



Assessment of arsenic contamination of milk and dairy products

Evaluación de la contaminación por arsénico de la leche y productos lácteos

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ABSTRACTS

Introduction: Milk and dairy products are nutritious and can play a significant role in a healthy diet. The safety of milk decreases with increasing concentration of arsenic. The Maximum Residue Limits of arsenic is 500 parts per billion (ppb).

Objectives: To evaluate the status of arsenic contamination of milk and dairy products produced and processed in some provinces and cities of Vietnam.

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Methods: A total of 367 samples were tested. Samples were digested before analysis to remove organic compounds, and the total arsenic content determined by atomic absorption spectrophotometry.

Results: The average concentrations of total arsenic in liquid milk were 139.32 ppb; in yogurt, 169.81 ppb; in cheese, 221.38 ppb; in milk cake, 232.80 ppb; and in milk powder, 35.43 ppb, respectively.

Conclusion: The arsenic concentrations in some samples are higher than the maximum permitted levels according to national regulations.

Keywords: arsenic; contamination; milk products; dairy products.

RESUMEN

Introducción: La leche y los productos lácteos son nutritivos y pueden desempeñar un papel importante en una dieta saludable. La seguridad de la leche disminuye con el aumento de la concentración de arsénico. Los límites máximos de residuos de arsénico son 500 ppb.

Objetivos: Evaluar el estado de contaminación por arsénico de la leche y los productos lácteos producidos y procesados en algunas provincias y ciudades de Vietnam.

Métodos: Se analizaron un total de 367 muestras. Las muestras se digirieron antes del análisis, para eliminar los compuestos orgánicos y se determinó el contenido total de arsénico mediante espectrofotometría de absorción atómica.

Resultados: Las concentraciones promedio de arsénico total en la leche líquida fueron 139,32 ppb; en el yogur, 169,81 ppb; en el queso, 221,38 ppb; en el pastel de leche, 232,80 ppb; y en la leche en polvo, 35,43 ppb, respectivamente.

Conclusión: Las concentraciones de arsénico en algunas muestras superan los niveles máximos permitidos según la normativa nacional.

Palabras clave: arsénico; contaminación; productos lácteos.

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INTRODUCTION

Milk and dairy products are part of a balanced diet because milk is food with a high nutritional value whose protein contains all the essential amino acids required by humans; and also supplies lipids with a high biological value. In Vietnam, milk has been included in the school food program to improve the stature of Vietnamese children, so there is great interest in the quality of milk. Milk and dairy products can be contaminated with heavy metals, making them hazardous to health. Arsenic (As) is a chemical contaminant deserving particular attention because of their toxicity and bioaccumulation in the food chain. Environmentalists have pointed out that As is considered toxic because it is harmful to human and animal health.⁽¹⁾

In Vietnam, the maximum concentrations of As in food have been prescribed by a national regulation (QCVN 8-2:2011/BYT) as 500 parts per billion (ppb), respectively, for liquid milk, yogurt, cheese, milk cake and milk powder.⁽²⁾

Heavy metals are known as one of the most toxic substances commonly present in food, and As is the most toxic to humans according to the ATSDR list (Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry) of 20 hazardous substances. Milk has been reported to be contaminated with As in some parts of the world. As is introduced in milk from human and other milk producing animals, by the intake of arsenic contaminated water or through the animal food.⁽³⁾ Heavy-metal-contaminated soils impose various adverse effects on groundwater and plant growth. Humans and animals, after eating polluted food, will be contaminated with heavy metals and can be transferred to milk. Therefore, the concentration of heavy metals in livestock and milk can be high and this is the cause of toxicity to humans.⁽⁴⁾

Contamination with heavy metals can also be caused by milking equipment, milk storage methods, and the habitat.⁽⁵⁾ A 2009 study demonstrated an association between As concentrations in fermented camel milk and animal feed.⁽⁶⁾ In addition, when cows, sheep, goats and camels are exposed to high concentrations of heavy metals, such as As, this will be present in their milk.⁽⁷⁾

