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ORIGINAL ARTICLE

Investigation of Heavy Metals, Aflatoxin M1 and Physicochemical Properties of Milk Used in Baby Dry Milk Formula in Shahrekord City

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KEYWORDS

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ABSTRACT: In this study, the characteristics of milk input to Pegah Shahrekord infant milk factory and the effect of season and month of lactation on fat percentage, protein percentage, acidity and density, the amount of lead, cadmium and aflatoxin M1(AFM1) in raw milk were measured. For this purpose, 690 milk samples were collected during the autumn and winter seasons (during September 2018 to March 2019). The level of toxic metals, aflatoxin with HPLC and acidity, fat percentage, dry matter and protein was measured according to standard. The results showed that Mean ± S.D lead in raw milk was 12.52±1.78 ppb and Mean ± S.D cadmium content was 10.8±2.83 ppb, which was lower than the allowable amount of codex for lead and cadmium in milk. AFM1 level in raw milk was 12.44±1.87ppt (Mean ± S.D). The milk input to the plant had mean ± S.D of 2.94±0.082% protein, 3.27±0.18% fat, 11.59±0.31% solids, density 1.0299±0.0008 and acidity 14.6±0.49. Lactation season significantly altered milk protein production (P <0.05). Mean ± S.D. milk production protein was 2.94±0.082%. Lactation season did not have a significant effect on milk fat percentage (P<0.05). Mean ± S.D. percentage of milk fat produced was 3.27±0.18%. Lactation season also had a significant effect on the acidity of milk produced (P<0.05). Milk produced had the average acidity 14.6 (14.6±0.49). Lactation season did not have a significant effect on the percentage of dry matter in the produced milk (P<0.05). Mean ± S.D. dry matter of milk produced was obtained 11.59±0.31. In general, the input milk to Pegah Shahrekord infant milk factory in terms of composition and quality is in line with the reports for milk compounds in Iran and the world.

INTRODUCTION

Supplementary or complete dry milk feeding on a global scale Due to concerns about their baby's development, the need for mothers to work and study living conditions and the problems of breastfeeding in the community, they are forced to use formula for infants [1, 2]. The process of producing, transporting, and storing baby food is more important than other food formulas because baby foods are more sensitive to reactions and interactions, as well as their physical characteristics and availability of nutrients change during storage. Therefore, these steps, particularly storage conditions, must be controlled to maintain the appropriate quality of the product. Many of the emerging contaminants of these products are on rise as a result of agricultural activities, industrial pollutants and the use of

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industrial effluents in agriculture. Therefore, concerns about the health of these products have attracted widespread attention [3]. Aflatoxins are highly toxic mycotoxins and are found in most plant products, mainly *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nominus*. When animals eat an aflatoxin-contaminated diet (AFB1), the toxin is metabolized in the liver and excreted as aflatoxin M1 in milk, urine, and feces. Aflatoxin has acute toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic impacts. To prevent aflatoxin M1, as one of the most important mycotoxins, from entering the human food chain through milk, its precursor, aflatoxin B1, should be prevented from entering the feed of dairy animals [4, 5]. Because this is currently very difficult and almost impossible to accomplish, the more immediate and practical action is to prevent the distribution and consumption of milk and contaminated products in amounts higher than the permissible limit of aflatoxin M1 in the community by measure the amount of the toxin in milk and dairy products. As with many countries, in Iran, regulations have been developed for the maximum permissible amount of aflatoxin M1 in milk and its products, according to which the maximum permissible amount of this mycotoxin in milk is considered to be 50 m m^{-1} [6]. A researcher [7] investigated the amount of aflatoxin M1 in milk and dry milk samples collected from markets in Jordan. The results showed that in 85% of the dry milk samples, the mean aflatoxin M1 concentration was higher than the maximum tolerance (25 ng kg^{-1}) accepted by the European Union and the United States. The study of Akhtar et al. [8] also showed that 30.76% of baby milk formulas distributed in Pakistani markets had aflatoxin M1 levels higher than the global standard.

