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Research Article

Evaluation of Heavy Metals Concentration in Milk Products by using Atomic Absorption Spectroscopy

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Abstract: Milk products are essential food in the human diet because it contains many essential trace minerals such as calcium, magnesium, copper, zinc, sodium, potassium and phosphorous. The utilization of, milk is increasing at a large scale throughout the world. It is essential to maintain the good quality of milk during production and manufacturing because the presence of toxic metals in the milk becomes the cause of health disturbance in human life. Therefore, the present investigation was carried out to determine concentrations of lead, nickel, iron, copper, and chromium in powdered milk and fresh milk products by Atomic Absorption Spectroscopy. Different milk samples of products (two fresh milks and two powdered milks) were purchased from the local market. For the decomposition of the organic substances in milk samples, wet digestion was used with a mixture of nitric acid and sulfuric acid in volume proportions 1:3 (v/v). The analytical curve for all metals covered the linear range from 0.5 to 4.0 ppm with correlation coefficients higher than 0.9994. The limit of detection (LOD) for Pb, Ni, Fe, Cu, and Cr were found to be 0.25, 0.023, 0.012, 0.0067, and 0.073 ppm, respectively. While the limit of quantification (LOQ) in the range of 0.02 to 0.76 ppm. Of all the metals determined, Pb, Ni, and Fe were the most abundant with concentrations between 1.233 and 1.677 ppm while Cr was not detected in all the samples. The results showed that fresh milk samples have a higher concentration of heavy metals compared to powdered milk samples.

Keywords: Heavy metals, milk products, Atomic Absorption Spectroscopy, correlation coefficients, limit of detection

Introduction

Increasing in population around the world from modern technology led to the rises of environmental pollution. Environmental pollution can occur because of many factors and one of the factors is because of the contamination of heavy metals. Heavy metals are referring to any metallic chemical element that is higher in density and toxic or poisonous at low concentrations [1]. Heavy metal can enter the body through food consumption in daily life. As for children, heavy metals entered the body through milk that they consumed for their growth and development during infancy. Daily consumption of milk products shows evidence that it provided a unique balance of nutrients to the human body.

Milk can be described as a vital component that gives benefits to the human diet. Milk has been categories as a complete food to human because contained important nutrients such as protein, vitamins, minerals, and essential fatty acid [2]. There are two groups of metal contents in milk which are essential elements and non-essential elements. Iron, copper, and zinc are essential elements while lead and cadmium are non-essential elements. Nowadays, there are many improvised milk productions that have been made by people such as powdered milk, fresh milk, and other dairy products such as yogurt and cheese. The heavy metals may enter the powdered milk through different machines that are involved in the processing and distribution and it also can contaminated powdered milk either through foodstuff and water or through manufacturing and packaging processes [3].

The excess presence of heavy metals in milk can give side effects on human health. Heavy metals in the body may cause drawbacks such as the damages of skeletal and cell, renal failure, osteoporosis, blood and lung cancer, hormonal disturbances, gastrointestinal problems, and anemia [3]. Plumbum (Pb) can cause the disorder of the central nervous system, damages to kidneys, liver, heart, and blood vessels, the immune system, and the development of cancer. Besides, Cadmium (Cd) may give side effects when it accumulates in tissues like the liver and kidneys. Cadmium (Cd) is carcinogenic especially in the lungs and prostate and caused the development of tumors.

The determination of trace inorganic constituents in milk is a challenging task due to their complex emulsions like matrices and low concentration levels of the metal ions [4]. Many digestion procedures to oxidize organic matrices of different samples have been reported in the literature [5,6]. The acid digestion procedures are the most popular sample pre-treatment techniques for elemental determination in biological and environmental samples.

The determination of heavy metals can be performed by several instrumental techniques [7] including indirect photometric chromatography [8], ion chromatography [9], flame atomic absorption spectrometry [10], and flame atomic absorption spectrometry [11]. One of the instruments that provide an accurate result is atomic absorption spectrophotometry (AAS). Due to the high sensitivity, this technique authorizes the establishment of sequential and simultaneous methods for determinations of many elements with efficient background correction [12].