When assessing the health risks to humans, As is considered being more unusual than other metals because it is present in a wide variety of chemicals and is toxic to humans.⁽⁸⁾ Humans are exposed to this



metalloid in a variety of chemical forms both inorganic and organic, but the organic form is highly stable and non-toxic.⁽⁹⁾ Long-term exposure to inorganic arsenic through inhalation, drinking water, or ingestion is one of the leading causes of skin cancer, lung cancer, and urinary bladder cancer.⁽¹⁰⁾ The above conclusions are mainly based on chronic exposure. Therefore, the presence of heavy metals in milk is of particular concern because milk is widely consumed not only in children but also in adults.

In Vietnam, the current situation on the level of contamination of cow's and goat's milk and dairy products by heavy metals has not yet been studied. Therefore, the present study aims to evaluate the level of As in liquid milk, yogurt, cheese, milk cake, and milk powder.

METHODS

Research location

Samples of milk and dairy products were collected in Hanoi, Hai Phong, Da Nang, Buon Ma Thuot, Ho Chi Minh City and Can Tho, Vietnam.

Heavy metals in samples were analyzed in the laboratory of the Department of Military Hygiene, Vietnam Military Medical University.

Chemicals

Standard solutions of As (1000 ppm) were purchased from Merck (Darmstadt, Germany). Acid sulfuric (H_2SO_4 98 %), acid nitric (HNO_3 65 %), sodium borohydride (NaBH_4), hydrochloric (HCl 37 %), potassium iodide (KI), ascorbic acid, hydrogen peroxide (H_2O_2), perchloric acid (HClO_4 70 %) were also purchased from Merck. All the chemical solutions were freshly prepared each day. The standard curve solutions were diluted with HNO_3 (1 %).

Sampling

All 367 milk samples were collected between July 2019 and June 2020. They were purchased at stores, markets, and supermarkets in Hanoi, Hai Phong, Danang, Buon Ma Thuot, Ho Chi Minh City and Can Tho. Ba Vi, Moc Chau and Bien Hoa, were visited to obtain samples of cow's and goat's milk from dairy companies, and also milk samples from households. After collection, the samples were stored at between



0 °C and 4 °C in sample storage boxes, then immediately sent to the laboratory of Department of Military Hygiene, Vietnam Military Medical University, then stored at – 20 °C until analysis.

Digestion of milk

For determination of total As content, the samples of milk and dairy products (1 mL or 1 g) were digested with HNO₃, H₂SO₄ and HClO₄ mixed together in the ratio of 10:1:1 with 5 mL H₂O₂ in the Multiwave PRO microwave reaction system (Anton Paar GmbH, Graz, Austria). First, the milk samples were placed into Teflon tubes, then the digestion mixture of HNO₃, H₂SO₄, HClO₄, H₂O₂ was added. After gentle shaking for 20 minutes to allow the digestion reaction to occur, the Teflon tubes were placed in the Multiwave PRO to complete the digestion of the samples.⁽¹¹⁾

The temperature of the Multiwave PRO was programmed as follows: heating to 170 °C for 5 minutes and hold for 10 minutes; increase the temperature to 200 °C and hold for 15 min, then cool to room temperature.

After digesting the milk, the sample solution should be transparent and free of residues and lipids. After digestion, the samples were diluted before analysis with HNO₃ (1 %) to a total volume of 25 mL. The dilution factor for As was therefore 25.

