methods on milk yield, milk flow rate, and milk composition in cow.

**Table 4.6a: Analysis of variance for raw and pasteurize milk for Protein**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Treatment** | 1 | 0.0081 | 0.0081 | $0.03^{NS}$ |
| **Specie** | 1 | 24.0901 | 24.0901 | 74.88** |
| **Treatment*Specie** | 1 | 0.0451 | 0.0451 | $0.14^{NS}$ |
| **Error** | 16 | 5.1476 | 0.3217 | |
| **Total** | 19 | 29.2909 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), NS = Non-Significant (P>0.05)

**Table 4.6b: Mean Value of raw and pasteurize milk for protein**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 4.2180 | 6.508 | $5.3630^{a}$ |
| **Pasteurize** | 4.353 | 6.453 | $5.4032^{a}$ |
| **G.M** | $4.2856^{b}$ | $6.4806^{a}$ | |

### 4.1.7. Solid Not Fat

Solids not fat contents of milk are the solid proportion without fat and moisture content, which represents good total solids profile.

Statistical results and mean values for SNF content in milk from cow and buffalo is shown in Table 4.7a and Table 4.7b. This ANOVA table shows that raw and pasteurize milk of buffalo and cow have significant difference in SNF content among each other this permits further study of this experiment. The mean values (Table 4.7b) of SNF content in milk samples showed a significant variation with maximum value of SNF content is 8.514 in buffalo raw milk followed by raw cow milk as 8.044 respectively. Whereas the table shows mean values of fat content in pasteurize milk of buffalo was 6.672 and in cow 6.128. These values are approximately comparable to the results found by Rehman *et al.* (2013) who worked on the comparative effect of different milking methods and udder hygiene on somatic cell count and milk quality in dairy cows.

**Table 4.7a: Analysis of variance for raw and pasteurize milk for Solid Not Fat**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Treatment** | 1 | 17.6532 | 17.6532 | 27.10** |
| **Specie** | 1 | 0.0067 | 0.0067 | $0.01^{NS}$ |
| **Treatment*Specie** | 1 | 1.2852 | 1.2852 | $1.97^{NS}$ |
| **Error** | 16 | 10.4239 | 0.6515 | |
| **Total** | 19 | 29.3691 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), NS = Non-Significant (P>0.05)

**Table 4.7b: Mean Value of raw and pasteurize milk for Solid Not Fat**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 8.044 | 8.514 | $8.2788^{a}$ |
| **Pasteurize** | 6.128 | 6.672 | $6.3998^{b}$ |
| **G.M** | $7.3576^{a}$ | $7.3210^{a}$ | |

### 4.1.8. Titratable Acidity

The acidity in milk is caused by lactic acid bacteria's action that convert the lactose into lactic acid. The quality attributes and further processing of milk depends on total acidity of milk. If the acidity exceeds the normal limit the quality of milk will be depreciated and with the further processing, contact with heat curdling and spoilage of milk will be caused.

Statistical results and mean values for Acidity content in milk from cow and buffalo is shown in Table 4.8a and Table 4.8b. This ANOVA table shows that raw and pasteurize milk of buffalo and cow have significant difference in acidity content among each other this permits further study of this experiment. The mean values (Table 4.8b) of acidity content in milk samples showed a significant variation with maximum value of acidity is 0.13998 in buffalo raw milk followed by raw cow milk as 0.13766 respectively. Whereas the table shows mean values of protein content in pasteurize milk of buffalo was 0.15460 and in cow 0.15260 His results displayed that fresh milk has pH levels between 6.5-6.9 and acidity varies between 0.22 to 0.24 percent. As the milk settled for long time the pH became decrease and acidity rise. The Haramay University dairy farm's pH and milk acidity of fresh milk were 7 and 0.198 percent

respectively. The appearance of mastitis and acidity respectively suggest a pH greater than or less than the normal range. The presence of bacteria in the milk is suggested by an acidity higher than the normal range. The influence of multiple methods was nearly negligible.

