Assessment of the chemical adulteration and hygienic quality of raw cow milk in the northwest of Iran

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Abstract

Milk adulteration and its hygienic quality has become a global concern particularly in developing and underdeveloped countries. So, the awareness of consumers about the common adulterations and the quality level of milk and dairy products are important. The present study was carried out to determine the common chemical adulterants added to raw cow milk by the suppliers from rural areas in the northwest of Iran. Furthermore, the hygienic quality of samples was investigated. A total of 100 samples of raw cow milk were randomly collected from different local markets of dairy products in the northwest of Iran. The results showed that formalin and sodium bicarbonate were present in 8 and 10% of the raw milk samples respectively. However, the adulteration with starch, sugar or skim milk powder, detergents, pulverized soap, chromate and dichromate, hydrogen peroxide were not determined in any of the samples. The mean pH values of collected samples were 6.46±0.11. The methylene blue reduction test also showed that only 44% of raw milk samples had proper hygienic quality. The results of Alcohol test and Clot-on-boiling test were positive in 20 and 43% of samples respectively. The total bacterial count of raw cow milk was in the range of 5.65 to 6.19 log cfu/ml. The results of present study showed that raw cow milk samples did not meet recommended standards, had poor hygienic quality and adulterated with formalin and bicarbonates which are harmful to the health of consumers.

Keywords: raw milk, hygiene, quality, adulteration, Iran

1. Introduction

Food adulteration is the act of intentionally or accidentally lowering the quality of food through the addition or substitution of inferior compounds or by the removal of some valuable ingredients at the stages of growth, harvesting, storage, processing, transport and distribution (Aishwarya and Duza, 2017). In recent years, Food adulteration has become a serious threat for food safety and public health (Spink and Moyer, 2011). Food adulteration is commonly done for economic gain throughout history. According to the definition of Food and Drug Administration (FDA) in 2009, economically motivated adulteration is 'the fraudulent, intentional substitution or addition of a substance in a product for the purpose of increasing the apparent value of product or reducing the cost of its production' (Wheatley and Spink, 2013). Unfortunately, the adulteration in food products is common throughout the world particularly in developing countries, which results in human health hazards. So, the awareness of consumers about the common food adulteration is important (Faraz *et al.*, 2013; Salih and Yang, 2017).

Milk and dairy products are the potential subject of fraud from old times. Milk is defined as the normal mammary secretion obtained by the complete milking of healthy milch animal without addition to or extraction from it, intended for consumption as liquid milk or for further processing (Dadgostar *et al.*, 2013; Shojaei *et al.*, 2008; Singh *et al.*, 2012). It contains 87% water, 5% milk sugar or lactose, 3.9% fat, 3.3% protein and 0.7% ash or minerals (Renny *et al.*, 2005; Salih and Yang, 2017). Milk is an important source of lactose, fats, proteins, carbohydrates, minerals, vitamins and energy for human body (Hamiti *et al.*, 2014;

Hossain and Dev, 2013; Mahmood and Usman, 2010; Salih and Yang, 2017). It also supplies the essential fatty acids, amino acids, calcium, phosphorus, riboflavin, one half of the protein, one third of vitamin A, ascorbic acid, thiamine and one fourth of calories needed daily by an average individual (Legesse *et al.*, 2017; Reddy *et al.*, 2017). So, it is known as an important food for adults, growing children, adolescents and patients (Legesse *et al.*, 2017; Reddy *et al.*, 2017; Renny *et al.*, 2005). Also, Milk as an 'ideal food' provides necessary nutrients for the growth of infants and children and for health maintenance in adults. After the detection of melamine contamination in infant milk products in China, milk adulteration has become a global concern. Due to the absence of adequate monitoring, lack of proper law enforcement and extensive use of milk and dairy products, the potential adulteration with financial gains by unscrupulous producers is high particularly in developing and underdeveloped countries (Nicolaou and Goodacre, 2010; Reddy *et al.*, 2017; Salih and Yang, 2017). Thus, the implementation of regulatory standards against the adulteration of milk and dairy product, and improvement of laboratory diagnostic methods has great importance (Salih and Yang, 2017).

