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Determination of Copper, Chromium, Nickel, and Lead Elements in Mozzarella Cheese by Flame Atomic Absorption Spectrometry Using Ultrasonic-Assisted Extraction

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ABSTRACT: Heavy metal contamination can occur in dairy products like mozzarella cheese, which is a significant concern due to its potential health risks to consumers. Traditional methods for the monitoring of heavy metals in dairy products can be time-consuming, arduous, and not always have satisfactory results.

In this study, we investigated the effect of ultrasonic waves on the extraction and determination of the concentrations of nickel, copper, lead, and chromium in packed mozzarella cheese samples through flame atomic absorption spectroscopy (FAAS). The proposed method uses ultrasound irradiation to extract heavy metals from cheese samples, employing a 5% nitric acid solution. The various influential parameters, including percentage and volume of nitric acid, and time of ultrasound irradiation to assess their impact on the responses and extraction recoveries of the analytes, were investigated.

After optimization, the limits of detection for Ni, Pb, Cu, and Cr were determined to be 2.18, 1.87, 0.41, and 1.42 µg L^{-1}, respectively. The obtained linear ranges were established in the ranges of 1.0–40 µg L^{-1} for Pb and Cu, 1.0–20 µg L^{-1} for Cr, and 4.0–50 µg L^{-1} for Ni. The practical applicability of the method was illustrated successfully through the determination of Ni, Pb, Cu, and Cr ions in different mozzarella cheese samples. The results and data achieved using this method were compared and checked out, and the outcome of the procedure was reliable and satisfactory. The method indicated dependable and appropriate results, suggesting practicable application for measuring heavy metal contamination in mozzarella cheese samples.

Keywords: Ultrasonic-Assisted Extraction, FAAS, mozzarella cheese, Heavy metals measurement.

1. INTRODUCTION

The presence of heavy metals in food, especially animal products such as dairy products, is one of the basic problems that is directly related to the health of consumers. This further reveals the need for continuous measurement and monitoring to evaluate the effects of heavy metals in food products [1].

Among milk products, chiefly cheese is an important part of the human diet, especially among children [2], and is a popular food. It is consumed alone or in combination with other foods, such as fast foods and ready meals. Also, cheese has widely been used for the texture and taste of food [3].

From a nutritional point of view, cheese can contain essential metals such as zinc, iron, and manganese, or toxic and nonessential metals, including cadmium, lead, mercury, aluminum, etc. [4]. Though essential metals are necessary for health up to a certain level, the existence of inorganic forms of chemical compounds such as heavy metals in food represents a severe risk to human health of its long-term toxicological effects [5]. The intake of heavy metals as a result of the consumption of contaminated milk [6] and milk products will harm health and trigger heavy metal toxicity [7].

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Milk and its products are contaminated with heavy metals in several ways. Animal’s intake heavy metals through the air, water, and especially contaminated feedstuffs, which are the source of metal residues in their milk [8]. Also, industrial methods of preparing, packaging, and preserving food can be the other important sources for the entry of these metals into food [9]. Indeed, accumulating these heavy metals in milk products poses a risk of human exposure to these substances.

The frequency of sound waves in the ultrasonic range is called ultrasonic waves, which have a mechanical nature and can propagate among the different states of matter [10]. Ultrasonic waves have been widely used in various scientific, research, and industrial fields, one of the most important of which is the application of ultrasonic technology [11] in the field of extraction and measurement of various chemical substances in the food and drug sector [12]. Ultrasound-assisted extraction is a new technique that has received much attention as a green technology in new research due to its many advantages compared to traditional methods, including less time, few instrumental requirements, less amount of solvent, and negligible environmental impact [13].

Measuring and quantifying heavy metals is of particular importance in dairy products [14], especially cheese, which presents various challenges due to the complexity of the matrix and the extremely low levels of concentrations of elements [15]. Several analytical methods have been reported in previous work to measure these metals in cheese; in most previous work, dry, and wet digestion techniques [16] or microwave-assisted digestion pretreatment techniques [17] have been reported for the measurement of these metals in cheese and dairy products. Devices such as flame atomic absorption spectrometry (FAAS) [18], inductively coupled plasma-optical emission spectrometry (ICP-AES) [7], electrothermal atomic absorption spectrometry [19], inductively coupled plasma-mass (ICP-MS) spectrometry [20], and inductively coupled plasma-optical emission spectrometry (ICP-OES) [21] have been used to measure results. Electrochemical methods have also been used to measure these metals in some work [22,23].

