

## Physicochemical properties and frauds in the samples of raw cow milk produced in Qazvin, Iran

Razzagh Mahmoudi<sup>1</sup>, Reza Norian<sup>2</sup>

*Journal of Research & Health*  
Social Development & Health Promotion  
Research Center  
Vol. 5, No.3, Autumn 2015  
Pages: 340-346  
Original Article

1. **Correspondence to:** Assistant Professor of Food Hygiene and Aquatics Department, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran  
Tel/Fax: +98 411 6378743  
Email: r.mahmodi@yahoo.com
2. MSc, Laboratory of Food Quality Control and Hygiene, Veterinary Medicine Office, Qazvin, Iran

Received: 1 Dec 2013  
Accepted: 12 Mar 2014

How to cite this article: Mahmoudi R, Norian R. Physicochemical properties and frauds in samples of raw cow milk produced in Qazvin Province in 2012. *J Research Health* 2015; 5(3): 340-346.

### Abstract

Additives that lower microbial load and hide defects of milk threaten milk hygiene and health, and always concern consumers. In this cross-sectional analytical study, 112 raw milk samples were randomly collected from 14 semi-industrial dairy farms across Qazvin province, Iran, on four occasions and examined in terms of physicochemical parameters and some fraudulent additives according to standard methods which involved an assessment of mean amounts of lactose, solid fat-free matter, and specific gravity of milk samples. Results showed that in most cases these parameters were in their average range. However, mean amounts of fat (2.48%) and protein (2.32%) in samples were significantly lower than their average standard range. Fraud determination tests in milk samples showed positive results for added water (16.07%), hypochlorite (0.89%), and hydrogen peroxide (1.78%) tests, and negative results in the remaining tests. Milk samples had significantly higher amounts of added water in warm seasons compared to cold seasons. It is highly important to control the quality of milk as an essential food item with high per capita consumption and a special place in human nutrition, especially in children in terms of physicochemical properties and residue of some additives.

**Keywords:** Fraud, Milk, Physicochemical, Iran

### Introduction

Milk is a complex mixture made up of fat emulsion, protein in colloidal suspension form, and a specific sugar or lactose in a true solution. In addition to these, milk also contains secondary elements such as minerals, vitamins, enzymes and organic compounds such as citric and lactic acids. Thus, milk is almost a complete food that can provide most of human nutritional requirements. Milk is one of the most consumed dairy products, and with high nutritional value, and a variety of nutrients, its consumption is recommended to all age groups [1, 2]. With knowledge of factors that

contaminate and spoil milk, contamination and corruption prevention methods may be addressed. Quality control of milk is highly important in all stages, from production and milking cattle to transportation, packaging, and consumption. Milk health involves control of cattle health, and where they are housed and milked. Observing principles of environmental health is very important such as cleanliness of sites and equipment, healthy water, hygienic disposal of waste, excrement, garbage, and dust since these can contaminate milk. External contamination sources should be kept away from all stages of milking,

collection and transportation. In the milk production stage, it is essential to consider farm building and equipment, and control pathogenic vectors in farm areas, water used, and storage of milk on the farm [1, 2, 3].

In a study on the composition of milk and the number of somatic cells in samples of raw milk collected in the Khorasan-Razavi Province, results showed no significant difference in acidity between different seasons. Fat content was the lowest in spring and the highest in winter. Milk samples contained equal amounts of protein in winter and autumn, but these amounts were higher in summer and spring. Results further identified a negative relationship of somatic cells with acidity and percentage of fat, yet their relationship with the amount of protein was positive and linear [4]. This valuable product has specific physical and chemical properties that are assessed at delivery to milk factories [1, 2].

Milk can be contaminated, first by microbial and chemical factors (fungal toxins, drugs, insecticides, preservatives, and so on), and second by staff, milking equipment, transportation to factory, and various processes. On the other hand, there is a variety of frauds in milk, and their knowledge is also beneficial to consumers [2]. Among major problems associated with health of this nutrient, and subsequently health of consumers, is the addition of compounds to lower microbial load and hide defects of milk which has always caused consumers' concern. According to definition provided by the International Milk Commission, raw milk delivered to factories should be fresh, pure, and clean with natural taste and smell, and free of colostrum. There should be nothing added or taken away, and any manipulation in composition of milk (including extracting fat, adding water, sugar, salt, dried milk, and so on), or addition of sourness-neutralizing agents (for example, baking soda), or preservatives (hydrogen peroxide, formalin, salicylic acid) is considered fraudulent [5].

