SAFETY ASSESSMENT OF MILK AND INDIGENOUS MILK PRODUCTS FROM DIFFERENT AREAS OF FAISALABAD

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ABSTRACT
The current study was designed to analyze the presence or absence of common adulterants in milk and milk products from six different regions of Faisalabad, Pakistan. Purposely, 54 samples from six different regions of Faisalabad were collected. Results revealed that milk of Faisalabad’s regions was adulterated with water, starch, urea, glucose, cane sugar, soap, synthetic milk and neutralizers. The results for starch, formalin, vegetable fat, artificial color, nitrates, sodium chloride, coal tar dyes, hydrogen peroxide, annatto, detergent, sulphate adulteration was found to be non-significant. The results for soap, synthetic milk, urea, sugar cane, neutralizers and glucose were significant. It was noted that R\(_2\) milk samples had more bacterial load (3.92x10\(^9\) CFU/mL) followed by R\(_1\) (3.83x10\(^9\) CFU/mL). Among milk products, cream samples collected from R\(_1\) had the maximum bacterial count (1.74x10\(^9\) CFU/mL). The milk samples were also analyzed for the presence of added urea by Fourier Transform Infrared Spectroscopy (FTIR).

INTRODUCTION
Food safety is a public health concern which encompasses the assurity of safe food throughout the supply chain. With the advent of technology and consumer awareness, the need of food safety is thriving now a day. It deals with all those hazards that can make food harmful for the health of the consumer. Important food hazards comprise of microbial hazards, pesticide residues, misuse of additives, biological pollutants and adulterants. The microbiological infection and chemical hazards have gained most attention of scientists as food adulteration and fraud could not be ignored (Annah et al., 2019; Wallace et al., 2018). Food fraud is becoming a major problem of the time. It involves the selling of the low quality food by deceiving the customers. Now a days a major food fraud involves the adulteration of food products. Adulteration is done in all foods including milk, oil, spices, cereals, confectionery, beverages and etc which ultimately result in decreased quality of all food commodities (Nayak, 2018). Milk is a complete diet, contains all the major elements like proteins, minerals, fat, vitamins and sugar etc. which are important for normal body growth and development. It is exceptionally important for the growing children. The 50% consumption of milk is either as it is after boiling or in the form of milk products i.e. yoghurt, butter, cream, khoa and cheese etc. The ever increasing greed has given way to a new type called synthetic milk which exactly looks like the natural milk and has same specific gravity, fat and Solid Not Fat (SNF) and is prepared by mixing water, detergents or soap, sodium hydroxide, vegetable oil, salt and urea. It is very dangerous from health point of view and found to have cancerous effects on human beings (Abhirami and Radha, 2015). In order to retain milk momentarily fresh, distributors frequently add ice to the milk, which results in reduction of milk solids. To compensate this, distributors need to add starch, sugar, whey powder and other constituents in order to maintain and increase the milk solids up to an acceptable level (Fakhari et al., 2006). The milk adulterated with ammonia develops regression, and disturb its sensory attributes (Memon et al., 2018). On the other side, formalin is added to enhance the shelf life of milk to be transported to a long distance. It causes liver and kidney damage (Kabariya and Ramani, 2018). Detergents are added to increase the foaming of milk to improve its color and thickness (Tangri and Chatli, 2014). Urea is added in milk to provide white color, increase the consistency and improves the SNF percentage of milk. It is exerting an extra load on kidneys to eliminate it out of the body. Hydrogen peroxide is added in milk to prolong its freshness. Peroxides affect the intestines resulting in gastritis and inflammation of the intestine. These also attract the antioxidants in body disturbing the natural immunity and making a person aged (Clare et al., 2003). Sugar is added in milk to give it the taste of natural milk but it causes irreversible damage to the patients suffering from diabetes (Tangri and Chatli, 2014).

