Environmental Health Engineering and Management Journal 2023, 10(2), 191–196
http://ehemj.com
doi 10.34172/EHEM.2023.21

Environmental Health Engineering and Management Journal

Original Article

Environmental Health Engineering and Management

Open Access Publish Free

Comparison of three digestion methods for determination of lead and cadmium in milk and dairy products

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Abstract

Background: Toxic metals enter the human food chain through water, soil, and plants. High consumption of dairy products makes it necessary to measure their concentrations in milk and its products.

Methods: In this study, four samples of dairy products, including milk, dough, yogurt, and cream were selected. They were spiked with concentrations of 0, 20, 40, and 60 µg/kg of lead (Pb) and cadmium (Cd) separately. In all samples, the concentration of these metals was determined using graphite furnace atomic absorption spectroscopy (GFAAS) after microwave, wet, and dry ashing digestion methods. To select the best digestion method, recovery percentage, linearity of increasing concentrations, relative standard deviation (RSD), and the limit of detection were used.

Results: According to the results, the RSD of all measurements was less than 5%. The instrument detection limit for Pb and Cd were 0.188 and 0.157 µg/L, respectively. The recovery efficiency of all digested samples by three methods was between 75.7% and 120%. According to the linearity index and R^2, the microwave digestion method with 90 to 110% efficiency was the best for Pb-spiked samples, and the dry digestion method was the best for Cd-spiked samples.

Conclusion: Considering all indexes, microwave digestion was the best method for Pb and Cd samples.

Keywords: Milk, Cadmium, Lead, Digestion, Spectrophotometry

Citation: Mehrasebi MR, Peyda M, Feizolahi R, Taromi A, Homayuni P, Fathi S, et al. Comparison of three digestion methods for determination of lead and cadmium in milk and dairy products. Environmental Health Engineering and Management Journal 2023; 10(2): 191–196. doi: 10.34172/EHEM.2023.21.

Article History:
Received: 5 July 2022
Accepted: 28 November 2022
ePublished: 27 March 2023

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Introduction

Milk and dairy products are one of the most consumed foods among children and adults (1). More than 6 billion people worldwide consume milk and dairy products. Dairy products have a special place in the food basket of families and are a rich source of calcium, protein, phosphorus, potassium, magnesium, and vitamins A, B2, B6, and B12 (2). The main constituents of milk are water, fat, and non-fat solids. The last part contains milk proteins such as casein, albumin, and globulin. It also consists of lactose, lactic acid, citric acid, and minerals (3). Lactic acid bacteria convert lactose to lactic acid through fermentation to produce yogurt. High-fat yogurt is diluted with water, excess fat is separated as butter, and the rest is consumed as dough. The cream is a fat-rich part of milk (4), this complex composition causes various challenges in analyzing toxic metals in dairy products. Today, with the industrialization of cities, human beings are more exposed to toxic metals than ever before. These metals are significant environmental pollutants that can be absorbed through the skin, respiration, and digestive tract. Their adverse effects on other pollutants can be related to their non-degradability and their accumulation in the body of living organisms. Heavy metals can pollute the air through natural and human resources (5,6). They enter water and soil after the deposition of total suspended particles (TSP) (7). The high amounts of heavy metals in water and soil are due to the lack of control over agricultural fertilizers, incineration of solid waste, and poor management of industrial waste. Heavy metals enter plants and the food chain through water and soil and enter the human body. Due to the high consumption of dairy products and meat in the diet, high amounts of these compounds can easily accumulate in the human body (8). Using phosphate fertilizers and their residues in plant products increases cadmium (Cd) concentration in agricultural lands, which can be a potential danger for humans (9). Plant roots easily absorb Cd metal. Its plant toxicity is several times higher than other toxic metals (10). This metal accumulates in living tissues with a long half-life of about 25-30 years. Epidemiological data suggest that Cd is a risk factor for osteoporosis. The liver and kidneys are susceptible to the