Preparation for measurement of total arsenic

Arsenic in milk samples includes divalent and trivalent. In order to quantify total As, it is necessary reduce it completely to one form. Similarly, the sensitivity varies with the trivalent or pentavalent state of arsenic. It is necessary to unify the arsenic in the sample to trivalent state beforehand. For this purpose, HCl, KI and ascorbic acid were used as a reducing agent. Sample Pre-treatment for reduction of As: 10 mL of diluted sample after digesting the milk, added 3 mL HCl (1 mol/L), 2 mL KI (20 %) and 0.4 mL ascorbic acid (10 %) then made up to 20 mL with HNO₃ (1 %). This solution was then reacted with NaBH₄ and quantified using an atomic absorption spectrophotometer. The concentration of the analyte was calculated from the absorbance peak. The results displayed are the average concentration of the 5 measurements.

Analysis of heavy metals

Atomic absorption spectrophotometry (AAS) (Model AZ3000, Hitachi Ltd., Tokyo, Japan) was used to quantify the heavy metals. As using Hydride Vapor Atomic Absorption Spectrometry at wavelengths



193.7 nm. The limits of detection (LOD) of arsenic 0.1 ppb. A standard curve was prepared using at least 5 points for each element.

Quality control

The blank sample method was used to control the quality of analytes using 2 blank samples: the first was prepared at the same time as the digestion of the milk sample, to check for contamination; and the second was prepared when creating the standard curve to check for heavy metal contamination from the chemicals prepared for analysis.

The standard curves for all analytical indicators were constructed using at least 5 different concentrations; the minimum value being set as the LOD of the instrument. The standard concentrations for As were 0; 0.1; 1; 10; 50 ppb. The correlation coefficient of 99.99 % indicated a highly linear relationship over the concentration range being studied.

To check the accuracy of the method, the percentage recoveries were analyzed and quantified (table 1). The recovery coefficients for As in the milk samples after adding the spiked sample were calculated, ranging from 84.00 % – 94.00 %. The values of these recovery coefficients indicated that the method was highly accurate and could be used for the analysis of heavy metals.

Table 1 - The percentage recoveries of As of the analytical method

Sample	As		
	1	10	20
Added (µg/L)	1	10	20
Found (µg/L)	0.84	9.11	18.80
Recovery (%)	84.00	91.10	94.00

Data analysis

The concentrations of heavy metals were calculated as means ± standard deviation (SD) based on 5 replicate measurements of As. The data were analyzed by ANOVA. All data were analyzed using SPSS (version 19.0, IBM Corp., Armonk, NY, USA).



RESULTS

Levels of As contamination

Table 2 shows the minimum, maximum, and the average concentration of arsenic in milk and dairy products. While the average concentration of arsenic in milk cake was the highest, in milk powder had the smallest value, with results of 232.80 and 34.43 ppb, respectively. Likewise, the second largest value for cheese was 221.38 ppb, followed by yogurt with 169.81 ppb and liquid milk with 139.32 ppb. Compared to the maximum permissible limit, the proportion of cheese was greater than milk cake with results of 19.00 % and 11.40 % respectively. The yogurt excess percentage was 6.3 %, which was almost double that of liquid milk with a result of 3.5 %. The research showed that all powdered milk samples conformed to this regulation.

Table 2 - Concentration of total As (ppb) and percentage exceeding the maximum permitted levels (QVCN) in samples of milk and dairy products on sale in Vietnam

Product	n	Min	Content Max	Mean ± SD	Percentage exceeding QCVN Limit (%)	QCVN Limit (µg/kg or µg/L)
Liquid milk	114	0	672	139.32 ± 140.91	3.50	500
Yogurt	158	0	842	169.81 ± 163.78	6.3	500
Cheese	21	0	595	221.38 ± 209.62	19.00	500
Milk cake	44	0	628	232.80 ± 191.87	11.40	500
Milk powder	30	0	208	34.43 ± 57.47	0	500

Comparison of levels of As contamination between different Vietnamese milk and dairy products

Table 3 shows the average concentration of As in milk samples taken in the study area. The average concentrations of As in milk and dairy products in descending order were milk cake > cheese > yogurt > liquid milk > powdered milk. Specifically, more than half of the milk powder samples had arsenic concentrations below the LOD of the instrument, followed by milk cake with a result of 11.36 %. Arsenic concentrations were less than LOD in 10.13 % of yogurt samples, 9.52 % of cheese samples and 8.77 % of liquid milk samples. In addition, while liquid milk had the largest percentage of



As contaminated samples at 91.22 %, the opposite result was for powdered milk with 46.44 %. In contrast, the percentage of samples contaminated with As of cheese was 90.49 %, slightly higher than that of yogurt and milk cake, with results of 89.87 % and 88.63 %, respectively.