Thus, this study aims to determine the heavy metal contents such as lead (Pb), chromium (Cr), nickel (Ni), copper (Cu), and iron (Fe) in different types of powdered milk and fresh milk products by using atomic absorption spectrophotometry.

Materials and Methods

The chemicals used in this study are nitric acid (HNO_3), sulphuric acid (H_2SO_4), deionized water, and the standard solution of Pb, Ni, Fe, Cu, and Cr. Atomic Absorption Spectrometer Analyst (Perkin Elmer) 700 equipped with high-intensity deuterium arc lamp for the correction of non-specific background and WinLab32 software for atomic absorption provides maximum productivity and compliance (Figure 1). Two different brands of milk which are fresh milk (A and B) and powdered milk (C and D) were purchased from the local market.

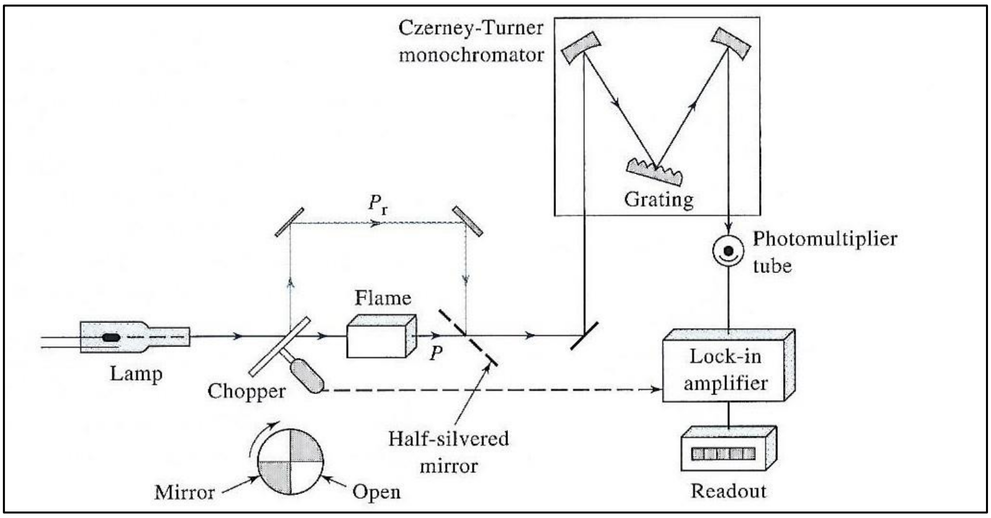

Figure 1. Basic atomic absorption spectrometer setup.

For the preparation of powdered milk sample, 0.5 g of sample was weighed by using an analytical balance. The sample was transferred into 100 mL round bottom flask and mixed with 5 mL of HNO_3 and 15 mL of H_2SO_4 with volume ratio of 1:3 (v/v). These mixtures were digested at 210 °C for 2 hours and 30 minutes in a Kjeldahl digested apparatus fitted with reflux condenser. Then, the digested sample was allowed to cool at room temperature. Deionized water was added into the digested residue and was filtered through syringe types membrane filter. The volume of the filtrate was made up to 100 mL of volumetric flask using deionized water and the solution was further diluted 10 times before determinations of Pb, Ni, Fe, Cu, and Cr by using AAS. The digestion was carried out in triplicate for both blank and samples. Digestion of a reagent blank was performed in parallel with powdered milk samples keeping all digestion parameters the same.

While, for preparation of fresh milk samples, 1 mL of milk was added into 100 mL of conical flask. Then, 10 mL of HNO_3 was added into the flask and heated for 20 minutes. The mixture was cooled down at room temperature. Then, 30 mL of H_2SO_4 was added into the mixture and was heated vigorously until the white fumes are appeared and reduced to 2-3 mL. The digested milk samples were diluted with deionized water up to 50 mL of volumetric flask.