The presence of some metal contaminants, especially cadmium and lead, facilitates entry into the food chain and thus increases the likelihood of their toxic effects on humans and animals. Many chemicals can be passed from the body's reserves and from the blood to mother breast milk [9]. There have been reports of serious susceptibilities in infants and children due to intake of food containing important heavy metals such as lead and cadmium, so that a study [10] and other study al. [11] have reported that infant vulnerability to these heavy metals are due to the underdevelopment of the renal system and the low tolerance level to them. Heavy metals can be present naturally in raw milk or raw materials, or can enter them through various ways, including production, processing, packaging, storage, supply, and even counterfeiting, or the products produced from them, including baby dry milk [12]. Akhtar et al. [8] investigated the milk powder of infants produced in Pakistan and also Bargellini et al. [13] studied the milk powder in the Italian market and showed that the toxicity of lead and cadmium metals was less than the standard set by Codex. Holstein cow milk has an average of 87.42% water, 12.77% dry matter, 3.5% fat, 3.1% protein, 4.8% lactose and 0.9% minerals and vitamins [1]. The composition of milk varies in cows of the same breed as well as different breeds. During the lactation season, different seasons and across geographical areas, there are differences in the production rate and composition of cow milk. Genetic factors, nutrition, environmental factors and lactation management are the most important effective factors on milk composition and quality [16]. Environmental temperature affects milk production and composition, and the breeding season affects milk and fat production and some chemical compounds in milk [14]. In a study in winter on the composition of dairy farms in Lordegan city of Chaharmahal and Bakhtiari province, Holstein cow milk contained an average of 87.42% water, 3.30% protein, 3.9% fat, and 57%. 12.1% of solids and had a density of 30.54. A researcher [15] studied the effect of lactation season on fat percentage, protein content, acidity and density of milk produced by Holstein cows and reported that lactation season had a significant effect on percentage of fat, protein percentage, acidity and density of milk produced. In our country, many milk price payment programs are still adjusted based on the amount of milk fat. The value of milk fat has now declined in many countries, and due to changes in consumption and market patterns, the amount of milk protein has become important as a parameter in determining the price of milk [3]. The nature and composition of milk have always been a topic of interest to researchers, and many factors affect the chemical quality and quantity of milk [1].

In order to protect the health of consumers in different countries, continuous monitoring of the quality of milk produced and the enactment of special laws are carried out in order to control the composition of milk. Therefore, the aim of this study was to investigate the amount**s** of heavy metals, aflatoxin M1 and physicochemical properties of milk used in infant milk powder produced by Shahrekord Pegah milk during September 2018 to March 2019.

MATERIALS AND METHODS

Sampling

This cross-sectional study was performed to evaluate the amount of heavy metals (lead and cadmium), aflatoxin M1 and physicochemical properties in 690 samples of the input milk to Pegah Shahrekord Infant Dry Milk Factory between September 2018 to March 2019.

The milk samples were collected in special sampling containers, and after recording the specifications, were transferred to the laboratory to record the quality parameters. Elimination of organic matter in the raw milk samples was performed using wet digestion method.

To this end, one mL of raw milk samples was homogenized separately with a digestive mixture [including concentrated nitric acid (65%) and oxygenated water (30%)] and heated for 5 hours at 130 °C. After the digestive mixture cooled down, its volume was reduced to 10 ml using deionized water [16].

Measurement of aflatoxin M1

First, the milk samples used to produce the infant dry milk were sampled according to the IDF 171: 1995 standard [8].

Then, the milk fat was isolated using centrifugation at 2000 rpm for 10 minutes, and passed through Whatman filter. Immunoaffinity columns were used to extract AFM1. Then AFM1 was removed from the column using pure acetonitrile.

The extracted solution was then concentrated and the amount of toxin was detected by high-performance liquid chromatography (HPLC) with a flowmetric detector.

To identify stock solution, 50 µl of stock solution, a calibrated solution containing 10 µL mL^{-1} AFM1 in chloroform was transferred into the vial using a Hamilton syringe and dried by nitrogen gas flow; then, the remaining 500 µl pure acetonitrile was dissolved and vortexed. The resulting solution was stored in a light proof vial under < -80 °C temperature.