**Table 4.8a: Analysis of variance for raw and pasteurize milk for Acidity**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Treatment** | 1 | 0.001092 | 0.001092 | 184.15** |
| **Specie** | 1 | 0.000023 | 0.000023 | $3.93^{NS}$ |
| **Treatment*Specie** | 1 | 0.000000 | 0.000000 | $0.02^{NS}$ |
| **Error** | 16 | 0.000095 | 0.000006 | |
| **Total** | 19 | 0.001211 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), NS = Non-Significant (P>0.05)

**Table 4.8b: Mean value of raw and pasteurize milk for Acidity**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 0.13766 | 0.13998 | $0.13882^{b}$ |
| **Pasteurize** | 0.15260 | 0.15460 | $0.15360^{a}$ |
| **G.M** | $0.14513^{a}$ | $0.14729^{a}$ | |

### 4.1.9. Moisture

Moisture content is the percentage of water in food stuff that may be in free or bound form. Highest proportion in milk is encountered by moisture which reveals that 81-88% of total milk is made up of water. Higher content of moisture makes the milk more prone to deterioration and spoilage, as higher the moisture content shorter the shelf life of food will be. Researchers have done a lot for calculating the overall chemical characteristics of milk so by comparison with the normal range of constituents, it can be judged whether there is anything wrong or not The analysis of variance for moisture content of cow and buffalo milk from 8 different regions of Central and Southern Punjab revealed that there was showed highly significant (P < 0.01) relation within the species and regions. It showed significant (P<0.05) relation between the regions and species. The analysis of variance for moisture content is represented in (Table 4.9a).

The mean value of moisture content of cow and buffalo milk samples collected from different regions show a slightly change in values.

Statistical results and mean values for Moisture content in milk from cow and buffalo is shown in Table 4.5 and Table 4.6. This ANOVA table shows that raw and pasteurize milk of buffalo and cow have significant difference in moisture content among each other this permits further study of this experiment. The mean values (Table 4.9b) of moisture content in milk samples showed a significant variation with maximum value of moisture content is 83.394 in buffalo raw milk followed by raw cow milk as 86.488 respectively. Whereas the table shows mean values of moisture content in pasteurize milk of buffalo was 83.124 and in cow 83.794. The current study results are in line with Ahmad *et al*., (2021) who disclosed milk at different farms do not justify the moisture content.

The present study results showed that the percentage of water, in samples, was increased that can be the evident of water adulteration, such a milk with higher moisture content surely be adulterated with water hence final consumer (Patients, doctors and especially children and infants) are depressed of greatly valued nutrient of milk.

**Table 4.9a: Analysis of variance for raw and pasteurize milk for Moisture**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Treatment** | 1 | 10.982 | 10.9816 | 11.72 |
| **Specie** | 1 | 17.710 | 17.7096 | 18.90 |
| **Treatment*Specie** | 1 | 7.345 | 7.3447 | 7.84 |
| **Error** | 16 | 14.989 | 0.9368 | |
| **Total** | 19 | 51.025 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), NS = Non-Significant (P>0.05)

**Table 4.9b: Mean value of raw and pasteurize milk for Moisture**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 86.488 | 83.394 | 84.941a |
| **Pasteurize** | 83.794 | 83.124 | $83.459^{b}$ |
| **G.M** | $85.141^{a}$ | $83.259^{b}$ | |

### 4.2. Microbial Examination

#### 4.2.1. Total Coliform Count

Coliforms are generally present in milk. Somatic cells are blood cells that fight infection and occur naturally in milk. The presence of mastitis (an infection of the mammary gland) in the cow will increase the somatic cell count. The somatic cell count can be determined by direct microscopic examination or by electronic instruments designed to count somatic cells. The TCC range varies in cow and buffalo milk samples.