Regarding the serious health risks arising from heavy metals, measurement and quantifying heavy metals are of particular importance in food studies. Concerning that there are a variety of methods for measuring heavy metals in types of cheese, we explored the development and optimized a method for the extraction and determination of heavy metals in mozzarella cheese using ultrasonic-assisted extraction followed by flame atomic absorption spectroscopy. Therefore, after optimizing the method with excellent sensitivity for Ni, Pb, Cu, and Cr, it proposes a simple, fast, and inexpensive pretreatment technique for the determination of these heavy metals in mozzarella cheese.

The practical application of this study was confirmed with the successful extraction and determination of these heavy metals in all packed mozzarella samples and returned dependable and satisfactory results. Additionally, this method was compared with conventional techniques for determining heavy metal contamination in mozzarella cheese samples, and the results were comparable and acceptable in terms of accuracy, precision, and reliability.

1. MATERIALS AND METHODS

1.1 MATERIALS

Analytical reagent-grade chemicals were used in this work unless otherwise stated. Deionized water was used at whole steps of processes and all dilutions. Standard solutions of metal salts, including $Cu(NO_3)_2$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, and $Cr(NO_3)_2$, each in 0.5 mol/L HNO_3 (all supplied by Merck, Germany, 1000 mg/L), were diluted daily for preparation of work and reference solutions. Also, one to ten percent solution of nitric acid was provided from HNO_3 (Merck, Germany, 65%).

The metal ions were determined with an atomic absorption spectrophotometer (PerkinElmer Pin AAcle900T, USA) and equipped with halo cathode lamps of copper, lead, and nickel and a 10 cm long slot-burner. A mixture of acetylene and air with flow rates of 3.30 L/min acetylene and 10.00 L/min air was provided for flame production.

An ultrasonic water bath instrument (Elmasonic P 180 H, Germany) was used to assist in extracting metal ions from the cheese samples. Laboratory glassware and plastics were cleaned by putting in a 10% HNO_3 solution and then rinsed with deionized water several times. The probationary work was performed at Ibrahim Majeed's laboratory for bee and food production tests, positioned in Sulaymaniyah.

Mozzarella cheese samples from several brands, packed in polyethylene containers, were purchased directly from the local markets in Sulaymaniyah and stored in a refrigerator below 4 °C.

1.2 ANALYTICAL METHODS

Mozzarella cheese packed in polyethylene boxes produced by the different companies was bought from local stores. The samples were stored in a refrigerator at a low temperature, 4 °C, before the analyses. First, the cheese sample was crushed with mortar and pestle. Five grams of mozzarella cheese were weighed, and it was placed into a polyethylene vial. Afterward, 10 ml of a 5% nitric acid solution was added to them and then inserted into the water bath ultrasonic apparatus. After 60 minutes, it was filtered with filter paper, and the solution was collected in other vials; eventually, the metal ions were determined by atomic absorption spectrometry. All steps are shown schematically in Fig. 1.

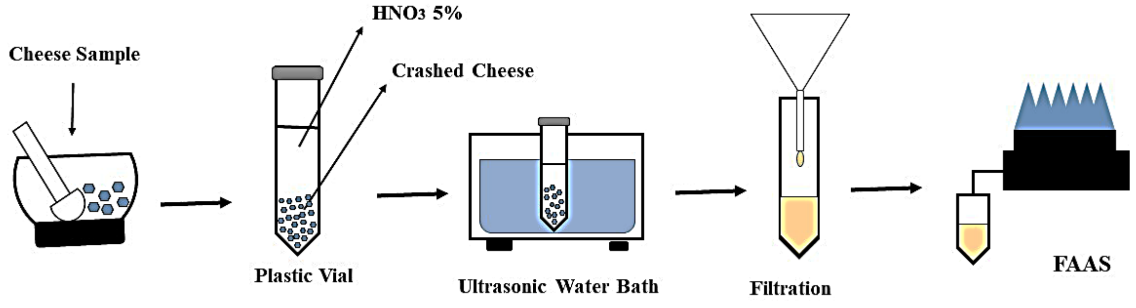

FIGURE 1. Schematic steps for the ultrasonic-assisted extraction method for determination of Copper, Chromium, Nickel, and Lead in mozzarella cheese.