The concentration of the Pb, Ni, Fe, Cu, and Cr present in the sample was determined by using AAS. The concentration of Pb, Ni, Fe, Cu, and Cr in sample was calculated from calibration curves. Three replicate determinations were carried out for each sample while comparing it on the respective standard calibration curve. The same analytical procedure was employed for the determination of elements in digested blank solutions.

Results and Discussion

Series of working standards and correlation coefficient of the calibration curves for determination of heavy metals in milk using AAS were obtained as shown in Table 1 below.

Table 1. The correlation coefficient of calibration curves for determination of heavy metals in milk by AAS.

Metals	Correlation coefficient, R^2
Pb	0.9999
Ni	0.9994
Fe	0.9997
Cr	0.9999

The linearity studied of Pb, Ni, Fe, and Cr were in the range between 0.5 ppm and 4.0 ppm. Calibration curve for all the metals showed good linearity with coefficients of determination (R^2) ranged between 0.9994 ppm and 0.9999 ppm. This showed that there is a good correlation between concentration and absorbance indicating good calibration of the instruments. Previous study has obtained R^2 0.9926 and 0.9979 for Fe and Cr, respectively [13].

Table 2 presents the limit of detection (LOD) and limit of quantitation (LOQ) for metals studied. LOD and LOQ obtained for each heavy metal were in the range of 0.012 – 0.25 ppm and 0.037 – 0.76 ppm, respectively. Low LOD and LOQ of metals using this method enable the detection and quantitation of these metals at low concentrations.

Table 2. Limit of detection (LOD) and limit of quantitation (LOQ) for heavy metals.

Types of heavy metal	LOD (ppm)	LOQ (ppm)
Pb	0.25	0.76
Ni	0.023	0.068
Fe	0.012	0.037
Cr	0.073	0.22

The previous study done by many researchers shows that the LOD obtained for each heavy metal has a different value compared to the LOD of heavy metals in our study [14]. As for Ni, it shows that LOD of Ni in our study is lower than the previous study reported by Ismail [15] which is 0.7 ppm meanwhile, for Fe, there is no difference in LOD between our study and the previous study.

Once the calibration curve is obtained, the sample is directly analyzed to determine the concentration of heavy metal in the samples. From the results in Table 3, no statistically significant differences in Pb were observed for A and B fresh milk. The only significant difference was observed for Pb concentration in D powdered milk where the concentration detected was 0.019±0.050 ppm which is slightly lower than other brands. However, the concentration of Pb in C powdered milk was not detected. The mean level of Pb in our study is lower than that stated by Imam et al. (2017) and Iftikhar et al. (2014) which is 1.87 ppm and 2.243 ppm respectively but, higher than the recommended value of 0.02 ppm by the Codex Alimentarius Commission as well as 0.025 ppm by World Health Organization (WHO) [16].

Table 3. Concentration of Lead (Means ± SD) in different types of sample.

Types of sample	Means (ppm) ± SD
Fresh Milk (A)	1.677 ± 0.018
Fresh Milk (B)	1.635 ± 0.044
Powdered Milk (C)	ND ± 0.048
Powdered Milk (D)	0.019 ± 0.050

ND: Not Detected

Nickel (Ni) being a cofactor for a number of hormones and enzymes is considered as an essential element for humans. However, excessive intake may result in cell damage, impaired reproductive system, altered hormonal, and other dangerous diseases [15]. Ni concentrations in fresh milks and powdered milks from commercial brands were obtained and summarized in Table 4 below.

Table 4. Concentration of Nickel (Means ± SD) in different types of sample.

Types of sample	Means (ppm) ± SD
Fresh Milk (A)	1.540±0.012
Fresh Milk (B)	1.458±0.014
Powdered Milk (C)	0.036±0.005
Powdered Milk (D)	ND±0.012

ND: Not Detected

The results that were obtained above show that there were slightly statistically significant differences between brand A and B fresh milk which is 1.540 ± 0.012 ppm for A and 1.458±0.014 ppm for B. The obvious significant differences were observed for the concentration of Ni in C powdered milk which is 0.036±0.005 ppm while the concentration of Ni was not detected in D powdered milk. This would be presuming because of the different packaging containers used, manufacturing practices, and raw materials quality [9]. The mean level of Ni in our study is slightly higher than the previous study reported by Ogabiela et al. [16], which is 0.130 ppm. However, the mean level of Ni is 3.013 ppm which is much higher compared to our study reported by Perveen et al. [17]. However, mean level of Ni from fresh milk samples in our study was higher than recommended value by WHO, which is 0.43 ppm.