Statistical results and mean values for TCC content in milk from cow and buffalo is shown in Table 4.10a and Table 4.10b. This ANOVA table shows that raw milk of buffalo and cow have significant difference in TCC content when compare to the pasteurize one among each other this permits further study of this experiment. The mean values (Table 4.10b) of TCC content in milk samples showed a significant variation with maximum value of TCC 2.604 in buffalo raw milk followed by raw cow milk as 3.320 respectively. Whereas the table shows mean values of TPC in pasteurize milk of buffalo was 1.2600 and in cow 1.0900 respectively. This shows a considerable difference in the value of TCC before and after pasteurization of cow and buffalo milk.

**Table 4.10a: Analysis of variance for raw and pasteurize milk for Total Coliform Count**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Treatment** | 1 | 15.9668 | 15.9668 | 366.25** |
| **Specie** | 1 | 0.9812 | 0.9812 | 22.51** |
| **Treatment*Specie** | 1 | 0.3726 | 0.3726 | $8.55^{NS}$ |
| **Error** | 16 | 0.6975 | 0.0436 | |
| **Total** | 19 | 18.0183 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), NS = Non-Significant (P>0.05)

**Table 4.10b: Mean value of raw and pasteurize milk for Total Coliform Count**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 3.320 | 2.604 | $2.962^{a}$ |
| **Pasteurize** | 1.2600 | 1.0900 | $1.175^{b}$ |
| **G.M** | $2.29^{a}$ | $1.847^{b}$ | |

#### 4.2.2. Total Plate Count

TPC is a count of microbial load in a sample i.e. it can enumeration of all heterotrophic bacteria that will grow in aerobic or micro aerophilic conditions that will grow at $35^{0}$C. This plate count will tell you how good your sanitization plan is, measure the safety of your product or water supply. This test is the most common test done in factories. Milk samples contain TPC depend on their quality.

Statistical results and mean values for TPC content in milk from cow and buffalo is shown in Table 4.11a and Table 4.11b. This ANOVA table shows that raw milk of buffalo and cow have significant difference in TPC content when compare to the pasteurize one among each other this permits further study of this experiment. The mean values (Table 4.11b) of TPC content in milk samples showed a significant variation with maximum value of TPC in buffalo raw milk is 2.0320 followed by raw cow milk as 2.834 respectively. Whereas the table shows mean values of TPC in pasteurize milk of buffalo was 1.0720 and in cow 1.1320 respectively. The current results are in line with Abd El Aal *et al*., (2015). This shows a considerable difference in the value of TPC before and after pasteurization of cow and buffalo milk.

**Table 4.11a: Analysis of variance for raw and pasteurize milk for Total Plate count**

| Source | DF | SS | MS | F-Value |
|---|---|---|---|---|
| **Treatment** | 1 | 8.8578 | 8.85781 | 180.18** |
| **Specie** | 1 | 0.9288 | 0.92881 | 18.89** |
| **Treatment*Specie** | 1 | 0.6882 | 0.68821 | 14.00* |
| **Error** | 16 | 0.7866 | 0.04916 | |
| **Total** | 19 | 11.2614 | | |

** = Highly Significant (P<0.01), * = Significant (P<0.05), NS = Non-Significant (P>0.05)

**Table 4.11b: Mean value of raw and pasteurize milk for Total plate Count**

| | Cow | Buffalo | G.M |
|---|---|---|---|
| **Raw** | 2.834 | 2.0320 | $2.233^{a}$ |
| **Pasteurize** | 1.1320 | 1.0720 | $1.102^{b}$ |
| **G.M** | $1.983^{a}$ | $1.552^{b}$ | |

