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Research Article

Physicochemical Characteristics of Various Milk Samples

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Abstract: Milk is an important source of all basic nutrients required for mammals including human beings. The study was conducted to evaluate physiochemical quality of milk samples in selected dairy plant of Iran. Various physiochemical properties of milk were analyzed by Lactostar analyzer and compared to the related standards. Also another major concern of this study was to consider the presence of melamine in milk samples by high-performance liquid chromatography. The method was performed using a C8 column and detection at 240 nm. Total 38 different milk samples were analyzed. The results for milk sample analysis showed fat 2.17±0.05%, protein 2.89%±0.01%, lactose 4.37%±0.02%, total solid 10.66%±0.06%, and mineral of 0.22%±0.05% and in infant samples fat 23.38±4.25%, protein 24.18%±3.86%, lactose 37.33%±6.26%, total solid 91.32%±6.26%, and mineral of 16.17%±7.25%. To conclude the occurrence of melamine in infant milk samples and pasteurized milk samples collected from Tehran-Iran revealed that melamine was detected in none of the studied samples. These findings didn't show awareness among consumers level in Iran and may be helpful for the concerned governmental regulatory bodies to monitor the quality of the commercial milk products in the market.

Keywords: Melamine, dairy product, HPLC, milk

INTRODUCTION

Milk is an important source of all basic nutrients required for mammals including human beings [1]. It is ideal for microbial growth and the fresh milk easily deteriorates to become unsuitable for processing and human consumption [2]. The presence of food-borne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal [3]. Although, it is very difficult to assure high quality and desirable physicochemical properties of raw milk designed for processing, the quality of raw milk encompasses such milk characteristics as chemical composition, physical properties, microbiological and cytological quality, sensory properties, technological suitability and nutritive value [4]. Therefore nutritionally enriched milk and its products with enhanced biological potential and without health risks are generally demanded [5].

Melamine (2, 4, 6-triamino-1, 3, 5-triazine) is a kind of triazine analog with three amino groups, which is widely used in plastic engineering and agriculture as an important industrial material in the early 1950s [6]. Melamine is forbidden to be used as an additive in food or related ingredients [7]. Because of its high nitrogen content (66%) and low cost, melamine was illegally adulterated in food products in order to increase apparent protein content [6]. Melamine made headlines in September 2008 because it was found to be the contaminant responsible for the deaths of several infants and making many more sick [8]. Some manufacturers illegally used melamine as an adulterant to increase the apparent protein content. It is used as an adulterant in milk and milk products because it causes a false positive value in a protein content measurement [9, 10]. Because milk is used in many other products for human and animal consumption, melamine is now detected in other food products in some countries such as China [11]. Melamine is classified by the World Health Organization as not posing a health risk [12]. The U.S. Food and Drug Administration (FDA) derived a tolerable daily intake (TDI) for melamine of 0.5 mg/ kg body weight [13].

The study was conducted to evaluate various physiochemical properties of milk including its fat, total protein, total solid non fat, lactose, freezing point, and minerals of branded milk samples in selected dairy plant of Iran were analyzed and compared to the valid Standards. Moreover determinations of melamine content in collected samples were a major concern of this study.

MATERIAL AND METHODS

Chemical and Reagents

All chemicals were analytical grade and purchased from Merck (Darmstadt, Germany).

Samples

A total of 38 samples of commercial milk including 8 infant milk samples and 30 pasteurized milk samples were purchased from supermarkets in Tehran-Iran. All samples were stored and analyzed before their expiry date.

Lactostar automatic analyzer

Fats, protein, lactose, solids non fat, and freezing point in milk samples were evaluated by milk content automatic analyzer (LactoStar, Funke Gerber, Berlin, Germany) which is a quick and reliable method to determine the constituent of milk. The measure resolution is 0.01% and the repeatability less than 0.04%. The unit is calibrated with two reference milks. The measurement is based on combined thermo-optical procedure i.e. the milk sample (12 ml to 20 ml, adjustable) is pumped in measuring cells.