2. RESULTS

2.1 EFFECT OF NITRIC ACID PERCENTAGE

For each test, 5 grams of the crushed cheese containing 2 micrograms of each of the target heavy metals standards were weighed and placed in a plastic vial. Then 10 ml of different percentages of nitric acid (0-8% HNO_3) were prepared to investigate the effect of nitric acid percentage on the recovery of the metal ions, keeping other conditions constant. (5 gr of cheese sample, 10 ml of the tested nitric acid with 0-8%, and 50 minutes of ultrasonic irradiation).

An increase can be seen in Fig. 2 in the recovery percent by increasing the percentage of nitric acid up to 5%, and then the relative stability can be observed in the graph.

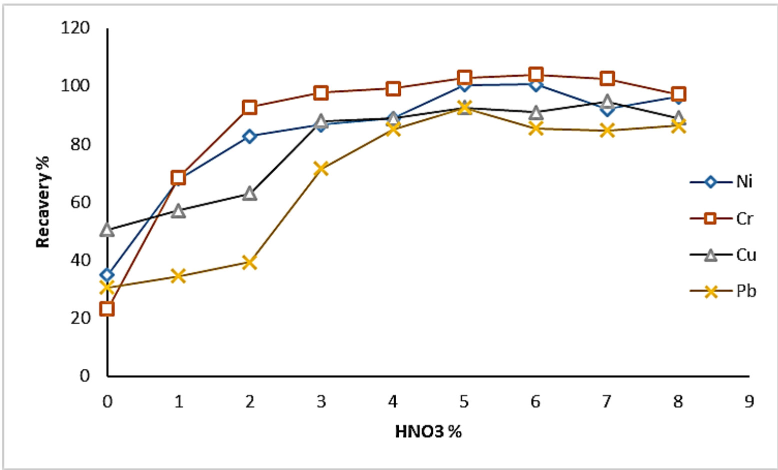

FIGURE 2. The effect of nitric acid percentage (0–8% of HNO3) on the extraction rate of the Ni, Cr, Cu, and Pb in 5 gr cheese.

2.2 VOLUME OF NITRIC ACID SOLUTION

Please The volume of nitric acid solution was the next factor that was optimized. To evaluate and optimize this factor, different volumes of 5% nitric acid solution (from 3 to 15 ml) were prepared and added to a fixed amount of cheese sample (5 gr) containing 2 micrograms of each of the target heavy metal standards; thereupon, they were placed in an ultrasonic apparatus for 50 minutes. The results showed that by increasing the volume of 5% nitric acid, the recovery of heavy metals was increased. The maximum recovery was in volume 10 ml, and in the upper volumes, the level of recovery for heavy metals was stable or declined. see Fig. 3. Subsequent factors were evaluated with 10 ml of 5% nitric acid.

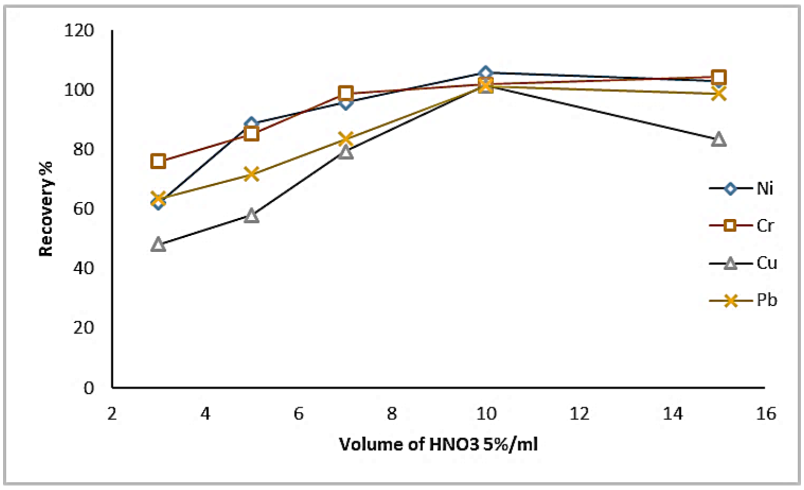

FIGURE 3. Effect of HNO_3 5% volume (3–15 ml) on recovery percentage of Ni, Cr, Cu, and Pb in 5 gr cheese.