Table 3 – Average concentrations of As in milk and dairy products

Product	Liquid milk	Yogurt	Cheese	Milk cake	Milk powder
n	114	158	21	44	30
Below LOD	10	16	2	5	16
% Samples below LOD	8.77	10.13	9.52	11.36	53.33
Number contaminated	104	142	19	39	14
Percentage contaminated	91.23	89.87	90.48	88.64	46.67
Meas ± SD (ppb)	139.32 ± 140.91	169.81 ± 163.78	221.38 ± 209.62	232.80 ± 191.87	34.43 ± 57.47

Conformity of levels of As contamination of Vietnamese milk and dairy products to national regulations

The average concentration of arsenic in milk and dairy products was 159.77 ppb. The results in table 4 clearly indicated that the arsenic concentrations in 49 samples of milk and dairy product were below the LOD of the instrument, however the presence of As in milk was found in the research. The results clearly showed that most milk samples were contaminated with As (86.65 %) and about 6 % exceeded the maximum permitted levels. Samples with As concentrations exceeding the allowable standard were yogurt and liquid milk samples, sampled at small companies and households in the North of Vietnam, with yogurt samples having a concentration of 842 ppb.



Table 4 - Concentration of As in milk and dairy products on sale in Vietnam compared with the maximum permitted levels

Heavy metal	As
Min (ppb)	0
Max (ppb)	842
$\bar{X} \pm SD$ (ppb)	159,77 \pm 164,15
Number contaminated	318
Percentage contaminated	86.65
Percentage exceeding QCVN Limit	6.27

DISCUSSION

Data on the levels of As contaminating milk in Vietnam are still limited, therefore, the results of the present study when comparing with those from other regions of the world, shows that were similar to those of *Castro-González et al.*⁽¹²⁾ The author *Licata P et al.*⁽¹³⁾ evaluated the concentration of As in goat milk and sheep milk and was much higher than the present study. *Khan N et al.*⁽¹⁴⁾ pointed out the level of As contamination in skim milk, drinking yogurt, mixed yogurt and are much lower than this study.⁽¹⁴⁾ The result in the powdered milk of *Ibrahim AS et al.*⁽¹⁵⁾ in Egypt was much higher than the present analysis;⁽¹⁵⁾ and they also analyzed the As concentration in dairy products as 214 \pm 8 ppb; this result is similar to milk cake samples presented.⁽¹⁵⁾

The differences between the results of the present study and other studies were probably caused by different levels of pollution in each region. As in emissions, waste and wastewater in industrial zones, and in the soil, water can contaminate the food consumed by animals, which, in turn, contaminates their milk and those consuming it. When the milk samples were collected at the dairy companies and households raising cows, there was no information on the level of As contamination of the animal food and drinking water, or in the soil, or water and air pollution where the grass was grown. In addition, the present study did not collect information on the level of As contaminating the water, air, soil and animal feed and drinking water on the dairy farms. Therefore, growing grass in an unpolluted area and filtration system to remove As in animal drinking water would play an important role in reducing the levels of As contaminating milk.