Milk Adulteration ultimately results in either the deception of consumers or threading of their health (Reddy *et al.*, 2017; Salih and Yang, 2017). Milk is a very spoilable product and its shelf life is few hours. So, chemicals like formalin, hydrogen peroxide, carbonates, bicarbonates, boric acid caustic soda and antibiotics are added to raw milk as preservative (Chanda *et al.*, 2012; Debnath *et al.*, 2015; Kandpal *et al.*, 2012). Adulterants such as starch, flour, skim milk powder, reconstituted milk, urea, melamine, salt, glucose, vegetable oil, animal fat and whey powder may also occur in raw milk to increase the thickness and viscosity and to maintain the level of total solids of milk (Salih and Yang, 2017).

Beside milk adulterations, its safety with respect to foodborne diseases is of great concern around the world especially in developing countries where the production of milk may occur under unhygienic conditions and poor production practices (Ashenafi, 1990). Due to its nutritional components, milk is an excellent culture medium for many microorganisms particularly for pathogenic strains (Henry and Newlander, 1977; Saeed *et al.*, 2009). The growth of microorganisms affects the quality of milk, thereby decreased its shelf life and threatens the public health (Barros *et al.*, 2011; Oladipo *et al.*, 2016). The microbiological quality of milk is directly associated with the sanitary level exercised during milking, cleanliness of the milking utensils, storage condition, manner of transport and cleanliness of the udder (Tassew and Seifu, 2011). The microbial load of milk indicates quality of milk handling from milking until consumption.

In Iran, milk and dairy production with the annual production about 7.6 million tons plays an important agricultural role on economy (FAO, 2019; Kalantari *et al.*, 2010). Despite the regulatory standards against food fraud in this country for decades, the adulteration of raw milk has not been controlled completely.

Since, there are limited studies in developing counties especially in the Middle East region regarding to adulteration of raw milk, the present study were aimed at determining the common adulterants added to raw milk by the suppliers in the northwest of Iran. Furthermore, the hygienic quality levels of samples were investigated.

2. Materials and methods

Chemicals and reagents

All of the chemicals and reagents used in current research had the analytical grades. Deionised distilled water was used throughout the study.

Sampling procedure

A total of 100 samples of raw cow milk (each sample containing 100 ml) were randomly collected from different local markets of dairy products in northwest of Iran during the period from April 2018 to September 2018. The samples were aseptically taken using sterile sampling bottles. After labelling, they were placed in cool box and transferred to the Laboratory of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tabriz. The collected samples were aseptically divided into two portions to be used for adulteration and microbiological analysis. All tests were carried out in triplicates at room temperature (25 °C).

Determination of chemical adulteration

Generally, it is possible to detect the adulteration of chemical materials in milk by a change in colour, residue and measurement of different parameters.

Neutralisers

Neutralisers like hydrated lime, sodium hydroxide, sodium carbonate or sodium bicarbonate may be added to milk to masks the developed acidity in milk (Mudgil and Barak, 2013). Rosolic acid test (soda test) was used for the detection of neutralisers in milk. For this purpose, 5 ml of alcohol was added to a test tube containing 5 ml of milk followed by 4-5 drops of rosolic acid (1% alcoholic solution). If the colour of milk changes to pinkish red, it indicates that the milk is adulterated by sodium carbonate or sodium bicarbonate. However, the pure milk shows only a brownish colour (Kamthania *et al.*, 2014; Singh *et al.*, 2012)

Formalin

Formalin (formaldehyde) is a poisonous preservative that can preserve milk for a long time (Singh *et al.*, 2012). For detection of formalin, Hehner test was used as follow: Ten ml of milk was taken in a test tube. Five ml of sulphuric acid (98%) with a little amount of ferric chloride were added without shaking. The appearance of a violet or blue ring at the intersection of the two liquids indicates the presence of formalin in the milk (Kamthania *et al.*, 2014).

Hydrogen peroxide

The hydrogen peroxide is another preservative which is used to prolong the preservation period of milk. Five drops of paraphenylenediamine (2%) were added to a tube containing 5 ml of milk and the tube was well shaken. Appearance of blue colour confirmed the adulteration with hydrogen peroxide (Azad and Ahmed, 2016; Singh *et al.*, 2012).