toxic effects of this metal, and exposure to occupational and environmental Cd may cause a variety of cancers, including breast, lung, prostate, pancreatic, and kidney cancers. (11,12). On the other hand, lead (Pb) is used to coat cables, paints, and stainless materials. Its widespread distribution in the environment due to industrial and mining activities causes increasing pollution of plants, animals, and humans. Children are more sensitive to Pb, and the rate of its absorption in children has been many times higher than in adults because the formation of the physical structure and mental development takes place in the primary years of life (13). Studies have shown that Pb can decrease IQ levels in children (14). Pb poisoning often occurs chronically. In this type of poisoning, complications such as weight loss, paleness, hand paralysis, memory loss, dizziness, decreased vision, depression, and irritability due to encephalopathy appear.

Pb poisoning has also been observed in herds of cattle, sheep, and goats in the areas, which are contiguous to Pb mines or sewage containing Pb that enters agricultural lands (15). In the world, especially in areas prone to soil and plant contamination with heavy metals, the amount of these metals is measured in food products (16-22), water (23), cosmetic products (24) and other materials and air (5).

To determine the concentration of heavy metals, atomic absorption spectrometry (A.A) and *inductively* coupled plasma are used, and chemical digestion is performed to prepare milk samples and dairy products. Due to the complexity of the milk composition and dairy products, the digestion method can separate all the metals in this composition. Several researchers use a particular digestion method. Microwave (13,25), wet ashing (13,26), and dry ashing digestion methods (27-29) have been widely reported. Some reports include the quality control indexes such as recovery percent of the used methods. However, in most studies, no quality control information and the reason for choosing the digestion used method have been provided. In this study, microwave, wet and dry ashing digestion methods were compared in terms of measuring Pb and Cd in milk and dairy products using quality control indexes. Furthermore, the most effective digestion method was determined for milk, dough, yogurt, and cream samples.

Material and Methods

Reagents and chemicals

Nitric acid (HNO_3) (65%) and hydrogen peroxide (H_2O_2) (30%) (Merck, Darmstadt, Germany) were analytical grades. All solutions were made with ultrapure water (0.055 μS/cm^2). Calibration solutions of Cd and Pb were prepared by diluting the standard stock solutions of 1000 mg/L purchased from Merck company (Darmstadt, Germany). The pipettes were class A. All glassware were soaked in 5% (v/v) HNO_3 for 24 hours and washed with ultra-pure water before use. The samples were shacked and homogenized with shakers before and after spiking.

Measurement of Pb and Cd

In this research, an atomic absorption spectrophotometer (VARIAN-240) equipped with a graphite furnace was used. High purity argon gas (99.99%) was used as the carrier gas. The measured wavelengths of Pb and Cd were 283.3 and 228.8 nm, respectively. Gain percentages were 30 and 42, and the correlation coefficient (R^2) for drawing standard curves for Pb and Cd were 0.998 and 0.999, respectively.

Digestion procedures

Dry Ashing method

10 cc of the sample was poured into a porcelain crucible and placed on a boiling bain-marie (100°C) until it dried completely. The sample was transferred to the oven at 103°C for 24 hours, and after drying, it was placed in the GTA-1200 electric oven for 4 hours at 500°C until a white or grey ash residue was obtained. The residue was cooled to room temperature and dissolved in 10 cc of HNO_3. The solution was transferred to a 50-mL volumetric flask and made up to a volume with 3% HNO_3. The control solutions were digested similarly (21,30).

Wet Ashing method

0.5 cc of Pb and Cd spiked samples were poured into a Falcon tube, and 5 cc of HNO_3 was added. The sample was placed in a water bath for 6 hours to obtain a clear brown solution. Next, the sample was passed through a paper filter, transferred to a 50-ml volumetric flask, and made up to a volume with 3% HNO_3. The control solutions were digested in the same way (31,32).