Iron (Fe) is one of the essential nutrients in human body. Excessive in this metal can cause severe damage. The result analysis of Fe concentration in the samples shown in Table 5 below.

Table 5. Concentration of Iron (Means ± SD) in different types of sample.

Types of sample	Means (ppm) ± SD
Fresh Milk (A)	1.233 ± 0.026
Fresh Milk (B)	1.331 ± 0.015
Powdered Milk (C)	ND ± 0.001
Powdered Milk (D)	0.024 ± 0.001

ND: Not Detected

From Table 5 above, the concentrations of Fe in A and B fresh milk are 1.233 ± 0.026 ppm and 1.331± 0.015 ppm, respectively. Compared to the concentration of Fe in D powdered milk, the obtained concentration of Fe is 0.024 ± 0.001 ppm. However, Fe concentration was not detected in C powdered milk. The mean level of Fe in our study is lower than that stated by Perveen et. al [17], which is 20.41 ppm, and other study reported by Ogabiela et al. [16], which is 1.580 ppm but slightly higher than recommended value by WHO, which is 0.2 ppm.

Chromium (Cr) is an essential nutrient for plant and animal metabolism, but increasing in accumulation of Cr in the environment from industrial outputs has caused great concern because long term exposure to Cr can cause kidney and liver damage as well circulatory and nerve tissue problems [13].

Table 6. Concentration of chromium (Means ± SD) in different types of sample.

Types of sample	Means (ppm)±SD
Fresh Milk (A)	ND±0.015
Fresh Milk (B)	ND±0.028
Powdered Milk (C)	ND±0.008
Powdered Milk (D)	ND±0.017

ND: Not Detected

From the results in Table 6, it shows that all the types of sample were free from Cr. This is due to the less pollution were exposed either directly or indirectly and less effecting the milk supplies. Compared to previous study done by Ogabiela et al. [16], they reported that mean level of Cr is 1.152 ppm which shows that the milk sample used was contaminated with this heavy metal. Since it was not detected, the concentration of Cr in milk can be considered as below the permissible limit of Cr.

Conclusions

In conclusion, in this present study the concentration of heavy metals such as (Pb, Ni, Fe, Cu, and Cr) were successfully determined by using Atomic Absorption Spectrophotometry (AAS). The concentrations of Pb, Ni, Fe, Cu, and Cr showed little variability with brand. Generally, Pb, Ni, Fe, and Cu, concentrations in milk samples (except Cr) exceed the maximum allowed values. The current study concluded that fresh milks and powdered milks distribute in Malaysia may carry potential health hazards for humans due to the majority of these examined samples contained heavy metals by levels exceeded the maximum permissible limits proposed for them. Fresh milk products (A and B) were found to be the richest in heavy metals (Pb, Ni, and Fe) compared to powdered milk products (C and D). Among the five metals studied, Pb concentrations were always the highest compare to Cr concentrations were very low.

The limit of detection (LOD) and limit of quantitation (LOQ) were successfully determined as the method validation for AAS. However, for fresh milk products (A and B), both show percent recoveries with the range between 52.0% - 58.00% which is quite low and needs to be improvised in further study. The value limit of detection (LOD) found were of 0.25 ppm (Pb), 0.023 ppm (N)i, 0.012 ppm (Fe), 0.0067 ppm (Cu), and 0.073 ppm (Cr). Meanwhile, LOQ values found were 0.76 ppm, 0.068 ppm, 0.037 ppm, 0.02 ppm, and 0.22 ppm for Pb, Ni, Fe, Cu, and Cr respectively. Based on LOD values, AAS was sensitive enough for analysis of these heavy metals because LOD values were lower than the maximum values of heavy metals allowed to be present in milk products.

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