Detergents

Five ml of milk sample was taken in a test tube and 0.1 ml of 0.5% bromocresol purple solution was added. Development of violet colour indicates the presence of detergent in milk. Pure milk shows only a faint violet colour (pale lavender).

Pulverised soap

The presence of pulverized soap was detected by a qualitative method as follows: Ten ml of milk was taken in a test tube and diluted with an equal quantity of hot water. Then, 2-3 drops of phenolphthalein indicator were added to the solution. The appearance of pink colour indicates the presence of soap in milk (Kamthania *et al.,* 2014).

Chromate and dichromate

Two drops of Ag (NO_3) was added to a tube containing 3 ml of milk. If the milk colour changes to red, it indicates that this adulteration has occurred in milk.

Sugar

Sugar is added to the milk to increase its dry matter content. This additive prevents the detection of extraneous water in milk. Ten ml of milk sample was taken into a test tube and 5 ml of concentrated hydrochloric acid was added along with 0.1 g of resorcinol. The test tube was then placed in the in boiling water for 5 min. In the presence of added sugar in milk, red colouration develops (Aishwarya *et al.,* 2017; Kamthania *et al.,* 2014).

Starch

Starch is added to increase the solids-not-fat content of milk to prevent the detection of added water (Kamthania *et al.,* 2014). In this study, 3 ml of milk was taken in a test tube and boiled. Then, the sample was cooled to room temperature and 2-3 drops of 1% iodine solution were added and mixed. Change of the milk colour to blue indicates the adulteration with starch (Azad and Ahmed, 2016).

Skim milk powder

Ten drops of nitric acid (65%) was added into the test tube containing 5 ml of milk. The appearance of orange colour indicates that the milk is adulterated with skim milk powder. A sample without skim milk powder shows the yellow colour (Debnath *et al.*, 2015; El-Loly *et al.*, 2013).

Determination of hygienic quality

The overall hygienic quality of raw milk samples was evaluated using the following experiments:

pH

The pH measurement was performed in duplicate using a digital portable pH-meter (ST300-B, Ohaus, Parsippany, NJ, USA) calibrated with pH 4 and 7 buffers. The pH of 6.6-6.8 was used as the standard for raw cow milk (Fernandes de Oliveira *et al.*, 2013; Gwandu *et al.*, 2018).

Alcohol test

Five ml of milk sample was mixed with 5 ml of ethanol (68%) in a test tube. After inversion for several times, the test tube was examined for formation of coagulation, clotting or precipitation (O' Connor, 1995).

Clot-on-boiling test

Five ml of milk was put in a boiling water bath for 5 min. Then, the test tube was checked for the presence of floccules (O'Connor, 1995).

Methylene blue reduction test

In this experiment, 1 ml of methylene blue was added to 10 ml of the milk sample in a test tube. The test tube was placed in water bath at 35 °C for 30 min. Milk samples were examined for decolourization after 30 min of incubation. The colour changes were checked at 30 min intervals. The dye reduction time was associated with the microbial load of the milk (Hawaz *et al.*, 2015).

Total bacterial count

One ml of each sample was dispensed in sterile test tube containing 9 ml peptone water. After thoroughly mixing, serial dilutions were prepared. 0.1 ml of each dilution was cultured on plate count agar (Oxoid, Basingstoke, Hampstead, UK) according the spread plate method. The plates were then incubated at 37 °C for 48 h. The colonies were counted using colony counter and the total bacterial count was calculated as cfu/ml (Oladipo *et al.,* 2016).

Data analysis

The data were statistically analysed using SPSS software (Version 18, SPSS Inc., Chicago, IL, USA). The results for total bacterial count were logarithmically transformed into $\log_{10}$ for statistical analysis. Measurements were made in triplicate. The mean, standard deviation, maximum and minimum values were obtained using descriptive statistics. The significant differences between means were evaluated using one-way analysis of variance (ANOVA) with significance level peg at 0.01.