Microwave method

Two milliliters of milk, dough, yogurt, and 1 g of cream were poured into a polytetrafluoroethylene (PTFE) container, then, 8 mL of 65% HNO_3 and 1 mL of 30% H_2O_2 were added to the PTFE container, and the lid was closed. Samples were placed on the microwave plate (SINEO model MSD-10) and digested according to the device method shown in Tables 1 and 2. At the end of the digestion process, the dishes were cooled in a 20-minute ventilation step. The residue was transferred to a 25-mL volumetric flask and made up with 3% HNO_3. For cream samples, first 10 ml of 65% HNO_3 was added. Then, 3 mL of HNO_3 and 1 mL of H_2O_2 were added after 30 minutes and digested according to Table 2. Control solutions were digested in the same way (33,34).

Preparation and spiking of samples

Milk, dough, yogurt, and cream samples were spiked with 0, 20, 40, and 60 μg/L of Pb and Cd (with three repetitions) and stirred on a shaker for 2 hours. Then,

Table 1. Temperature program of microwave for milk, yogurt, and dough

Step (N)	Temp (°C)	Time (min)	Power of single vessel (W)
1	130	10	400
2	150	5	400
3	180	10	400

Table 2. Temperature program of microwave for cream

Step (N)	Temp (°C)	Time (min)	Power of single vessel (W)
1	130	10	400
2	150	5	400
3	180	5	400
4	200	10	400

all samples were digested by microwave, wet and dry ash digestions. Pb and Cd concentrations were measured with graphite furnace atomic absorption spectroscopy (GFAAS). To draw the calibration curve of the device, the recommended concentrations of GFAAS device were used and with relative standard deviation (RSD) less than 5%, the standard curves of Pb and Cd were prepared.

Validation of the methods

In this study, validation indexes of recovery (accuracy), RSD and linearity (precision), limit of detection and limit of quantification were evaluated (35,36).

The linearity of the measured concentrations of the incrementally spiked samples indicates the accuracy of the results. To determine the linearity of the results, linear regression and regression coefficient (R^2) were used.

To determine the precision of the methods, the method RSD was calculated. Each sample was digested three times, and after measuring of metals, the RSD was calculated using the following formula:

$$\%\text{RSD} = \frac{100 \times SD}{\bar{X}} \quad (1)$$

Where $\bar{X}$ is the mean of the data and SD is the standard deviation.

Also, the RSD of the device was presented by the device automatically for measuring the calibration solutions and samples.

To determine the accuracy of the results, recovery in spiked samples was calculated using the following equations:

$$\%\text{Recovery} = \frac{Cm - Co}{Cs} \quad (2)$$

Where C_m is the concentration measured in spike samples, C_s is the concentration of spike samples, and C_0 is the initial concentration of the samples.

Before measuring by GFAAS, the calibration curve was drawn with the concentrations shown by the device. The device determined the RSD of all concentrations.

The value of the instrument detection limit was obtained. To determine the limit of detection (LOD), the blank sample was analyzed seven times, and then, the LOD was calculated using the following equation:

$$\text{LOD} = \text{SD} \times \text{T} \quad (3)$$

The standard deviation (SD) of the adsorption results was calculated, and with a degree of freedom of 6 and a confidence interval of 99%, T values equal to 3.143 were obtained from the t-test table.

The limit of quantitation (LOQ) was calculated through the following equations:

$$\text{LOQ} = \text{LOD} \times 3 \quad (4)$$

Results

In the beginning of analysis, the digested samples, 2 calibration curves were prepared for measuring Pb and Cd concentrations. After injection of each 50 samples, a new calibration curve was prepared. Each standard solution was injected 3 times. The RSD values were less than 5% and the R^2 was more than 0.998 in all cases.

The digested spiked samples were injected into the GF-AAS instrument and after measurement of Pb and Cd concentrations, the RSD and recovery percent were calculated and the linearity of the responses of the instrument against increasing concentrations was evaluated by regression method. The results are shown in Table 3.