The columns were allowed to reach ambient temperature and 50 ml of sample was added to the tank syringe and the sample was gently passed through the column at a rate of 2-3 ml per minute and after removing the syringe with distilled water, the column was washed and dried under air pressure, and AFM1 was removed from the column by passing 4 ml of pure acetonitrile at a constant flow. The acetonitrile exposed to the column for at least 60 seconds.

Then, the resulting solution was transferred to a conical tube and dried with dried nitrogen flow, and the residue was dissolved with 200 µl mobile phase (25% aqueous acetonitrile solution).

High Performance Liquid Chromatography (HPLC) Specifications

1) Column: C18 column, 5 µm, 4.6 mm, 250mm

2) Guard Column: Guard Column, Novapak C18 Waters

3) Fluorescence detector: Waters 474, Fluorescence detector

4) HPLC system, Waters 616 HPLC pump

• Waters column heater

• Waters 717 plus Auto Sampler

• Waters Detector Status

• Waters 600S Controller

• Waters Millennium software

Required materials include distilled water and nitrogen gas and HPLC ethanol, chloroform and acetonitrile and aflatoxin M1 (R-Biopharam France) 2475 Water fluorescence detector with 360 nm excitability and 4.6 mm 440 nm output and ODS column) 250 plus a protector column. The rate of the mobile phase that was 0.8 mL min^{-1} by a pump (Water 1525), and the calibration curve and the stability of the chromatographic system were also examined. The calibration curve was drawn and included 0.1, 0.2, 0.5, 1, 2, and 2.5 5, 7.5, 10 that were injected in ng and aflatoxin M1, and the calibration curve was plotted according to the area under curve.

200 microliters of the prepared solution was injected into the device. The area under curve and the placement in the calibration curve for the poison concentration were recorded in mg mL^{-1}.

Measurement of heavy metals lead and cadmium

In order to determine the concentration of lead and cadmium in the studied samples, atomic absorption spectrophotometer (Australia Varian AA 200) was used. Cathode lamps at wavelengths of 288.3 and 288.8 nm were used to read lead and cadmium, respectively. The device was calibrated with standard lead and cadmium solutions and a standard curve was drawn for each element. Furthermore, the recycling rate for the studied elements by the device was calculated using the reference chemical sample [NRCC sample prepared from (LUTS-1)] and applied separately in the measurement of the elements. Cadmium was 0.1 ppb and 0.02 ppb, respectively.

The concentration of the elements was calculated by considering the ratio of the data obtained from the device to the weight of the digested initial sample, and then concentrations of the elements in the samples were expressed as ppb. Each sample, including milk and dry milk, was tested in triplicate, and then the mean values were determined and to ensure the accuracy of the experiment, the recycling percentage was performed, and finally the recycling percentage for lead and cadmium in the samples was estimated to be 97.8% and 98.1.5%, respectively [12].

Measurement of physicochemical properties

Acidity measurement was performed according to standard 2852 based on the Dornic degree. Milk protein measurement was performed according to the standard 639 [17]. The density measurement was done according to Standard 638 [18]. Measurement of milk dry matter was performed using a hygrometer device in the chemical laboratory of Shahrekord Pegah Baby Milk Powder Factory [HR83-P (Halogen), Switzerland]. The percentage of milk fat was measured using the Gerber method (Gerber butyrometer) and in accordance with the Iranian National Standard No. 384.

Statistical analysis

For statistical analysis of data obtained from the analysis of milk samples, SAS software (2001) was used.

RESULTS

Lead and cadmium levels in input milks to the factory

Tables 1 and 2 show the amounts of lead and cadmium in Pegah milk. As can be seen in the results, the amount of cadmium and lead in the milk of Pegah Dry Milk Factory complies to the national standard of Iran. The Mean ± S.D. amount of cadmium and lead in input raw milk to the factory was 10.8±2.83 ppb and 12.52±1.78 ppb, respectively. The permissible limit of cadmium in liquid milk has been proposed to be 10 ppm according to the Codex standard. The authority states that the daily limit for cadmium intake is 10 ng kg^{-1}.bw.d [19].

