Melamine levels in food products of animal origin in Iran

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SUMMARY
During recent years, an increasing number of infants have been sickened by dairy products contaminated with melamine. As a result, many countries have adopted additional testing requirements for food products of animal origin; however, Iran has not yet adopted regulations to control levels of melamine. The present study is to assess levels in poultry meat (50 samples), hen eggs (50 samples), ultra-filtered cheese (50 samples), and dairy cream (50 samples) from Iran. Results showed that melamine was detected in all of 200 samples that 90th percentile levels were 1.31, 1.41, 1.29 and 1.30 (mg/kg), respectively; determined by high-performance liquid chromatography was defined. The limit of quantitation (LOQ) was 0.2 μg/mL for this method. Results were within International Standards (FDA), confirming low health risks for these four products. Considering the sources of contamination, melamine levels in other food products should also be carefully monitored.

Keywords: Food of animal origin, Iran, Melamine, HPLC

RÉSUMÉ
Niveaux de mélamine dans les produits alimentaires d’origine animale en Iran
Au cours des dernières années, un nombre croissant de nourrissons ont été malades du fait de produits laitiers contaminés par la mélamine. En conséquence, de nombreux pays ont adopté des exigences supplémentaires en matière d’essais pour les produits alimentaires d’origine animale. Cependant, l’Iran n’a pas encore adopté de réglements pour contrôler ces niveaux. La présente étude vise à évaluer la mélamine dans la viande de volaille (50 échantillons), puis dans les œufs (50 échantillons), le fromage (50 échantillons) et la crème (50 échantillons) en provenance d’Iran. Les résultats ont montré que la mélamine a été détectée dans tous les 200 échantillons et que les niveaux du 90e percentile étaient respectivement de 1,31, 1,41, 1,29 et 1,30 (mg/kg). La limite de quantitation (LOQ) est de 0,2 µg/mL pour cette méthode. Les résultats sont conformes aux normes internationales (FDA), ce qui confirme que les risques pour la santé de ces quatre produits sont faibles. Compte tenu des sources de contamination, les niveaux de mélamine dans d’autres produits alimentaires devraient être surveillés de près.

Mots-clés : Aliments d’origine animale, Iran, Mélamine, HPLC

Introduction
Melamine contamination in food, especially in food and feed products of animal origin has become a public health incident [10]. Melamine (2,4,6-triamino-1,3,5-triazine, C₃H₆N₆) is a corollary of the charcoal industry [21]. It is a chemical compound with much industrial utilization, containing the production of plastic plates [20], coatings, commercial filters, cookery, trade smoothness, fertilizer, insecticide—adhesives [32], dishware, kitchenware, flame retardant materials [1] and glues [11]. This general use of melamine and its formatsives means that it has penetrated the environment and as a result, influences the human food line [1]. Whereas melamine contains 66% nitrogen, the deliberate addition of melamine to food is known to raise the apparent amount of protein [11, 18].

Melamine contamination has been reported in food products of animal origin such as milk and milk powder [3] and dairy products [32], meat and meat products, hen eggs, fish and fishery products [1, 4] and non-animal food products such as wheat gluten, rice protein concentrate and beverages [12, 19]. Unfortunately, melamine can result in the formation of insoluble melamine cyanurate crystals in kidneys thus causing renal failure, kidney stones and urinary problems [14].

Median lethal dose (LD50) of melamine was determined as 3.16 mg/kg body weight and 3.83 mg/kg body weight for male and females rodents, respectively [28]. The tolerable daily intake (TDI) of melamine in humans might be equal to 0.63 mg/kg body weight per day [13].

The percentage of melamine contamination varied widely in food in Asia, such as dairy products [4, 9, 16]. An increased incidence of renal failure in babies has been reported in China, which was believed to be associated with the ingestion of infant milk-based formula contaminated with melamine [13, 20]. It appeared that nitrogen-high melamine was added to raw cow’s milk to raise its outward protein mass [31, 32]. Many countries have set especially high standards for the maximum permissible melamine amounts in many food products [7, 10]. In the USA, the level of standard limits for...
MELAMINE LEVELS IN FOOD PRODUCTS OF ANIMAL ORIGIN

milk and dairy products was agreed at 2.5 mg/kg, while in Europe, the Food Safety Authority (FSA) has set the limit at 2.5 mg/kg for all products [6, 8]. These standard limits are considered using research laboratory survey on animals and it’s clear that the consequences change from animal model studies to human hazard assessment [21]. Iran still has not accepted legislation to control levels of melamine in many foods [20].

High performance liquid chromatography (HPLC) is one of the most accurate techniques for the detection of melamine in many foods [18, 22, 23, 25]. It is very important to establish amounts of melamine in milk products, some foods, animal feed and sediment in order to monitor food contamination and this has been the main consideration in studies related to the subject in recent years.

The objective of the present study is to assess amounts of melamine in food products animals’ origin from famous brands such as poultry meat, hen eggs, ultra-filtration cheese and dairy cream in all of the Iranian markets.