In addition, raw milk that is not polluted with heavy metals can be contaminated during production, storage and packaging.⁽¹⁶⁾ The average concentration of AS in cheese in this study was 221.38 ppb, of which, 4 samples exceeded the maximum permissible concentration. Samples with high concentrations may be due to air pollution in the cheese production area. Most of the liquid milk samples were contaminated with As and the samples exceeding the maximum permissible levels, had an average concentration of 139.32 ppb. High levels of As in milk samples may be due to the use of veterinary drugs to kill tapeworms in cattle, and chemicals use to disinfect the environment for livestock, all containing As compounds).⁽¹⁷⁾ Samples with high As concentrations also could be explained by the fact that the cattle farm was raised near an industrial area or near a traffic route.

Drinking water is also one of the main causes of As contamination in livestock because the daily water intake of animals can be up to 75 L. As can be accumulated in animals and be transferred to milk.⁽¹⁸⁾ In Vietnam, due to geological structure, As is present in soil, surface water and underground water; and there are differences in content in different regions. Research by *Ngoc NTM et al.*⁽¹⁹⁾ reports the average concentration of As in surface water and well water, and it was 190 and 60 ppb, respectively. More than two thirds of samples were higher than the maximum permissible levels of Vietnam. Cattle farms use ground water and surface water as the main source of water for livestock. In the present study there were 23 samples of milk and dairy products with As content exceeding the maximum permissible levels, this result may be due to cattle raised in areas with high concentrations of As in water, animal food, milk preservation process or polluted environment.

Many authors have studied the human health risk assessment data for As. As in the organic form, monomethylarsenic (MMA), dimethylarsenic (DMA), arsenobetaine and arsenocholine, were observed to be relatively non-toxic compared with inorganic As.

Inorganic As (Arsenic III) and (Arsenic V) are classified as “group 1 carcinogens” by the Agency for Research on Cancer (IARC).⁽²⁰⁾ Previous data have reported As exposure causing systemic fatigue, cardiac arrhythmias, bruising, and neurasthenia. Other non-cancerous health effects may include Blackfoot disease, hyperpigmentation, and hyperkeratosis. Long-term exposure to inorganic As increases the risk of skin cancer, liver cancer, urinary bladder cancer and lung cancer.⁽²¹⁾



Another reference value for As levels in milk is the Codex standard (Codex Alimentarius Commission), most recently revised in 2019. FAO/WHO recommends a TDI (Total Daily Intake) for As in food, drinking water and air of 3 µg/kg of body weight/day.⁽²²⁾ This means that a daily intake for 60-kg persons of less than 180 µg would not affect their health. Judging by the As content found in this study, some samples were above or below the maximum allowed by Codex standards.

The average concentration of As in liquid milk, yogurt, and powdered milk in this study was 139.32; 169.81 and 34.43 ppb, respectively. This indicates that if only consuming liquid milk, yogurt or milk powder contaminated with As, it did not affect health. However, milk is not the only source of As contamination, but also through daily food and drinking water. Hence, the health effects of liquid milk, yogurt, and powdered milk cannot be concluded. The mean concentrations of As in cheese and milk cake were 221.38 and 232.8 ppb, respectively, which was greater than the Codex maximum allowable recommendation. Therefore, samples of cheese and milk cake with As content higher than the maximum allowed by Vietnamese and Codex standards need attention because they pose a danger to consumers' health. In addition, it is necessary to assess As exposure in food, water, and the atmospheric environment to assess the health risk to consumers.

This is the first study to assess the current state of heavy metal contamination in milk and dairy products in Vietnam, based on 367 samples. One research limitation was that it could not assess the health risks of other heavy metals. Further studies are therefore needed to investigate the Food Ingestion Rate (FIR) per day, the Target Hazard Quotient (THQ) and the Hazard Index (HI), and the effect of heavy metals in milk on human health.

The arsenic concentrations in some samples are higher than the maximum permitted levels according to national regulations.

Therefore, control measures must be enforced to reduce these sources of contamination. Further research should examine the causes of arsenic contamination in milk, such as environmental pollution in livestock farms, animal feed and drinking water sources, there by suggesting measures to reduce arsenic concentration in milk.



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