3. Results and discussion

Detection of chemical adulteration

The evaluated adulterants in raw milk collected from different local markets in the northwest of Iran are presented in Table 1. It was found that all examined cow milk samples were free from starch. These results agree with the findings of El-Loly *et al.* (2013) and Faraz *et al.* (2013) in Egypt and Pakistan respectively. However, Ahmad (2009) in a study on 300 milk samples in Sudan found that 35.5% of samples were adulterated with starch. Solid materials like starch can increase the concentration of diluted milk to increase the profit (Chanda *et al.*, 2012). Excessive starch in the milk may cause diarrhoea due to the effects of undigested starch in colon. Furthermore, its accumulation in the body may be fatal for diabetic patients (Rideout *et al.*, 2008; Singuluri and Sukumaran, 2014).

Table 1. The percentage of raw cow milk samples containing adulterants collected from local markets in the northwest of Iran.

Adulterants	Positive (%)	Negative (%)
Starch	–	100
Sugar	–	100
Skim milk powder	–	100
Formalin	8	92
Hydrogen peroxide	–	100
Carbonate/bicarbonate	4	96
Pulverised soap	–	100
Chromate/dichromate	–	100
Detergents	–	100

The results of present study indicated that all of collected samples contained no sugar or skim milk powder. However, Chanda *et al.* (2012) evaluated the raw milk samples in the rural areas of Barisal district of Bangladesh and detected 26.0 and 14.0% of samples as positive for sugar and powdered milk respectively. Also, in the study of Singuluri and Sukumaran (2014), the extent of adulteration for sucrose and skim milk powder in Hyderabad (India) were 22 and 80% respectively. These two adulterants may be added to either increase the weight or relative mass of milk. Sucrose may be used in diluted milk to mimic the natural sweetness of milk (Chanda *et al.*, 2012; Faraz *et al.*, 2013).

Since milk fat is an expensive food product, some producers of dairy products, substitute the milk fat with non-milk fat such as vegetable oil for additional financial gain. Detergents can emulsify and dissolve the oil in water giving a foamy appearance to milk, mask fat value of milk and enhance its cosmetic nature (Azad and Ahmed, 2016; Debnath *et al.*, 2015; Singuluri and Sukumaran, 2014). Some manufacturer may also add potassium chromate/dichromate to the spoiled milk, to prevent from its coagulation during the thermal process. But, it is known that potassium dichromate can cause skin irritation, rhinitis, and allergic contact dermatitis (Singh and Gandhi, 2015). In the present study, the adulteration with detergents, pulverized soap, chromate and dichromate were not detected in the samples. However, Debnath *et al.* (2015) reported that 9.68% of raw milk samples from Kolkata and its suburban areas were adulterated with detergents.

The raw milk may adulterate with hydrogen peroxide to prolong its freshness. Hydrogen peroxide is used to increase lacto-peroxidase activity but peroxides damages the gastro intestinal cells which can result in gastritis and inflammation of the intestine (Debnath *et al.*, 2015; Singuluri and Sukumaran, 2014). Fortunately, in this study hydrogen peroxide was not detected in any of the samples. However, this result is a bit different from the findings of Debnath *et al.* (2015) and Faraz *et al.* (2013) who found hydrogen peroxide in raw milk.

In the present study, 8% of the milk samples were detected as formalin positive and carbonate/bicarbonate was detected in 4% of the samples (Figure 1 and 2). These results are comparable with the findings of Chanda *et al.* (2012) who found that 20% of formalin and 10% of sodium bicarbonate as added preservative in raw milk samples in Bangladesh. In raw milk, formalin is added to increase the shelf life of milk. This additive has the potential to cause serious hazards for health (Chanda *et al.*, 2012; Salih and Yang, 2017). Also, neutralisers such as carbonates and bicarbonates of various alkalis are generally added to mask the pH and acidity of

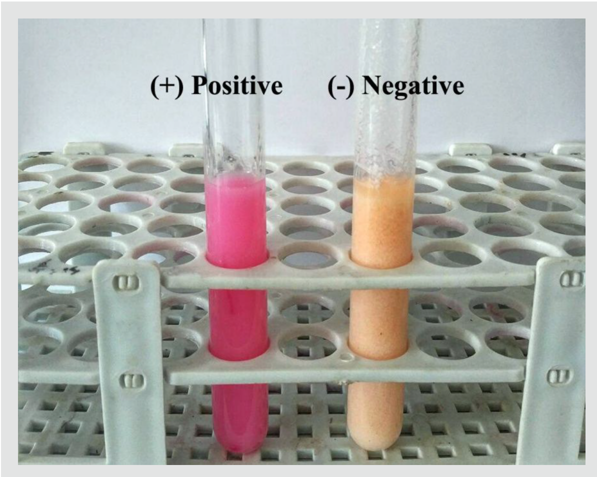

Figure 1. Difference between the pure milk and adulterated milk with neutralisers in the Rosolic acid test (soda test). Appearance of a pinkish red in milk indicated the presence of neutralisers.