Milk from healthy animals fundamentally does not build the bacterial heap in milk. After that bacterial contamination of milk begins. The basic reasons behind the contamination of milk with microbes are interior ofudder, exterior ofudder, milking environment, health status and state of the animals. Temperature and time of capacity likewise influence microbial nature of milk. Furthermore, the transportation of milk in Pakistan is ordinarily completed with bicycles, vans, animal trucks and bicycles. The component of a food either physical or chemical can be measured by a new simple, non-destructive technique called Fourier transform infrared spectroscopy. This is found to be an advance and so rapid technology (Souhassou et al., 2018). The instrumentation of the FTIR is so developed that it has an internal data analysis system (Rodriguez-Saona and Allenendorf, 2011).

Pakistan is honored with hereditarily high yielding dairy creatures, for example, Nili-Ravi Buffaloes and Sahwal Cows. Yearly 45 million tons of milk is produced in Pakistan. The dairy sector of Pakistan is not so developed and as a result, the percentage of milk that is further processed is only 3% (FAO, 2002). Raw milk is cheaper than the processed milk so consumers in Pakistan mostly prefer the raw milk. In Pakistan, as population increases, the demand of milk also increases and in the future quality of milk is likely to be reduced due to the gap in supply and demand of milk. To fill this gap different adulterants are in practice.
As adulteration is becoming a burning issue of the present time, so there was a need to develop a study regarding all possible types of adulterants practiced in raw milk. Therefore, the current study was designed to screen out the adulterants in milk and milk products. Keeping in view the significance of FTIR as a rapid and non-destructive technique, it was used to detect urea in milk samples.

**MATERIAL AND METHODS**

The present research work was conducted at the National Institute of Food Science and Technology (NIFSAT), University of Agriculture Faisalabad, Pakistan. All the reagents and chemicals used for physical analysis, adulteration tests, microbiological analysis and FTIR were obtained from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan) and Merck (Germany). The other materials used for work were procured from reputed scientific store of Faisalabad, Pakistan.

**Collection of milk samples**

Total six regions of Faisalabad named as Nishatabad (R1), Mansorabad (R2), Khurianwal (R3), Jinnah colony (R4), Madina town (R5) and Gulberg (R6) were selected for obtaining randomized and representative samples. The total 54 milk samples as 9 samples from each region (9x6 =54) were taken early morning in sterilized bottles and then brought to the laboratory for analysis.

**Physical examination of milk**

The color, general appearance, odor, sediments and consistency of the milk were observed by following the methods as described by Eckles, (1986).

**Adulterants in milk**

Milk samples were evaluated for the presence or absence of different adulterants listed below:

- **Starch**
  - 3 mL milk sample was taken in a test tube, boiled and cooled to room temperature. Afterward, a drop of 1% iodine solution was added and color was observed. Blue color indicated the presence of starch (Kaur and Kaur, 2018).

- **Water**
  - Milk sample was taken in a measuring cylinder and the lactometer was placed in it. Lactometer reading was observed and reading less than 26 was taken as an indicator of water addition in the milk sample (FFSSAI, 2012).

- **Cane sugar**
  - 10 mL milk, 1 mL conc. HCl and 0.1 g resorcinol were added in it and mixed well. Test tube was placed in water boiling water for 5 min. Red color indicated the presence of cane sugar (sucrose) (Miralles et al., 2000).

- **Glucose**
  - 1.5 mL of milk from each sample was taken in a test tube. A strip of diastase was dipped in it for 30 sec. Change in color from blue to green indicated that the milk is adulterated with glucose (Kaur and Kaur, 2018).

- **Sodium chloride**
  - For detection of sodium chloride, 2 mL of milk, 0.1 mL of 5% potassium chromate and 2 mL of 0.1 N silver nitrate were added in it. Yellow precipitate showed the occurrence of sodium chloride, while the appearance of brick red precipitate indicated the absence (Abhirami and Radha, 2015).

- **Urea**
  - For detection of urea, 5 mL milk, 0.2 mL urease (20 mg/mL) was added in it and shaken well at room temperature, followed by addition of 0.1 mL Bromothymol Blue (BTB) solution (0.5%). Blue color after 10-15 min indicated the presence of urea in milk. Normal milk showed faint blue color due to natural presence of urea in milk (Kaur and Kaur, 2018).