2.3 TIME OF ULTRASOUND IRRADIATION

The duration of ultrasonic irradiation was another parameter that was measured and optimized in this work. For this purpose, an identical number of samples (5 gr) were tested separately for a constant value of the heavy metal standards (2 micrograms of each of the target heavy metals) at different periods. As shown in Fig. 4. the recovery of sample metals increased with increasing duration of ultrasonic radiation, and the most recovery was in 60 minutes. Hereafter, with the increasing duration of the ultrasonic radiation, there was stability or a relative decrease in the recovery.

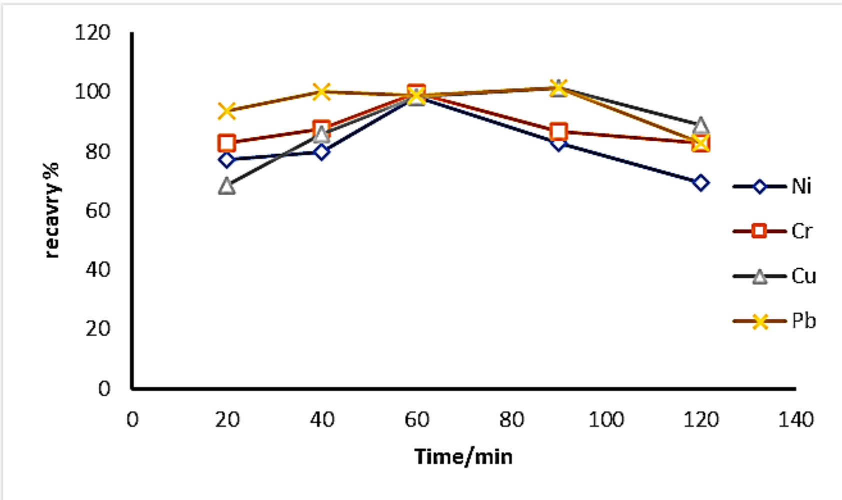

FIGURE 4. Influences of the duration of the ultrasonic radiation on the recovery percentage of Ni, Cr, Cu, and Pb (5 gr cheese in 10 ml HNO_3 5%).

2.4 ANALYTICAL PERFORMANCE

The analytical characteristics and sensitivity parameters of the proposed method, including limits of quantification (LOQ), limits of detection (LOD), standard deviations (SD), and other factors, are calculated and shown in Table 1. The LOQ and LOD were specified as LOQ = 10 SB/m and LOD = 3.3 SB/m, respectively. The standard deviation (SD) was found by using six replicate measurements of standard solutions of nickel, chromium, copper, and lead [24].

Table 1. Analytical characteristics of the calibration curves of the Copper, Chromium, Nickel and Lead in cheese samples.

Analyte	LOD (µg/g)	LOQ (µg/g)	Linear range (µg/g)	RSD%
Ni	2.18	6.62	4 – 50	9.8
Cr	1.42	4.75	1 – 20	7.3
Cu	041	1.25	1 – 40	6
Pb	1.87	5.67	1 – 40	6.0

3. DISCUSSION

Considering the shortcomings of traditional methods, in this research, an alternative method for measuring heavy metal contamination in mozzarella cheese was investigated. Attempts to improve the extraction and detection of heavy metals (Ni, Cu, Pb, Cr) using ultrasonic waves in mozzarella cheese samples achieved acceptable results. Ultrasound radiation was also used to enhance the extraction of heavy metals from mozzarella cheese, which is faster and possibly more effective than traditional extraction methods.

Various parameters, such as duration of exposure to ultrasound, concentration, and volume of nitric acid, were optimized to achieve the best extraction rate. Then, suitable and low detection limits for heavy metals were obtained (Ni: 2.18 µg/L, Pb: 1.87 µg/L, Cu: 0.41 µg/L, Cr: 1.42 µg/L), indicating acceptable and high sensitivity.

This method works effectively in certain concentration ranges for each heavy metal, and according to these results, it can be used as a reliable tool to measure different levels of pollution.

3.1 COMPARISON WITH THE OTHER METHODES

Analytical characteristics of the other reported measurement methodologies for the determination of Cu, Pb, Ni, and Cr in some types of cheese are shown in Table 2, compared to the analytical performance of this work. Comparatively, this method shows a low detection limit and an appropriate number of other characteristics for the determination of Cu, Pb, Ni, and Cr in cheese samples. Additionally, the recovery rate in this work is very appropriate and more acceptable compared to other reported methods.