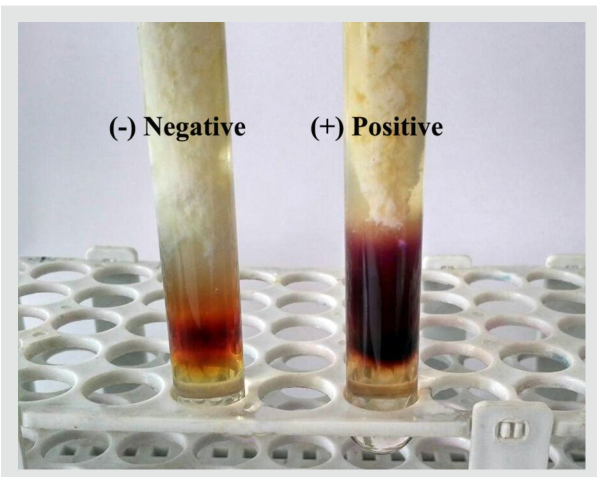

Figure 2. Difference between the pure milk and adulterated milk with formalin in Hehner test. Appearance of a violet colour at the intersection of two liquids indicated the presence of formalin in milk.

badly preserved milk (Singuluri and Sukumaran, 2014). The presence of carbonates in milk may lead to gastrointestinal complications like diarrhoea, gastric ulcer, colon ulcer and electrolytes disturbance (Reddy *et al.*, 2017). In addition, carbonate and bicarbonates can disturb hormone signalling that regulate development and reproduction in the body (Azad and Ahmed, 2016). According to these results, it is very unfortunate that the consumers in the northwest region of Iran are at the risk of the consumption of formalin and neutraliser contaminated milk.

Neutralisers like hydrated lime, sodium hydroxide, sodium carbonate or sodium bicarbonate may be added to milk to masks the developed acidity in milk (Mudgil and Barak, 2013). Rosolic acid test (soda test) was used for the detection of neutralisers in milk. For this purpose, 5 ml of alcohol was added to a test tube containing 5 ml of milk followed by 4-5 drops of rosolic acid (1% alcoholic solution). If the colour of milk changes to pinkish red, it indicates that the milk is adulterated by sodium carbonate or sodium bicarbonate. However, the colour of pure milk changes only to brown (Kamthania *et al.*, 2014; Singh *et al.*, 2012).

Detection of hygienic quality level

The pH values of collected raw milk samples were in a range of 6.16-6.71 and the mean of value was 6.46±0.11. About 87% of samples had pH lower than the acceptable levels (6.6-6.8). The analysis of data showed that there were significant correlation between the pH value and total bacterial count of samples ($P<0.01$, $r^2=-0.621$). These results are in agreement with findings of Legesse *et al.* (2017), Dadgostar *et al.* (2013) and Imran *et al.* (2008). During the storage period, the production of lactic acid by the growing microflora (mainly psychrotrophic bacteria) has an important effect on the decreasing of milk pH value (Malacarne *et al.*, 2013).

The results of Clot-on-boiling test showed that 20% of the tested raw milk samples were likely to clot on boiling. Also, it was found that 43% of the samples were positive for Alcohol test. All of the positive samples in boiling test were positive in alcohol test too. There was a significant correlation between the results of boiling test and alcohol test ($P<0.01$, $r^2=0.525$). In addition, all of the positive samples in both tests had an average pH value of 6.32 and this value was lower than acceptable levels (6.6-6.8) (Fernandes de Oliveira *et al.*, 2013; Gwandu *et al.*, 2018). These findings are in accordance with the results of Tassew and Seifu (2011), who reported 23 and 58% of cow milk samples in Ethiopia were positive in Clot-on-boiling and alcohol tests respectively. Clot-on-boiling test evaluates the same properties like the alcohol test but it is somewhat less rigid than the alcohol test (O'Connor, 1995). According to these results, it can be concluded that the alcohol test is more sensitive than the clot-on-boiling test.