- **Sulphates**
  - 10 mL milk from each sample was taken in a 50 mL stoppered test tube. 10 mL of Trichloro acetic acid (TCA) solution was added. The coagulated milk was filtered through Whatman filter paper (grade 42). 5 mL of clear filtrate was taken and few drops of barium chloride solution were added in it. Milky white precipitate indicated the presence of milk adulteration with sulphates like ammonium sulphate, sodium sulphate, zinc sulphate and magnesium sulphate etc (Sharma & Rajput, 2012).

- **Formalin**
  - 10 mL milk from the sample being tested was taken in a 50 mL test tube and 10 mL of TCA solution was added in it. The resultant coagulated milk was filtered through Whatman filter paper grade 42. 5 mL of clear filtrate was taken and few drops of barium chloride solution were added in it. The formation of a violet or blue color indicated the formaldehyde adulteration in milk (Kaur and Kaur, 2018).

- **Detergents**
  - For the detection of detergents, a 5 mL milk, 0.1 mL Bromocresol Purple (BCP) solution (0.5%) was added in it. Violet color indicated detergent in the milk while pure milk shows faint violet color (Nayak, 2018).

- **Soap**
  - 10 mL milk, 10 mL hot water and 2-3 drops of phenolphthalein indicator were added in it. Formation of red/pink color shows the incidence of soap in milk (Reddy et al., 2017).

- **Synthetic milk**
  - 5 mL milk, 0.2 mL urease (20 mg/mL) was added in it. Test tube was shaken well followed by addition of 0.1 mL of Bromothymol blue (BTB) solution. Dark blue color indicated the presence of synthetic milk (Kaur and Kaur, 2018).

- **Neutralizers**
  - A volume of 5 mL raw milk was taken in a test tube, followed by addition of 5 mL alcohol and a few drops of rosalic acid. The solution was mixed thoroughly. Red color indicated the existence of sodium carbonate or bicarbonate in milk while pure milk showed brown coloration (Miralles et al., 2000).

- **Artificial color**
  - A 10 mL milk, 10 mL diethyl ether was added in it and shaken vigorously. Test tube was allowed to stand for 5 min. Incidence of any color is indicated by yellow color of the ethereal layer (Reddy et al., 2017).

- **Annatto**
  - A 5 mL milk sample and sodium bicarbonate (8%) solution was added in it to make it alkaline. A drop of filter paper was dipped in it for 2 hr. Red yellow color on filter paper indicated the adulteration of annatto (Nayak, 2018).

- **Coal tar dyes**
  - A 5 mL volume of the milk sample was taken followed by the addition of a few drops of HCl. Formation of pink color in milk samples indicated the presence of coal tar dyes (Souza et al., 2000).

- **Hydrogen peroxide**
  - A 5 mL raw milk sample and 5 drops of paraphenylenediamine (2% solution) were added in it. Formation of blue color indicated the incidence of hydrogen peroxide (Reddy et al., 2017).

- **Nitrates**
  - A 10 mL milk and 10 mL mercuric chloride solution (5%) was added in it. The mixture was mixed well and filtered through Whatman filter paper. Then 1 mL filtrate was taken in a test tube and 4 mL of diphenyl amine sulphate was added. Formation of blue color indicated the existence of nitrates (Kabariya and Ramani, 2018).

- **Vegetable fats**
  - A 5 mL volume of melted milk fat, 5 mL conc. HCl and 0.4 mL furfural solution (5%) were added in it. The mixture was shaken well for 2 min and allowed it to separate. Formation of red or pink color in acid layer indicated the incidence of sesame oil/Vanaspati, which was further confirmed by the addition of 5 mL water. The persistence of color in acid layer, confirmed the presence of sesame oil/ Vanaspati (Recio and Olieman, 1996).
Adulterants in milk products

Starch in khoa and cream

Starch adulteration was determined by following the method as mentioned above and described by Kumar et al. (1998).