One of the factors affecting health and quality of milk is changes that occur in various stages of collection, storage, transportation,

and consumption of milk. Knowledge and assessment of these factors and their effects on quality and health of milk and its products, and how milk is corrupted help producers, distributors and consumers, and will substantially affect improvement in community health and prevention of contamination of this important food source [1,5]. Hence, this study aims to investigate physicochemical properties of raw milk samples collected from semi-industrial dairy farms in Qazvin Province, together with potential frauds using standard methods.

### Method

This cross-sectional study was conducted to determine physicochemical quality and frauds in raw cow milk samples. One hundred twelve raw milk samples were randomly collected from 14 semi-industrial dairy farms across Qazvin Province, Iran, 2012 on four occasions, and examined in terms of physicochemical parameters and some fraudulent additives according to standard methods (the first six months of the year were considered warm season, and the second six months as cold). Sampling was conducted according to a random cluster sampling method, and sample size was determined according to equation below:

According to milk samples tested in Iran, proportion of frauds (P) was reported between 0 and 21.66% [6]. In the present study, sample size was estimated as 112 considering the mean prevalence of about 8% and error of 5%. Samples were transported to the laboratory at 2 °C (kept on ice) immediately after sampling. After homogenization of samples, Milkoscan system model 400 (Foss-Electric A/C Hillerod, Denmark) was used to assess physicochemical parameters of fat, protein, sugar, specific gravity, and fat-free dry matter according to standards [7]. Added water fraud was assessed through specific gravity using Thermolac-densimeter [8].

For detection of sourness-neutralizing agent, 10 ml of suspect milk sample was poured into a beaker, and its acidity was determined

after titration with 0.1 N NaOH. Then, 10 ml of the same sample was taken and boiled for 1 minute, and acidity was found in the same way. A significant drop in acidity following boiling (more than 1 Dornik degree) meant the probable addition of baking soda [9].

Added salt was determined through titration with 0.1 N silver nitrate.

Quaternary ammonium compounds were detected using the Eosin-based qualitative method [8]. Hypochlorites were detected using the potassium iodide-based qualitative method [8]. Added sugar was detected using saturated solution of ammonium molybdate and hydrochloric acid [8, 9].

Formalin turns milk purple with a peculiar smell when assessing percentage of fat in the Gerber method. Formalin was detected using condensed hydrochloride acid and chloroferric 2.5% [8].

Hydrogen peroxide was detected using Dupouy reaction, which is based on the presence of peroxidase in milk. This test was performed using Guaiacol solution [10].

In this study, variables consisted of sampling

seasons (warm and cold seasons), and presence or absence of frauds. Physicochemical parameters in milk samples were examined according to seasons. Data collected were analyzed in SPSS-17 using Chi-square test at significant level  $P < 0.05$ .

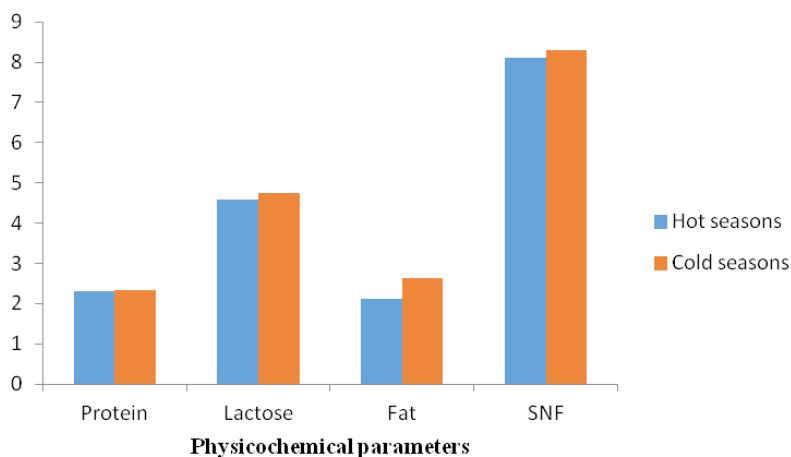
**Results**

Results of assessment of physicochemical parameters in milk samples are presented in Table 1. According to chemical tests, mean values in samples tested were found in the average range. Mean values of fat (2.48%) and protein (2.32%) were found significantly lower than their standard average range (3.75% and 3.4% respectively) ( $P < 0.05$ ). However, no significant difference was found between warm and cold seasons (Figure 1).

Fraud determination tests in milk samples showed positive results in added water (16.07%), hypochlorite (0.89%), and hydrogen peroxide (1.78%) tests, and negative results in the remaining tests (Table 2). Accordingly, milk samples had significantly higher amounts of added water (Figure 2) in warm

**Table 1** The mean values of physico-chemical parameters of raw cow milk samples Qazvin province, 2012

Physicochemical properties	Mean±SD	Min–Max	Average in cow’s milk
Fat	2.48±0.35	1.87-3.85	3.70
Protein	2.32±0.07	2.00-2.52	3.40
Lactose	4.67±0.42	3.90-7.49	4.75
SNF	8.02±0.25	6.98-8.68	8.50
Density	31.56±1.8	27.30-34.40	28-34



**Figure 1** Compare chemical parameters of raw cow milk samples during warm and cold seasons

seasons compared to cold seasons ( $P < 0.05$ ). Hypochlorite was detected in only one milk

sample in warm seasons (Figure 3).