Since methylene blue reduction test has a strong relationship with standard plate count, this test is recommended as a cost effective method to evaluate the quality of raw milk at the factory (De Silva *et al.*, 2016). For the evaluated samples in the present study, 56% of them were such in which the methylene blue got decolourised within 2 h. The dye was reduced within 0.5-1 h and 1-2 h in 25 and 31% of samples respectively. However, only 44% of samples had the proper hygienic quality in this test (with the methylene blue reduction time between 2-6 h) (Table 2). Analysis of data showed that there was a positive correlation between the result of methylene blue reduction test and total bacterial count ($P<0.01$, $r^2=0.904$). The results of this test showed that the hygienic quality of the most of collected raw milk

Table 2. The percentage of collected milk samples that reduced methylene blue dye at various time intervals.

	Methylene blue reduction time (h)				
	0.5-1	1-2	2-6	6-8	>8
Percentage of raw milk samples	25%	31%	44%	0%	0%
Hygienic quality	very poor	poor	good	very good	excellent

samples were in poor condition. Similar result was also reported by Hawaz (2015) about raw cow milk samples collected from Harar Milkshed, Eastern Ethiopia.

The total bacterial counts of raw cow milk samples evaluated in the study area ranged between 5.65-6.19 log cfu/ml. The overall mean total bacterial count of raw cow milk was 6.19±0.48 log cfu/ml which is in agreement with the average value (6.25±0.87 log cfu/ml) reported by Hawaz *et al.* (2015). Oladipo *et al.* (2016) also found that the total bacterial counts of the raw cow milk samples in Ogbomoso, Nigeria ranged from 5.30 log cfu/ml to 6.62 log cfu/ml. However, the mean total bacterial count of cow milk produced in Ethiopia was 7.58 log cfu/ml (Tassew and Seifu, 2011). These variations in the results of various studies are related to different sanitary condition during milking, collection, storage and transportation (Hawaz *et al.*, 2015). The total bacterial count in the most of raw milk samples obtained in the present study was higher than the acceptable level of 10^5 cfu/ml (O'Connor, 1995). Poor milk handling practices during milking and poor animal health services and quality of used water for rinsing are the most frequent cause of high total bacterial count (Hawaz, 2015). After milking, the milk residues on surfaces of equipments provide enough nutrients for the growth of bacteria that contaminate milk. Failure to cool the milk rapidly to the temperature lower than 4.4 °C and extremely hot and humid weather can also contribute in the increasing of the total bacterial count in raw milk (Bereda *et al.,* 2012).

Generally, lack of knowledge about sanitary milk production, contamination of udder surface and the use of unclean milking utensils would be some of the factors which contributed to the poor hygienic quality of produced milk (Bereda *et al.,* 2012; Oladipo *et al.,* 2016; Tassew and Seifu, 2011). Contamination of teats with manure, mud, feeds or bedding can increase contamination of raw milk as well as the risk of developing mastitis (Ruegg *et al.,* 2002). The results of total bacterial count in the current study indicated that there was not a proper hygienic condition during the production and handling of raw milk in the northwest of Iran.

4. Conclusions

Since, milk has relatively lower cost and higher rate of consumption than other protein sources; it is often exposed to highest levels of fraudulent activity. Milk adulteration is often done for economical gain that ultimately led to a decrease in its nutritional value and increase safety hazards. Hence, the consumption of adulterated milk may cause serious risks for public health. Such types of fraud are a common issue in many regions throughout the world especially in developing countries like Iran, which have inadequate monitoring and regulatory practices. Thus, a reliable and efficient quality control system is needed to regular monitoring of the quality of raw milk, which requires collaboration between the scientific community and regulatory authorities. Consumers must be well aware of the common adulterants and hygienic quality of raw milk. Also, producers of milk and dairy products should realise the importance of regular inspection from their products to ensure whether they meet the minimum quality standards. They should be aware of the necessary required sanitary measures during handling, milking, processing, transportation and storage.

Conflict of interest

The authors declare that they have no conflict of interest.

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