Gelatin in cream

10 mL cream, 20 mL water and 20 mL of stokes reagent were mix together. Mixture was filtered and an equal volume of picric acid solution was added into the filtrate. Yellow precipitate formation indicated the existence of gelatin (AOAC, 2006).

Coal tar dyes in khoa

Coal tar adulteration was determined by following the method as mentioned above and described by (FSSAI, 2012).

Blotting paper in rabdi

1 mL of rabdi, 3 mL of HCL and 3 mL of distilled water were added. The contents were stirred with a glass rod. The rod was removed and examined.

Cellulose in rabdi

10 g of rabdi, 50 mL of hot water was added and mixed well for 2 min. The mixture was filtered and residues were washed with 50 mL of hot water. After that, the residues were placed in a spotting plate. A part of residues was washed with Iodine-Zinc Chloride reagent and another part with iodine solution. Formation of blue color in Iodine-Zinc Chloride reagent and absence of blue color in iodine solution confirmed the presence of cellulose (Toteja et al., 1990).

Microbiological analysis of milk and milk products

Total plate count of 18 milk samples and 3 milk products was determined according to the method described by (Yasmin et al., 2019). The MRS agar were prepared according to the manufacturer’s instructions. Media was autoclaved at 121 °C for 15 min at 15 psi. Media was poured into petri plates after proper cooling. Samples were prepared through serial dilution in sterilized test tubes. 100 µL from each dilution were transferred on agar plates followed by gentle spreading through spreaders. The plates were placed in the incubator at 37 °C for 24-36 hr. After incubation, microbial count was counted using colony counter and numbers were expressed as colony forming units (CFU/mL).

Detection of urea by using FTIR

FTIR was performed by following the method as described by Souhassou et al. (2018). Pure milk sample (control) was taken from dairy farm. Three standards were made by adding the urea at different concentration of 700, 1400 and 2000ppm. A disposable pipette was used to place 1 to 50 µl of trans-free reference fat on ATR horizontal surface and ensured that the surface of ZnSe or diamond crystal was completely covered. After that 128-scan single-beam FTIR spectrum was collected. The ATR crystal was cleaned thoroughly by wiping as many times as needed to ensure the absence of cross-contamination. The cleaning was repeated during each standard and samples run. For each of the calibration, standards and milk samples, the absorbance spectra were displayed in the expanded wave number in the range of 650 to 4500 cm⁻¹ corrected

Statistical analysis

The data collected for the adulteration parameters was analyzed statistically by using Chi Square test while other parameters were analyzed by using Analysis of Variance (ANOVA) under completely randomized design (CRD) according to the methods described by Montgomery (2008). However, ANOVA and chi-square test were applied through JMP v10 (Statistical Discovery Software) from SAS.

RESULTS AND DISCUSSION

Physical examination of milk

Milk samples collected from six different regions of Faisalabad were examined physically for general appearance, order, color, consistency, sedimentation and results presented in Table 1. The effect of regions on the consistency and sedimentation was significant while general appearance, order and color was found to be non-significant. It was noted that the milk samples from R8 had 82% and 88% clear. The odor of the milk was 77% and 79% normal in R6 and R7 respectively while 30% and 27% samples from R1 and R5 had very mild order. The 19% samples were yellow color in R1 and 89% samples were white color in R6. 88% samples from R1 and R6 region had watery consistency while 70% samples from R8 were found without sediments.