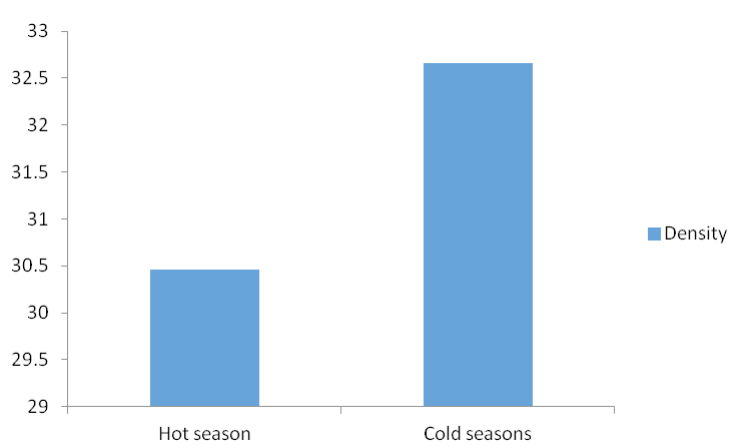


Figure 2 Compare Density of raw cow milk samples during warm and cold seasons

Table 2 Added fraud in raw cow milk samples, 2012

Type of fraud	Positive samples (N)	Positive samples (%)
Water	18	16.07
Salt	0	0
Quaternary ammonium compounds	0	0
Hypochlorite	1	0.89
Carbonate	0	0
Formalin	0	0
H <sub>2</sub> O <sub>2</sub>	2	1.78

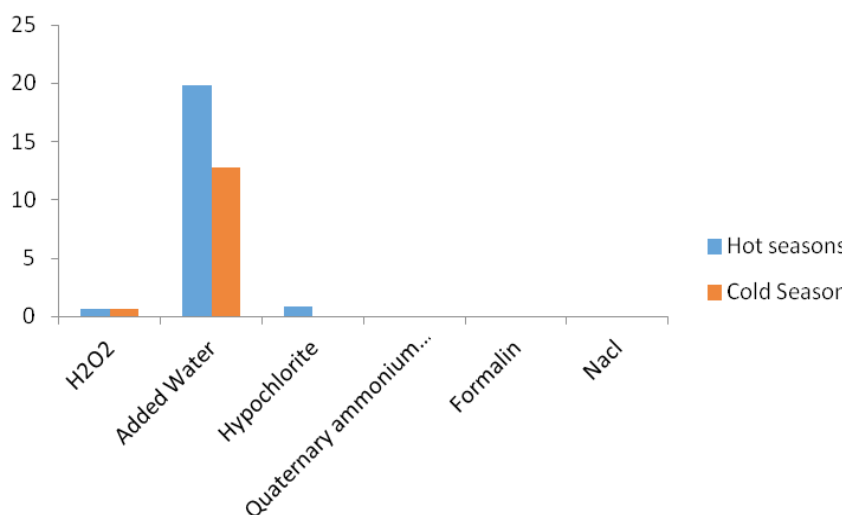


Figure 3 Compare the percentage of positive cases of fraud have milk samples during warm and cold seasons

### Discussion

Milk is one of the most essential and complete natural foods, and is used to make various dairy products that are consumed by people of all ages. Given the characteristics of milk, controlling this valuable product and measuring its composition are essential for milk producers

and the dairy industry since the quality of milk directly affects milk processing and quality of dairy products. Quality of milk is affected by environmental factors and livestock characteristics. Thus, it is important to maintain quality of milk to maintain its

competitive place in the market. Any changes in composition seriously threaten milk producers (price cuts), dairy industry (production costs), and consumers (dietary and health aspects) [3]. In this study, physicochemical characteristics of milk together with possible fraudulent additives were investigated using standard methods. Results obtained showed a gradual increase in fat content of raw milk from the warm season, reaching the highest in the cold season. However, no significant difference was observed in fat content of milk samples collected between warm and cold seasons. Generally, seasonal effect on fat content is largely attributed to environmental temperature changes. In warm seasons, percentage of fat is lower, but the difference in the mean seasonal difference is only about 0.4%. Various researchers have reported that the percentage of fat reaches the lowest in warm months and the highest in cold months [10, 11], which agrees with the present study results.