Liquid Chromatographic Analysis

Samples were analyzed by a Knauer HPLC (Germany) system which consisted of a pump (Maxi-Star K-1000, Knauer, Germany), a degasser, an automated injector, a column oven, and a UV detector. The system was controlled by EuroChrom 2000 software (Version 1.6, Knauer Co., Germany). Chromatographic conditions were evaluated and optimized in Eurospher-100 C8 column (5 μm, 4.6 × 250 mm). Mobile phase consisting of 92% (v/v) of citric acid (1.05 g) and also heptanes-1-sulfonic acid (0.9 g) in 500 ml of distilled water, with 8% acetonitril. The flow rate was set at 1 mL/min. The injection volume for all samples was 20 μL and the chromatographic detection was monitored at 240 nm. Melamine was identified according to retention times as a comparison with the related standard. The concentration melamine compound was measured from peak area according to calibration curves.

Method validation

Based on the ICH method [14], the detection limit (LOD) and quantitation limit (LOQ) were expressed as: $DL = 3.3\ \sigma / S$ and $QL = 10\ \sigma / S$, where σ is the standard deviation of the response and S is the slope of the calibration curve of the analyte. The estimate of σ was carried out by using the standard deviation of blank. In this study, blank samples were analyzed three times, and the magnitude of the analytical background response was measured. Then the standard deviation of responses was calculated. In order to verify the feasibility of the method, sample recovery was used by analyzing samples before and after the addition of known quantities of each compound.

Data analysis

All measurements were replicated three times to improve the reliability of the results. Data were analyzed using statistical program for social sciences, version 21 (IBM SPSS Inc., Chicago, USA), Data are expressed as mean ± SD.

RESULT AND DISCUSSION

Some factors such as fat, total protein, total solid non fat, lactose, freezing point, and minerals are important parameters in studying the physicochemical compositions and nutritional aspects of milk (Table 1).

Table 1: Physicochemical characteristics of various milk samples (%)

No	Fat%	Protein%	Lactose%	SNF%	%F.P	Minerals%
Milk Samples	2.17±0.05	2.89±0.01	4.37±0.02	10.66±0.06	-0.53±0.00	0.22±0.00
Infant Samples	23.38±4.25	24.18±3.86	37.33±6.26	91.32±15.20	0.45±0.08-	16.17±7.25

In current study the measured lactose content of milk samples was 4.37±0.02 %. It could be worthy to note that total solids represent the components that remain after the complete removal of water from milk. The concentration range of total solids was from 7.25% to 11.83% with average of 10.66±0.06% as given in Table 1. The level of mineral elements in the tested milk samples (0.22±0.05%) was also reported. The determined amount of fat materials in samples was 2.17±0.05%. Additionally, the amount of total protein was found to be in the range of 1.97% to 3.2% with the average of 2.89±0.01%. Lactose milk sugar is found only in the milk of mammals and is formed by a condensation reaction between carbon 1 of a galactose molecule and carbon 4 of a glucose molecule [15]. Lactose is a reducing sugar, because the aldehyde group

on the glucose molecule is not tied up in a glycosidic link [16]. The composition of milks in the present study was compared favorably with the composition of milk in northern Europe, which contained fat of 4.3%, total protein of 3.4%, lactose of 4.65%, and total solid of 13.3% [17]. The present study revealed lower mean values for lactose (%) than that reported by mentioned Europe reference. The lower lactose may be due to the effect of psychotrophic bacteria [18]. This result also agrees with that reported by El Zubeir *et al.* (2005) for raw milk. Further, Imran et al claimed that the buffalo milk contained the highest amount of lactose among all the tested milk samples [5]. Both fat and total solid non fat investigated of the samples were within the recommended values of Iran-standard (No: 93 and 2012) [19].

In chromatography analysis of melamine the limits of detection and quantification of melamine were 0.124 and 0.376 ppm, respectively. The precision of the HPLC method is expressed as recovery of spiked melamine standard in milk at concentrations of 0.25, 0.5, 1, 5, 10 ppm. The analysis was performed in replicates of three at each level. Concentrations in the samples were calculated based on the external standard calibration curves. The chromatograms of the spiked standard (10 ppm) are shown in Figures 1.