Table 1. Physical examination of milk samples collected from six different regions of Faisalabad

<table>
<thead>
<tr>
<th>Physical Examination</th>
<th>Region scores (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Appearance</td>
<td></td>
</tr>
<tr>
<td>Clear</td>
<td>81</td>
</tr>
<tr>
<td>Dirty</td>
<td>19</td>
</tr>
<tr>
<td>Odor (%)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>70</td>
</tr>
<tr>
<td>Very mild</td>
<td>30</td>
</tr>
<tr>
<td>Color (%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>81</td>
</tr>
<tr>
<td>Yellow</td>
<td>19</td>
</tr>
<tr>
<td>Consistency (%)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>17</td>
</tr>
<tr>
<td>Watery</td>
<td>83</td>
</tr>
<tr>
<td>Sediments</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>65</td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
</tr>
</tbody>
</table>

Adulterants in milk

The results for the detection of soap, synthetic milk, urea, cane sugar, formalin and glucose in the milk samples were significant (P≤0.05). The results for the presence of neutralizers, water in milk samples were found to be highly significant. It was noticed that no sample was adulterated with starch, detergent, sulphates, artificial color, vegetable fat, annatto, hydrogen peroxide, coal tar dyes and nitrates (data not given). Results of adulterants showed that 33% of the milk samples collected from R1 and R6 were found to be adulterated with soap. Starch adulteration results were positive for R1 and R6 which had 22% samples showing presence of starch. The samples adulterated with synthetic milk were 33% from R1, R2 and R4 whereas R3 demonstrated 67% adulteration (Figure 1). It was noticed that no sample was adulterated with starch, detergent, sulphates, artificial color, vegetable fat, annatto, hydrogen peroxide, coal tar dyes and nitrates (data not given). The results for the detection of these adulterants in the milk samples were found to be non-significant (P≥0.05).
Adulterants in milk samples collected from six different regions of Faisalabad (number of sample adulterated out of 54 sample from 6 regions)

Khoa was tested for the presence of three types of adulterants named starch, sugar and coal tar dyes. The results for starch adulterant named starch were found to be significant (P<0.05) (Figure 2a, b) whereas for coal tar was noted as non-significant (data not given). It means that 44% samples were adulterated with starch while no sample is adulterated with coal tar, starch and cellulose. Another milk product (cream) was also tested for two types of adulterants named starch and gelatin. The statistical results for the presence of starch were found non-significant (P>0.05) whereas for gelatin, it was found to be significant (Figure 2c). According to result presented, starch adulteration results was found to be non-significant. On other side, gelatin was found positive in 33% samples and negative in 67% samples. The results of rabri were found to be significant and non-significant (P>0.05) for the presence of blotting paper and cellulose, respectively. Blotting paper was found positive in 21/54 samples and negative in 33/54 samples. It means that overall 39% samples exhibit blotting paper and 61% without any adulteration of blotting paper were noted during study (Figure 2d).

Microbiological analysis of milk and milk products

The statistical results for Total plate count in tested milk samples was presented in Table 2. The results showed that TPC in R1 was found to be non-significant (P>0.05). The microbial load in raw milk was maximum in R2 (3.92×10⁸ CFU/mL) followed by R3 (3.38×10⁸ CFU/mL) and demonstrated that the significant effect of regions. The least microbial count was observed in TPC in samples of R3 and R4 7.65×10⁷ CFU/mL and 9.89×10⁷ CFU/mL, respectively. Among different indigenous milk products, the bacterial load of rabri ranges between 1.11×10⁷- 2.41×10⁸ CFU/mL. The maximum bacterial load was observed in cream 1.74×10⁸ CFU/mL. While the bacterial load of khoa ranges between 1.432×10⁴-2.71×10⁴ CFU/mL.
Table 2: Enumeration of Total Plat Count (TPC) of milk and milk products

<table>
<thead>
<tr>
<th>Regions</th>
<th>TPC (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw milk</td>
</tr>
<tr>
<td>R1</td>
<td>2.33x10^7</td>
</tr>
<tr>
<td>R2</td>
<td>3.92x10^8</td>
</tr>
<tr>
<td>R3</td>
<td>7.65x10^6</td>
</tr>
<tr>
<td>R4</td>
<td>9.89x10^5</td>
</tr>
<tr>
<td>R5</td>
<td>3.38x10^8</td>
</tr>
<tr>
<td>R6</td>
<td>8.34x10^7</td>
</tr>
</tbody>
</table>

**DISCUSSIONS**

The findings for appearance (color, consistency and sedimentation) of milk samples are in line to those reported by Lateef et al. (2009). They analyzed milk samples collected from the canteries of various hospitals in Faisalabad city and found that 66% samples had dirt. Javaid et al. (2009) in his study, found that attributes of physical quality of milk supplied by different vendors could be significantly varied than direct seller. Conclusively, the results observed in current study were in accordance with the findings of Lateef et al. (2009).