Table 2. Comparison of the proposed ultrasonic-assisted extraction method with other methods used in the determination of Ni, Cu, Cr, and Pb in cheese samples.

Analyte	Method	Sample	LOD (µg/g)	LOQ (µg/g)	RSD%	R%	Ref
Ni	Microwave digestion followed by graphite furnace atomic absorption spectrometry.	Mozzarella Cheese	20.1	-	7.8	79.4	[9]
Cr			2.14	-	2.4	100	
Cu			5.9	-	0.41	98.9	
Pb			0.930	-	15	100	
Ni	ICP-OES after microwave digestion	Turkish white cheese	10	-	6.26	-	[21]
Cr			6.6	-	9.1	-	
Cu			210	-	37.9	-	

Pb			25	-	28.3	-	
Ni	Dry digestion followed by atomic absorption spectrometry.	Deferent kinds of cheese	2.65	8.83	3.37	106	[25]
Pb			1.28	4.28	3.81	105	
Ni Cr Cu Pb	Wet digestion ICP – OES,	Oaxaca cheese & ranchero cheese	0,1	0,3	-	104	[26]
Cu	Digestion followed by atomic absorption spectrometry	Kareish cheese	1	-	29.88	-	[27]
Pb			12	-	6.74	-	
Ni	Ultrasonic-assisted extraction method using FAAS	Mozzarella Cheese	2.18	6.62	9.8	99.6	This work
Cr			1.42	4.75	7.3	99.9	
Cu			0.41	1.25	6	98.5	
Pb			1.87	5.67	6.0	98.6	

3.2 ANALYSIS OF DIFFERENT MOZZARELLA CHEESE SAMPLES

To illustrate the reliability and practicality of the proposed method, it was used for analysis of Ni, Pb, Cr, and Cu in some different packed mozzarella cheese samples. All specimens were identified and measured according to the proposed method and at the concentrations shown in Table 3 three times. The results of the survey of samples of cheese packed in different companies are shown in Table 3.

As can be seen in the results, the measurement of specific concentrations of heavy metals in different samples of cheese did not have a significant effect on the efficiency of the proposed method.

Table 3. Addition and recovery test for extraction and determination of Ni, Cr, Cu, and Pb in three different packed Mozzarella cheese (10 ml HNO_3 5%, 60 min ultrasonic irradiation, 2 micrograms of Ni, Cr, Cu, and Pb).

Element	Added (µg/5g)	Sherif		Naza		Tek sut	
		Found *(µg/5g)*	Recovery %	Found *(µg/5g)*	Recovery %	Found *(µg/5g)*	Recovery %
Ni	0	7.47	-	10.36	-	1.88	-
	5	4.94	98.7	4.57	91.4	4.66	93.2
	10	9.11	91.1	9.31	93	9.8	98
Cr	0	3.8	-	3.3	-	8.66	-
	5	5.44	108	5.22	104.4	5.04	100.8

	10	10.44	104	9.91	99.1	9.6	96
	0	2.7	-	2.82	-	1.14	-
Cu	5	4.66	93	4.57	91.4	4.43	88.6
	10	9.29	92.9	9.31	93.14	9.4	94
	0	8.9	-	11.2	-	1.75	-
Pb	5	5.58	111	5.24	105	5.42	108
	10	10.4	104	11.04	110	10.47	104.7

4. CONCLUSION

This study presents a new, simple, and efficient sample pretreatment method for simultaneous extraction and determination of nickel, lead, copper, and chrome in cheese samples by flame atomic absorption spectrometry (FAAS). The proposed method, in addition to simplicity, presents other advantages such as cheapness, ease of operation, and reducing the risk of contamination and consumption of toxic and hazardous chemicals. An investigation of the effect of different factors on the extraction and measurement of these metals in cheese samples showed they exhibited almost similar behavior, which enables simultaneous pretreatment with less time and cost.

Generally, the validation results demonstrate that the presented procedure provides an easy and accurate measure of expressed metals in various cheese samples. Due to providing reliable and accurate results, this method can be a suitable alternative to traditional methods, which could potentially make it a better method for routine analysis of heavy metals in dairy products.

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