The LOD values for Pb and Cd were 0.188 and 0.157 µg/L, respectively. By tripling the mentioned values, LOQ values for Pb and Cd were obtained 0.564 and 0.471 µg/L, respectively.

Discussion

The minimum concentration for drawing the calibration curve was 5 times more than the LOD values; therefore, the obtained concentrations could be analyzed with high confidence. RSD values for all samples containing Pb and Cd were less than 5%, indicating the measurements' accuracy. This study considered the optimal recovery value between 90 and 110%. According to the recovery values and R^2 values presented in Table 3, the acceptable method for digesting samples of dairy products can be summarized as follows.

For measuring Pb in all dairy product samples, the microwave digestion method had a good performance with recoveries between 102–109% and acceptable R^2. Therefore, it is the best method for digestion of dairy product samples for Pb analysis. For Cd measurement, all three digestion methods were acceptable, but considering linearity index, microwave methods and wet ashing digestion showed better results

Table 3. % Recovery, regressions equation, R^2, and RSD for three digestion methods of Pb and Cd

		Samples	Mean of % recovery	Regressions equation	Regression coefficient (R^2)	Method RSD
Microwave Digestion	Pb	Milk	102.6	y = 1.185x + 3.7	R^2 = 0.9688	< 5%
		Dough	109	y = 1.13x + 20.1	R^2 = 0.9937	< 5%
		Yogurt	107.7	y = 1.1725x + 35.45	R^2 = 0.9779	< 5%
		Cream	108	y = 1.07x + 20.4	R^2 = 0.9995	< 5%
	Cd	Milk	107	y = 1.01x + 9.2	R^2 = 0.9977	< 5%
		Dough	117	y = 1.1a5x + 4.5	R^2 = 0.9996	< 5%
		Yogurt	93	y = 1.01x - 0.2	R^2 = 0.9783	< 5%
		Cream	109.3	y = 1.17x + 6.4	R^2 = 0.9930	< 5%
Wet Ashing	Pb	Milk	81.6	y = 0.765x + 2.8	R^2 = 0.9813	< 5%
		Dough	75.7	y = 0.755x + 28.1	R^2 = 0.9994	< 5%
		Yogurt	101	y = 1.05x + 44	R^2 = 0.9839	< 5%
		Cream	92	y = 0.923x + 26.36	R^2 = 0.9846	< 5%
	Cd	Milk	92.6	y = 1.045x + 5.9	R^2 = 0.9906	< 5%
		Dough	104.3	y = 1.13x + 3.6	R^2 = 0.9849	< 5%
		Yogurt	89.7	y = 0.907x - 0.16	R^2 = 0.9916	< 5%
		Cream	105	y = 1.055x + 14.1	R^2 = 0.9952	< 5%
Dry Ashing	Pb	Milk	80	y = 0.605x + 8.6	R^2 = 0.9281	< 5%
		Dough	75.7	y = 0.91x + 9.7	R^2 = 0.9941	< 5%
		Yogurt	120	y = 1.185x + 40.2	R^2 = 0.9985	< 5%
		Cream	105.7	y = 1.075x + 15.5	R^2 = 0.9925	< 5%
	Cd	Milk	96	y = 0.78x + 10.6	R^2 = 0.9611	< 5%
		Dough	96.7	y = 0.895x + 5.4	R^2 = 0.9958	< 5%
		Yogurt	98.3	y = 0.9925x + 0.5	R^2 = 0.9567	< 5%
		Cream	106.6	y = 1.045x + 8.4	R^2 = 0.9997	< 5%

The microwave method had an acceptable recovery and linearity in 7 out of 8 treatments; except for dough and Cd, the recovery values were between 90 and 110%. Wet and dry ashing methods had acceptable performance in 5 out of 8 treatments. In addition, the wet ash method is not recommended for milk and dough digestion in measuring Pb and yogurt digestion in measuring Cd. The dry ashing method showed a weak recovery in the digestion of milk, dough, and yogurt in measuring Pb.