The amount of aflatoxin M1 in input milk to Pegah factory. In the present study, the mean aflatoxin content in raw milk was 12.44±1.87 ppt (Table 2).

The experimental results showed that the studied milk contains an average of 11.95% of solids.

The effect of lactation season on density, protein, dry matter, fat and acidity is shown in Table 3. The results showed that the density of the produced milk was not affected by the season (P <0.05). Lactation season significantly altered milk protein production (P<0.05). Mean ± S.D. milk production protein was 2.94±0.082%. Lactation season did not have a significant effect on milk fat percentage (P<0.05). Mean ± S.D. percentage of milk fat produced was 3.27±0.18%. Lactation season also had a significant effect on the acidity of milk produced (P<0.05). Milk produced had the average acidity 14.6 (14.6±0.49). Lactation season did not have a significant effect on the percentage of dry matter in the produced milk (P<0.05). Mean ± S.D. dry matter of milk produced was obtained 11.59±0.31.

Table 1. Investigation of cadmium and lead levels (ppb) in input milk to Pegah Shahrekord factory.

Raw milk raw material	Number of samples	Mean ± SD	
		Pb	Cd
Raw milk	50	12.52±1.78	10.8±2.83

Table 2. The amount (ppt) of aflatoxin M1 in input milk to Shahrekord Pegah Factory

Raw milk raw material	Number of samples	Mean ± SD
Raw milk	50	12.44±1.87

Table 3. Investigation of chemical properties of delivered milk to Pegah Shahrekord factory

Compounds	Mean ± S.D.	The most	The least	P- value
Density (g cm^{3-1})	1.0299±0.0008	1.033	1.027	
Dry matter (g)	11.59±0.31	12.73	10.67	
Protein (g)	2.94±0.082	3.27	2.7	P <0.05
Fat (g)	3.27±0.18	3.7	2.8	
Acidity	14.6±0.49	16.2	13	

DISCUSSION

As can be seen in the results, the amount of cadmium and lead in the milk of Pegah Dry Milk Factory complies to the national standard of Iran. Various studies have been done across the world; for example, in the study of Shakerian and Karim [16], contamination of milk and some dairy products with lead and cadmium was investigated by atomic absorption spectrophotometry in Isfahan. The results showed that the mean lead concentration in raw milk was 0.245 ppm, in fat-free milk was 0.118 ppm and in cream was 0.292 ppm. Besides that, the mean concentration of cadmium in raw milk was 0.057 ppm, in fat-free milk 0.015 ppm, and in cream 0.031 ppm, which is higher than the results reported in the current study for input milk to the Pegah Factory. In a study conducted by Bonyadian et al. [3] on raw and pasteurized milk samples in Shahrekord, the mean cadmium and lead concentrations were 2.87 ppb and 60.72 ppb, respectively that are higher than the results of the present study.

Najarnezhad and Akbarabadi [20] reported the amount of lead and cadmium in cow's milk in Khorasan Razavi province, as being 12.9±0.6 µg kg^{-1} and 0.3±0.3 µg kg^{-1}, respectively. Gardener et al. [21] examined the concentrations of lead and cadmium in baby dry milk in the United States. Ninety-one samples of baby dry milk did not exceed the FDA's permissible lead intake, but 22% of the samples had over the recommended amounts, 23% had more cadmium than permissible amount, and 14% had more than the WHO tolerable amount of cadmium for 4-month children.

The potential risks of aflatoxin to humans due to the use of milk and dairy products have been demonstrated by several studies [7,8]. Its risks to human health, especially for liver cancer, due to AFM_1 in milk and dairy products are noticeable.