Mean protein content of milk is 3.4%, which may be determined in the Kjeldahl method, or faster by titration. The present study results showed no significant difference in mean protein content between warm and cold seasons (Figure 1), yet mean value of this parameter was slightly higher in warm season. Low protein content in the cold season is attributed to temperature changes and has been reported about 0.2% [10, 11].

Sugar content in milk samples (table 1) was in the right range (4.67%) and comparable to the standard amounts (4.75%) [1]. Despite slightly higher sugar content in the warm season, no significant difference was found between seasons.

Specific gravity of natural milk is normally between 1028 and 1034, which means that one liter of milk at 15 °C weighs between 1028 and 1034 grams. Removal of fat increases the specific gravity of milk, so that specific gravity of churned milk (fat removed) is about 1033 to 1036 grams. Conversely, addition of water reduces the specific gravity of milk, so that the specific gravity is reduced by about 3 grams for every 10% addition of water. However, if

both water and churned milk are added to milk, the specific gravity remains constant, and other tests should be used to detect this double fraud. Assessment of mean values of specific gravity of milk samples showed these values were within the right range, and specific gravity in the cold season (32.66) was significantly higher compared to the warm season (30.46) (figure 2). Changes in specific gravity are affected by factors such as type of fodder, lactation period, fat removal, and addition of water [1]. In the present study, significantly higher frequency of added water was found in milk samples in the warm season compared to the cold season.

Consumers have the right to receive healthy, fraud-free milk in exchange for their payment. Milk fraud may be intentional, or may occur accidentally in the process of production. Regarding added frauds in raw milk samples, results obtained were positive in added water, hypochlorite, and hydrogen peroxide tests, and negative in the remaining tests.

Addition of soda (carbonate) to milk causes digestive problems such as stomach ulcer, diarrhea, colon cancer, and impaired balance of body fluids. Hydrogen peroxide causes early aging by causing dysfunction of the natural body antioxidant system [9].

Addition of chlorides also causes imbalance in blood acid, base, and pH [13].

Ammonia compounds in milk cause regression and sensory and speech disorders [14].

As discussed, raw milk delivered to milk factories should be free of additives. Yet, results obtained showed higher prevalence of additives in samples collected in the warm season (added water and hypochlorite) compared to the cold season. Due to high temperatures in the warm season and difficulties in storage of milk under standard conditions, it is highly likely for milk to become spoiled, and therefore, addition of sourness-neutralizing additives is also more probable. There was no significant difference between seasons in frequency of positive hydrogen peroxide containing samples.

Results of a study on residue of some



additives in raw milk samples collected from Pakdasht region in 2009 showed no significant difference between the two seasons in terms of positive samples containing microbial growth-inhibiting and acidity neutralizing substances, formalin, and hydrogen peroxide. Yet, positive cases of hydrogen peroxide and formalin were higher in the first six months compared to the second. Moreover, this also applied to cases with sugar content ( $P < 0.05$ ). In all samples, the amount of salt was in normal range. The present study results showed higher cases of fraud (especially added water and hypochlorite) in the warm season.

Results of a study by Abtisa et al. in Sudan on 240 raw milk samples over one year revealed five cases of added formalin and one of hydrogen peroxide [16]. The present study results also showed the presence of hydrogen peroxide in samples studied (1.78%), but no cases of formalin contamination were found.

Results of a study by Abogeh et al. (2000) in Kenya investigating residue of anti-microbial substances in 212 raw milk samples and 222 pasteurized milk samples confirmed contamination of raw (9.4%) and pasteurized (5.7%) milk samples, and the only test showing a greater number of positive samples in the second six months compared to the first was microbial growth-inhibitor residue test [17].

### Conclusion

The present study results showed a significant seasonal effect on amounts of fat and protein in milk, and these amounts were found higher in the cold season compared to the warm. Mean amounts of fat and protein were significantly lower than the average range for cow milk. Hence, given nutritional and technological value of fat and protein contents, controlling factors affecting changes can play a decisive role in improving quality of dairy products. Milk is an important nutritional source for humans, and since it contains nutrients, it is vulnerable to corruption by growth and proliferation of microorganisms. Thus, the addition of substances for profiteering and hiding defects such as sourness, and lowering

microbial load threatens the health of this nutritional substance and subsequently the health of consumers. Therefore, it is essential to control the quality of this important food product, with its high per capita consumption and special place in human nutrition, especially in children, in terms of physicochemical properties and residue of some additives.

### Acknowledgements

This work was financially supported by University of Tabriz, Tabriz, Iran.

### Contributions

Study idea and design: MR, NR

Data collection and analysis: MR, NR

Writing, compiling, and editing of article: MR

### Conflict of interest

"The authors declare that they have no competing interests."

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