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Determination of copper, zinc, lead and cadmium in chicken liver by atomic absorption spectroscopy

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ABSTRACT

Animal liver is a storage organ for many important nutrients (vitamins A, D, E, K, B12 and minerals such as copper and zinc). However, liver also might contain higher amounts of heavy metals such as lead, cadmium and arsenic which tend to accumulate in liver tissues. This study was conducted to determine the content of copper, zinc, lead and cadmium in chicken liver samples. The samples of chicken liver were digested using wet digestion technique. Effects of several digestion parameters such as types of solvent, concentration of solvent and sample weight have been optimized. Determination of copper, zinc, lead and cadmium in chicken liver were performed by dissolving 0.5 g of the sample in 2.0M sulphuric acid and then measured directly by using flame atomic absorption spectrometer. Results showed that the concentrations of copper (2.285 -2.505 µg/g),zinc (23.450 -24.475 µg/g), lead (0.095 -0.230 µg/g)and cadmium (2.145 µg/g)in the chicken liver samples. It is proven that chicken liver contain trace metals which are essential for the body as well as heavy metals which are toxic.

Keywords: chicken, liver, heavy metal.

1 INTRODUCTION

During the last decades, the increasing demand of food safety has stimulated research regarding the risk associated with consumption of food stuffs contaminated by pesticides, heavy metals and/or toxins. The implication associated with heavy metal contamination is of great concern. Heavy metals, in general are not biodegradable, having long biological half-lives and having the potential for accumulation in different body organs leading to unwanted side effects [1]. Heavy metals constitute a very heterogeneous group of elements which are widely varied in their chemical properties and biological functions. The term "heavy metal" can be defined as metals which have specific weights more than 5 g cm^{-3} [2]. The term heavy metal has been called a "misinterpretation" in an International Union of Pure and Applied Chemistry (IUPAC) technical report due to its contradictory definitions and lack of a "coherent scientific basis". Thus, an alternative term "toxic metal" has been proposed, but no consensus of exact definition exists either [3]. They do not degrade or are not destroyed; they generally do not breakdown into less harmful constituents .They accumulate where they are released. As trace elements, some heavy metals are essential to maintain the metabolism of human body.

Contamination by heavy metals is a major concern worldwide, regional and local level and influences the functional and structural integrity of an ecosystem. The ingestion of plants produced in the contaminated area is another principal factor contributing to heavy metal of exposure for population. It has been recognized that food crops can be an important source of heavy metals for humans and animals [4]. Bird populations are particularly susceptible to the effects of anthropogenic activities on the environment. Several biological and physiological processes, such as eating habits, growth, age, breeding, moulting may influence metal concentration and distribution in birds [5]. The concentration of heavy metals in internal tissues of chicken has been extensively determined by several researchers [6,7]. However, data on the trace element levels in chicken and other domestic bird in Malaysia are still scarce. Bioaccumulation of heavy metals in tissues of birds has received attention because of the lethal and sub-lethal effect of their accumulation, apart from the fact that birds are often located in high levels in the food chain which makes them suitable for use in bioaccumulation studies [8]. The risk of heavy metal contamination in meat is of great concern for both food safety and human health because of the toxic nature of these metals at relatively minute concentrations [9]

2 EXPERIMENTAL

2.1 Sample collection

The chicken liver samples are collect from four different premises in University Teknologi Malaysia. The samples were collected in polyethylene bags (all estimations are made in triplicates) and transported to the

laboratory for analysis. The samples were cut into small pieces and heated in an oven at 110°C for 2 hour, then grounded to a powder and stored in a container at room temperature until further use.

2.2 Sample analysis

An amount of 0.5 g of dried powdered chicken liver was weighed accurately in four beakers labeled as A, B, C, and D. Then, 7 mL of 2.0 M sulphuric acid, H_2SO_4 and 3mL deionized water was added into beakers A, B, C and D respectively. The *digestion* was carried out on a *hot plate.* The solutions were then stirred. Once the digestion is completed, there should be no solid at the bottom of the beaker. After cooling, the solutions are filtered into 25 mL volumetric flask and diluted with deionized water up to the mark. The samples were analyzed by using flame atomic absorption spectrophotometry to measure the concentration of heavy metal in the samples. The results were reported as the average of three repeated measurements, and all digestions were conducted in triplicate.

2.3 Optimization of type of solvent

There were three different solvents used to digest the sample which were 3.0 M nitric acid, HNO_3, 3.0 M hydrochloric acid, HCl, and 3.0 M sulphuric acid, H_2SO_4. Then the concentrations of the heavy metals were measured by using the F-AAS. The best solvent was selected in such that digesting the sample well for the next experimental parameters.

2.4 Optimization of concentration of solvent

As the best acid solvent was selected from the previous parameter, then it was prepared in three different concentrations which were 1.0 M, 2.0 M, and 3.0 M. The samples were dissolved in the solvents of varied concentration. The concentrations of the heavy metals then were measured by using F-AAS. The solvent with the best concentration then were used for the next parameter.