Milk color ranges from bluish-white to golden yellow, mainly depending on the breed, feed, the amount of fat and total solids. Milk is appeared entirely opaque when in large quantity while in case of thin layers it is appeared somewhat transparent. Skim milk shows a bluish tint. Due to the reflection of light milk appears white in color while the yellow color of milk is due to the carotene pigment associated with xanthophyll. The milk quality can be improved by eliminating the middle men from milk supply chain (Lateef et al., 2009).

The findings procured about adulterants in milk are consistent with the results reported by Catradi et al., 2003; Renny et al., 2005; Borin et al., 2006; Luykx et al., 2007; Lateef et al., 2009. These researchers reported that milk sold at various canteries of educational institutes and public places were extensively adulteration with water, starch and synthetic milk.

To strengthen present study, Sharma et al. (2005), also reported about adulteration of paneer, cream, rabri and khoa samples. For their research, samples were being collected from states of northern India. During study, 50 samples were collected from different Indian states and analyzed for the different adulterants in milk based products. It was concluded that the analyzed samples were adulterated with starch (10%), blotting paper (45%) and gelatin (25%). It was also noticed that no sample was adulterated with coal tar dyes.

The observations about microbial load in raw milk and its products suggested that milk of the healthy animal has count of microbes in an acceptable range. There is least microbial load present in milk at the time of the milking (Reyher et al., 2011). The increase risk of microbial contamination through hand milking is more as compare to machine milking (Olofsson, 2013). Hand milking, milk pouring from one container to another container, dirty milk utensils all these factors contribute towards increase bacteria contamination (Annab et al., 2019). The presented results for TPC are also consistent with the results of Bramley & McKinnon (1990). Furthermore, it was suggested to educate the farmers and milk vendors about the clean milk production practices on urgent basis (Murphy and Boor, 2000; Tangri and Chatli, 2014). The personal hygienic status of the person is very important in determining the total bacterial contamination in milk and milk-based products (Lohumi, 2014; Anoop et al., 2014; Chaudhary and Rashmi, 2015).

The results presented for FTIR depicted that clear contrasts were found in the spectra of milk with/without urea supplementation. Greater part of crests relating to ingestion frequencies of urea were in the ghostly area of 1600-1600cm^-1. The other worldly district 1670-1564cm^-1 relating to retention top due CO, CN and NH vibrations accessible in urea. It is reported that the most sensitive region observed for the urea is 1700-1600 cm^-1. As carbonyl bond showed up at extent 1630-1600cm^-1 as urea is a diamide. This study results are in line with Jha et al. (2015) and Sanyam et al. (2015). They analyzed 210 spectra of milk for the detection and quantification of added urea in milk. The 210 samples of the milk were collected for the study. The standards of 700 and 1400ppm were selected to get the spectrum of the milk samples against them.

**CONCLUSION**

Milk and milk products play vital role in addressing nutritional challenges, especially in developing countries like Pakistan. Although milk and milk products are nutritious and easily accessible but also they possess a huge challenge in terms of consumer’s safety. The outcomes of current research concluded that milk and indigenous milk products of Faisalabad city and its surrounding areas were adulterated to fill the gap between supply and demand. They added water, starch, urea, glucose, cane sugar, soap, synthetic milk and neutralizers to maintain its nutritional and compositional profile. It is obvious from the results that microbial quality of milk and milk products is also questionable and special consideration at every step of food supply chain is required to meet the standards. The data suggests that the relevant authorities should conduct regular inspection to ensure milk safety and hygienic requirements for the improvement of public’s health. Moreover, there is need to conduct further studies to explore other factors contributed toward microbial contamination of milk throughout the supply chain.

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