Generally, it can be concluded that all three digestion methods had acceptable capability for measuring Pb and Cd in dairy products; however, the results of all three digestion methods in Table 4 show that some methods are more specific. Choosing an effective method depends on the equipment, time, and digestion solutions. The microwave digestion method requires the purchase of this device, which is not available in all laboratories, though, according to the results, alternative methods should be used. The advantage of this method over other methods is that the human errors are few because the digestion is conducted machinery. Dry ashing methods require more time, but less acid is used in the wet ashing method, and the amount of acid consumed is 50% of the acid consumed in other methods. Therefore, laboratories are recommended to select the digestion method according to better recovery. It is worth mentioning that adding some additives to digestion solutions can also enable dry and wet ashing methods to provide better recovery.

The results of the present study are consistent with the results of other studies, as shown in Table 5.

Conclusion

The measuring results with GFAAS showed that the microwave digestion method had an acceptable recovery for determining Pb in milk, dough, yogurt, and cream samples and Cd in all studied dairy products except in dough samples. Moreover, dry ashing digestion method had a high recovery in all samples spiked with Cd in milk,

Table 4. Results of comparison of three methods of digestion

		Samples	Mean of % recovery (90-110)	(R^2) > 0.95	Method RSD <5%
Microwave Digestion	Pb	Milk	√	√	√
		Dough	√	√	√
		Yogurt	√	√	√
		Cream	√	√	√
	Cd	Milk	√	√	√
		Dough	-	√	√
		Yogurt	√	√	√
		Cream	√	√	√
Wet Ashing	Pb	Milk	-	√	√
		Dough	-	√	√
		Yogurt	√	√	√
		Cream	√	√	√
	Cd	Milk	√	√	√
		Dough	√	√	√
		Yogurt	-	√	√
		Cream	√	√	√
Dry Ashing	Pb	Milk	-	-	√
		Dough	-	√	√
		Yogurt	-	√	√
		Cream	√	√	√
	Cd	Milk	√	√	√
		Dough	√	√	√
		Yogurt	√	√	√
		Cream	√	√	√

Table 5. The comparison of the results of this study with similar results

Matrix	Digestion method	Recovery (metal)	Reference
Milk	Dry ash	95% (Pb), 59% (Cd)	(27)
Biological material	Dry ash	82% (Pb), 98% (Cd)	(28)
Milk	Dry ash	92%-97% (Pb)	(29)
Dairy products	Dry ash	75.7%-120%(Pb), 96%-106.6%(Cd)	Present study
Milk	Wet ash	91.6%(Pb), 99.8%(Cd)	(26)
Milk	Wet ash	98%(Pb), 99%(Cd)	(13)
Dairy products	Wet ash	81.6%(Pb), 92.6%(Cd)	Present study
Milk	Microwave	101%(Pb), 97%(Cd)	(25)
Milk	Microwave	97%(Pb), 100%(Cd)	(13)
Dairy products	Microwave	102.6%(Pb), 107%(Cd)	Present study

dough, yogurt, and cream. Considering all indexes and aspects, microwave digestion seems to be the best method for measuring Pb and Cd in all dairy products.

Acknowledgments

This study is a part of the MSc. thesis funded (Grant No. A-11-357-1) by the School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran. The authors would like to thank the Vice-Chancellor for Research and Technology of Zanjan University of Medical Sciences, Zanjan, Iran.

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Competing interests

The authors declare no conflict of interests.

Ethical issues

The authors hereby certify that all data collected during the research are as expressed in the manuscript, and no data from the study have been or will be published elsewhere separately.
This research was approved by Vice-Chancellor for Research Affairs of Zanjan University of Medical Sciences, Zanjan, Iran (Ethical code: IR.ZUMS.REC.1399.022).

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