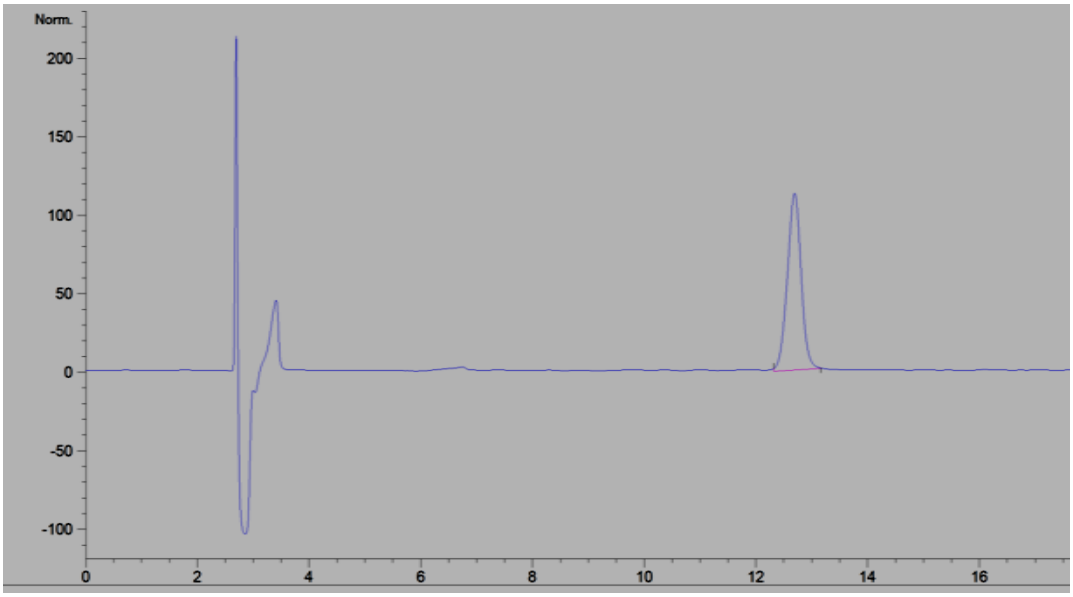

Fig-1: The chromatograms of the spiked melamine standard (10 ppm)

The recovery and reproducibility data are shown in Table 2.

Table 2:The recovery and reproducibility of studied samples

Concentration (ppm)	Area	Mean
10	938.45	940.91
	943.38	
5	458.95	456.84
	454.73	
1	100.96	101.59
	102.22	
0.5	51.55	51.59
	51.63	
0.25	27.82	27.24
	26.67	

According to the achieved results the occurrence of melamine in infant milk samples and pasteurized milk samples collected from different regions of Iran showed that melamine was detected in none of the samples with varying concentrations. In addition, due to validate data, the achieved results were verified by detection and identification process based on mass-HPLC. Consumption of foods containing these

low levels of melamine does not constitute a health risk for consumers.

Other studies also are in well agreement with this issue. In Filazi et [20] a simple, precise, accurate, and validated reverse-phase HPLC method was developed for the determination of melamine in pasteurized and UHT milk milk and dairy products including powdered infant formula, fruit yogurt, soft cheese, and milk powder purchased from major retailers in Turkey. Melamine was not found in infant formulas and pasteurized UHT milk, whereas 2% of cheese, 8% of milk powder, and 44% of yogurt samples contained melamine at the 121, 694 ± 146, and 294 ± 98 μg/kg levels, respectively. Same as our study, these findings were below the limits set by the Codex Alimentarius Commission and European Union legislation [21]. Analytical surveys performed during the 2007 pet food and 2008 milk product contamination episodes implicate the typical by-products of melamine synthesis and degradation as the agents most consistently detected with melamine that are also present at comparable levels [11]. In Mohamed et al study [22] samples were analyzed and determination for melamine. Melamine was detected in 28 out of 32 samples with varying concentrations [22]. In Vachirapatama et al study [23] the micellar electrokinetic chromatography was developed for the simultaneous determination of melamine which showed that melamine and cyanuric acid were found in UHT milk samples.

It would be a great interest if further investigations are to be carried out to examine other dairies such as cheese. Although no contamination was detected in the studied samples, the standard range of melamine in milk and other milk production could be seriously suggested since till now there isn't any reported limitation in Iranian standard for this element.

CONCLUSION

There have been problems with global national standards for regulating the use of melamine in terms of determining food additive types and quantitative limitations. Therefore it is necessary to revise standards and introduce a reliable quantitative method to control food quality. Quantitative analysis of milk properties resulting from this study has demonstrated that no manufacturers have used melamine to improve the appearance of their product against available standards.

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