Materials and Methods

SAMPLING

A total of 200 samples from major and important brands that were high consumption in Iran that produce from industrialized food, 50 each, of poultry meat (only the muscle), hen eggs, ultra-filtration cheese, and dairy cream were purchased from major retailers that produced from Iran (during 2015-2016). Samples were immediately transported to the Food Hygiene Research Center Laboratory, Iran, in a cooler at 4°C with ice packs.

REAGENTS AND CHEMICALS

Melamine standard (95% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was obtained by distillation through a Milli Q water purification system (Millipore Ltd., Bedford, MA, USA). Methanol (MeOH, HPLC grade), acetonitrile (ACN, HPLC grade), concentrated formic acid (98-100%, reagent grade) and potassium dihydrogen phosphate (93%, HPLC grade) were purchased from Merck (Germany). All chemicals used in this study were reagents of the highest grade and used without further treatment.

PREPARATION OF STANDARD SOLUTIONS OF MELAMINE

A 1000 µg/mL melamine stock standard was prepared by accurately weighing 100 mg of melamine into a 100-ml volumetric flask. Melamine was dissolved with aqueous methanol (50%, v/v). The calibration curve was created by plotting peak parts in contradiction of the concentrations of melamine and was used to determine the concentration of melamine in all subsequent analysis. The linear range was determined to be between 62.5, 125, 250, 500, and 1000 µg/mL with an R² of 0.9585. The calibration curve confirms the validity of the method in determination of melamine.

EXTRACTION OF MELAMINE

One gram of each of the samples (poultry meat, hen eggs, ultra-filtration cheese and dairy cream) was separately transferred to 50 ml volumetric flask, then to each sample, a mixture of Acetonitrile: Buffer (8:92, v/v) was added. The tube was mixed for 1 min and sonicated for 30 min in an ultrasonic cleaning bath (DSA150, USA). The homogenate was centrifuged (kind Sigma 2-16 P22R, Germany) at 4500 rpm for 15 min, and the supernatant was filtered through a 0.45-µm syringe filter into a 2 mL vial and injected into the HPLC system.

EQUIPMENT AND CHROMATOGRAPHIC CONDITIONS

Melamine composition is determined by the HPLC Waters system equipped with 1000 pump from (Knauer, Germany) stainless steel filter, guard column Symmetry C18(5 µm, 3.9 × 20 mm), Symmetry C18 column (5 µm, 250 × 4.5 mm) was used through this study. Symmetry C18 column (5 µm, 250 × 4.5 mm) was used through this study. Waters column heater module with waters temperature control module, waters 486 tunable absorbance detector, Waters automated gradient controller associate with computer system using EZ-Chrom software was applied to run and control all the calculations for the instrument. The oven temperature was set up to 50°C. The injection loop was set up to 20 µL with Knauer injector (model 14163, Germany).

The concentration of the products was determined from the peak areas under the curve using EZ-Chrom Elite software for instrument control and data collection and processing. All solvents were filtered through a 0.45-µm Millipore filter before use and degassed in an ultrasonic bath. The HPLC system operated at a Mobile phase: Buffer 80:20 Acetonitrile, the flow rate of 1.3 mL/min, the injection volume was 20 µL with Knauer injector (model 14163, Germany). The Retention time (Rt) was 10 min, the pH 4.5. The UV detector was operated at a 240 nm and the external temperature control column oven was set at 40°C.

QUALITY CONTROL

The accuracy of the method was evaluated at three-concentration levels of melamine (0.3, 1 and 10 µg/mL) using 1 g of the samples. The percentage recovery of melamine in poultry meat, hen eggs, ultra-filtration cheese and dairy cream samples showed a recovery value larger than 97.2%, with some as high as to 99.9%. This method shows reliable recovery.

The limit of detection (LOD) and limit of quantification (LOQ) values are 0.1 µg/mL and 0.2 µg/mL, respectively. Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, Washington, USA) for analysis.
Results

The occurrence of melamine in poultry meat, hen eggs, ultra-filtration cheese and dairy cream samples produced from different geographic locations in Iran is presented in Table I. Results showed that melamine was detected in all of 200 samples, but with different concentrations. The lowest and highest concentrations of melamine were found to be 0.96 and 1.72 mg/kg in poultry meat, 0.96 and 1.98 mg/kg in hen eggs, 0.45 and 1.62 mg/kg in ultra-filtration cheese and 0.81 and 2.18 mg/kg in dairy cream, respectively (Table I). Figure 1 showed the results obtained, and depicts the chromatogram for melamine extraction from ultra-filtered cheese samples and HPLC analysis. The highest concentration of melamine in poultry meat, hen eggs and ultra-filtration cheese samples were 1.72 mg/kg, 1.98 mg/kg and 1.62 mg/kg respectively, from the central of Iran and the highest in dairy cream samples was 2.18 mg/kg from the south of Iran.