## CONCLUSION

Milk is a complete food. Milk is used in each and every home. Milk safety is important because otherwise microbial contamination causes serious health problems to humans. Milk is a healthy drink that should be consumed by everybody. Milk contains a wide range of the vitamins, minerals and nutrients in it those are very important for the healthy life and growth and development of children and young ones. A lot of dairy products are also available in the market. To take healthy milk, milk microbial safety is very necessary. Due to the absence of knowledge to the milk handlers, milk is not related hygienically. That's why consumers are not safe if they drink the milk with bacteria and other microbes. Due to this contamination foodborne pathogens grow into the milk that causes food borne illness. These food borne illnesses may be nausea, fever, dehydration, cholera, vomiting, abdominal cramps and syndrome. To prevent these diseases milk should be safe. These microbes enter into the milk by different sources. These sources may be the bared handed use or no use of gloves, milk collection in the contaminated utensils, instruments used for the evaluation are not sterilized, air contamination if the sample is not covered ,improper storage and storage location, during transportation without using hygienic measures, milking of the milk without washing hands, pouring the milk from one bottle or utensil to the other, improper handling, contamination with contact to the contaminate things, animal udder may be diseased, food used for the animal. Present study is important because it gives knowledge about the raw and pasteurized milk and milk products safely from the milking to the consumption. The series wise contamination causes very serious disease as mentioned above. Milk and milk products are used in each part of the world. The purpose of the study was to evaluate the possible sources of the contamination and pasteurization effect was also assessed on the milk of the buffalo and cow. The physicochemical characteristics were also measured and the effect of the pasteurization on all of the main pathogenic bacteria was tested. During study, it was analyzed that raw milk consumption is not good. It contains a lot of microbes even the milk is fresh. So the milk should be properly pasteurized otherwise improper pasteurization also carries a lot of microbial load in it. After analyzing the pasteurized milk, mostly pathogenic bacteria were killed that resulted that pasteurization was safe for the milk consumption. The pasteurization heat treatment is safe to kill all the vegetative bacteria from the milk to level at which they do not cause any harm to the human health. Physicochemical characteristics of all the species were different. Specific gravity, pH, titratable acidity, ash, protein and Lactose content were measured. Pasteurization killed most of the pathogenic microbes. A few microbes like Listeria and Campylobacter were not killed completely but were present in small limit after the pasteurization. Quality and safety of milk is extremely important. The major factors which are involved include unhygienic milking techniques, transportation, and animal mishandling, feeding and storage conditions. In Pakistan large quantity of the milk is wasted due to the improper channels of delivery system and storage. To improve the economy of country production of clean and hygienic milk is important and that is possible by using safe techniques and following food safety standards. In current study the safety and quality status of cow milk and buffalo's milk was checked. Physical examination, microbial status of milk and chemical composition was determined. Chemical composition like percentage protein, fat, SNF and total solids were determined. Milk was assessed for physicochemical properties like acidity, pH, and average specific gravity. Milk samples of 5 cows and 5 buffaloes were taken for analysis. Mean resulted values of percent protein for the milk samples in raw milk of cow were 4.2188 and for pasteurized milk were 4.353 whereas protein mean value for raw milk of buffaloes were 6.508 and for pasteurized were 6.453. The mean resulted values for fat in raw cow milk were 3.588 and for pasteurized it was 4.744. Whereas for raw cow milk it was 5.832 and for pasteurized milk it was 5.974. Mean values for the total solids by cow in raw milk was 12.102 and by pasteurized it were 10.872.whereas in buffaloes raw milk it were 13.876 and for pasteurized it was 12.646. The mean value of SNF percentage for raw milk of cow was 8.044 and for pasteurized it was 6.128. Whereas in buffalo's raw milk it was 8.514 and for pasteurized it was 6.672. The microbial count (TCC) for raw and pasteurized milk of cow was 3.320 and 1.2600.whereas for buffaloes it was 2.604 and 1.0900. In case of TPC it was 2.834 and 1.1320 from raw to pasteurized milk of cow whereas it was 2.0320 and 1.0720 in buffaloes. The conclusion of this study is that milk that is unpasteurized have high microbial load when compared with pasteurized milk as in both cases either for, milk of cow or for buffaloes.

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