Rastogi [22] in India measured the AFM_1 levels of milk and baby milk products collected from supermarkets using competitive ELISA, and found a contamination of at least 87%, so that approximately 99% of samples had a contamination exceeding the permissible limit of the European Committee and the Food Codex (50 ng l^{-1}). However, 9% of the samples were reported to have above the standard limit (500 ng l^{-1}). Omar et al. [7] examined milk contamination with AFM_1 using ELISA. Their results showed that the contamination rate of milk samples with AFM1 was 9.71-288.68 ng/l. In a study, Yilmaz and Altinci [23] examined the prevalence and incidence of aflatoxin AFM_1 contamination in 104 milk samples in the Turkish city of sacaria by chromatography. The results showed that 61.5 of milk samples contained AFM_1. In 53 samples (50.96%), the AFM level was less than the detection limit. The amount of AFM_1 in 51 samples (49.04%) varied from 2.4 to 47.8 ng l^{-1} kg^{-1}. The AFM_1 level did not exceed the permissible limit according to Turkish regulations (50 ng kg^{-1}); however, this is a potential risk to the health of customers, especially children and infants.

Factors that affect the production of mycotoxins are divided into physical, chemical and biological. Physical factors include factors that affect the environmental status of fungal such as pneumonia, relative humidity and insect infestation. Chemical effects include the use of a variety of fungicides, and biological effects include the effects of other fungal species during *Aspergillus* growth [24]. The composition of the studied input milk to the factory is shown in Table 3. The mean value of milk protein in this study was estimated to be 2.94%. This value is higher than the amount reported by Bauman et al. [25]. The difference in milk protein content and milk fat percentage can be due to various factors such as genetics, type of nutrition and milk production [26].

Differences in dry matter may be due to race, nutrition, and management. The researcher reported that breast tissue, factors related to lactation and milk production, genetic factors, nutritional factors, environmental factors and lactation management are among the factors affecting the composition and quality of milk produced. Therefore, the high amount of solids in the studied milk can be related to one of the mentioned factors. Hosseini et al. [15] reported that season had a significant effect on the milk density of Holstein cows and reported the minimum and maximum density for spring and summer, respectively. Mlynek et al. [27] found that daily milk production has a significant effect on the content of milk components and body condition. The researchers also reported that the average fat and dry matter of domestic cow's milk was 3.25% and 12.82%, respectively [28], which corresponded to the composition of input milk to the factory. The samples of the milk had average density and acidity of 1.029 and 14.6, respectively. Auldist et al. [29] reported that the percentage of milk protein is affected by the amount of milk produced and the month of lactation, and reaches the lowest in summer and the highest in winter [4]. The percentage of milk protein produced by Holstein cows in the winter was reported to be 3.2%, which is higher than the amount obtained in the current study. Hosseini et al. [15] reported that the season had a significant effect on the acidity of Holstein cow's milk, so that the highest acidity was reported in winter milk and the lowest in summer.

Milk production and composition are affected by environmental factors, so changes between different seasons that are associated with changes in temperature, humidity, food resources and management factors can cause differences in milk production and composition. Microbial and chemical contamination of food is one of the nutritional problems [30-38]. The use of herbs and plant antioxidants [39-41] is one of the useful solutions to reduce the effects of heavy metals and harmful chemicals. According to the results of this study and also by comparing the average residual amount of toxic metals (lead and cadmium) and aflatoxin M1 in the studied samples with Codex standard which is normally followed in Iran, the average of the studied elements in raw milk was obtained below the standard limit. Therefore, all samples were within the permissible range in terms of cadmium, lead and aflatoxin. Due to the numerous side effects of these metals in infant dry milk, it is necessary to conduct studies in different parts of the country regarding the degree of contamination of powdered milk with these toxins and determining the amounts of heavy metals and aflatoxins; and in case of its contamination with these metals, necessary measures should be taken to reduce its contamination with these toxins. In general, it can be argued that the milk entering the Shahrekord Pegah baby milk powder factory is equal to the available reports for milk compounds in Iran and the world in terms of composition and quality characteristics, and the acidity of the raw milk entering the factory is affected by breastfeeding season, but the composition characteristics of milk have not changed substantially. Therefore, the lack of large changes in the two seasons of autumn and winter is associated with small changes between temperature, humidity, food resources and management factors. These large changes affect the composition characteristics of raw milk are effective on various factors in milk, the most important of which are genetic, nutritional, and environmental factors, lactation, mastitis and management. Therefore, it may be argued that in terms of raw milk composition, the conditions are favorable, and with more research and studies, this optimal state of milk can be improved with basic and logical methods.

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Conflict of interest

The authors declare no conflicts of interest.

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