Discussion

The Iranian National Standards Organization (INSO) has not yet accepted legislation to control levels of melamine in food products of animal origin [20], therefore ours results were compared with levels of concern in many countries [16]. The U.S. Food and Drug Administration and many countries set 2.5 mg/kg as the concern level [8]. According to these result mentioned in Table I, all of the poultry meat, hen eggs, ultra-filtration cheese and dairy cream samples collected from different regions of Iran contained melamine levels lower than the Food and Drug Administration and European Commission recommended levels, and in many other countries [13]. In evaluation with the published concern levels for melamine in the some of countries [1, 26], totals of melamine in 100% the poultry meat, hen eggs, ultra-filtration cheese and dairy cream samples were calculated as not allowed. Results showed that melamine was detected at levels greater than 1 mg/kg compared with the control limit set in China in powdered infant formula (1 mg/kg) and lower than compared in other foods (2.5 mg/kg). This result is in agreement with other studies obtained by Deabes and El- Habib (2012) [4], Feng et al. (2012) [7], Filazi et al. (2012) [9] and Poorjafari et al. (2015) [16]. Because melamine was detected in all of the samples, this may be due to migration of melamine from the packaging material [24]. Melamine-formaldehyde containers [15] and resin dishes made of melamine formaldehyde resin [29], plastic plates [7] and coverings [10] on the ultra-filtration cheeses and dairy cream samples are used under hot and acidic conditions [31]. One of the most important origins is migration of melamine from pesticide substances [22]. Melamine is a metabolite of a pesticide called cyromazine, which is approved for application in a few countries. Cyromazine has also been used in animal farms, particularly as a larvicide additive in laying hen feed and fly control in cattle dung, harvests and spuds [2, 27]. Contamination can also occur from the environment, and in nitrogen supplements used in animal feeds [5]. The transfer of melamine from feed to meat and hen eggs and can be found as a metabolite and degradation product of veterinary medicine and fertilizers [30]. The residue of melamine from

![Figure 1: Chromatogram for identification of melamine positive ultra-filtered cheese sample](image)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean ± SD</th>
<th>Maximum</th>
<th>Minimum</th>
<th>50th percentile</th>
<th>90th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry meat</td>
<td>1.28±0.11</td>
<td>1.72</td>
<td>0.96</td>
<td>1.27</td>
<td>1.31</td>
</tr>
<tr>
<td>Hen eggs</td>
<td>1.30±0.17</td>
<td>1.98</td>
<td>0.96</td>
<td>1.30</td>
<td>1.61</td>
</tr>
<tr>
<td>Ultra-filtration</td>
<td>1.24 ± 0.17</td>
<td>1.62</td>
<td>0.45</td>
<td>1.25</td>
<td>1.29</td>
</tr>
<tr>
<td>Dairy cream</td>
<td>1.45 ± 0.31</td>
<td>2.18</td>
<td>0.81</td>
<td>1.23</td>
<td>1.30</td>
</tr>
</tbody>
</table>

Fifty samples of each food animal’s origins products

**Table I:** Levels of melamine (mg/kg) in food animal’s origins products collected from Iran.
animal feedstuff to food products of animal origin is an important topic for risk assessment determinations [32]. In two studies, conducted by Bermudez et al., [2] and Mariana et al., [15], melamine residue concentrations were found in broiler chickens’ muscle [24]. Also, Wang et al., [27] and Valat et al., [24] found melamine residue concentrations in hen eggs, which are in agreement with our results. To ensure the food supply is not affected by melamine adulterated products, the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) has taken proactive steps by increasing sampling of food products of animal origin for monitoring of melamine contents [6, 28].

We accept that the lower totals of melamine were the consequence of contamination during the preparation of ultra-filtration cheeses and dairy cream products and that the higher totals of melamine contents may be the consequence of intentional additions. The most important causes for the detection of melamine in all of the samples in different geographic locations in Iran may be due to milk and milk products such as cheese and cream which were manufactured using components prepared from melamine-contaminated milk.

According to previous study the addition of one gram of melamine to one Liter of raw milk falsely increases the protein content by 0.4%. When melamine is dissolved at the 25°C, 3.1 g of melamine can be liquefied in water without making precipitate, and protein content will incorrectly increase by 1.2%. This can approximately lead to an overestimation of the protein content in milk by 30% [11]. Dairy products, poultry meat and hen eggs are likely contaminated through animal feedstuff adulterated with melamine [17]. Furthermore, a melamine metabolite is an herbicide for use in the growth of many root vegetable and insecticides for use in animals, especially in the poultry industry [24, 27, 32].

In conclusion, food fraud using melamine adulteration is a growing reality in the marketplace which can be prevented using simple detection measures and important community monitoring [32].

Conclusion

Based on the results, melamine was present in all samples analyzed. Consumption of food products of animal origin containing these low levels of melamine does not constitute a health risk for adult consumers. However, monitoring of melamine residue is necessary in order to prevent excessive consumption of contaminated food products. It is necessary that maximum residue levels and tolerable daily intake values for melamine to be defined and monitored daily in Iranian food products. As a result, if melamine is identified in samples of milk and milk products, these food products of animal origin must be inhibited from the marketplace.

Conflict of interest

None.

Acknowledgments

The authors are thankful to Iranian National Standards Organization (INSO), Chaharmahal va Bakhtiari province and Islamic Azad University Shahrekor Branch for grants, that supported this study. The Grant Number is 388.

References