2.5 Optimization of weight of the samples

Chicken liver samples were weighed at three different weights, which were 0.3 g, 0.5 g, and 1.0 g. Then the samples were dissolved in sulphuric acid, H_2SO_4 with the concentration of 2.0 M. Next, the concentrations of heavy metals were measured by using F-AAS. The ideal sample's weight for digestion was determined.

3 RESULTS AND DISCUSSION

3.1 Optimization of type of solvent

One of the objectives of this study is to find the best solvent used to extract desired heavy metals in the chicken liver sample. The best solvent is selected based on the extraction that can give the highest absorbance by F-AAS. Three different solvents were used for this purpose, which were nitric acid, HNO_3, hydrochloric acid, HCl, and sulphuric acid, H_2SO_4. The concentration of each solvents used were fixed at 3.0M. The best solvent used in extracting the desired heavy metals was sulphuric acid, H_2SO_4 as Figure 1.

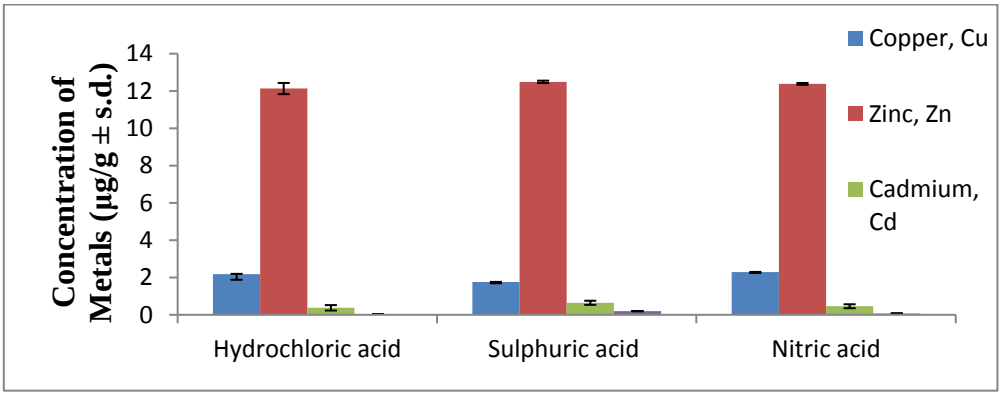

Figure 4.1: Concentration of metals in different types of solvent.

3.2 Optimization of concentration of the solvent

The concentration sulphuric acid, H_2SO_4 was varied from 1.0 M, 2.0 M, and 3.0 M. The optimum concentration chosen based on the extraction that can give the highest absorbance by F-AAS. Figure 2 show that 2.0 M of sulphuric acid, H_2SO_4 was giving the best result for the digestion of the desired heavy metal. Sulphuric acid concentration only showed a small influence on the digestion of the metals. There are only small different on the reading of the metals concentration obtained. In this study, the optimum concentration of sulphuric acid, H_2SO_4 used is 2.0 M.

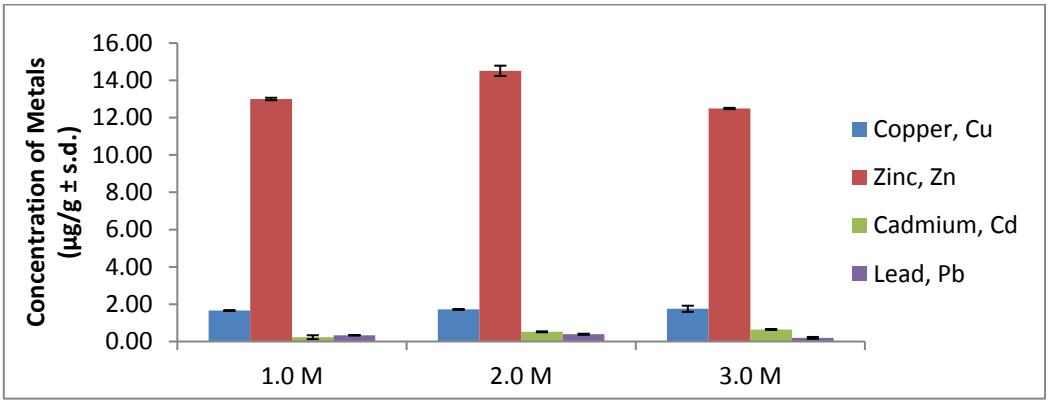

Figure 2: Concentration of metals in varied concentration of sulphuric acid.

3.3 Optimization of weight of the sample

There will be three different weights of chicken liver sample that will be stressed on, which are 0.3 g, 0.5 g, and 1.0 g. The reasonable absorbance of desired heavy metals were detect from the sample that having weight 0.5 g. Figure 3 shows the results obtained from the different weight of chicken liver sample varied in the experiment. The result shows that 0.5 g of sample used gave a reasonable absorbance of desired heavy metal compared to 1.0 g. The concentration given was slightly higher when the weight of sample was increased to 1.0 g but some desired heavy metal cannot be detected. This might be because of the sample were not fully digested when higher weight was used. In addition, the ratio of the sample to solvent must be kept constant in order to get better digestion result. Therefore, 0.5 g was chosen as the optimum condition because it has higher concentration with moderate amount of samples as compared to 0.3 g and 1.0 g.

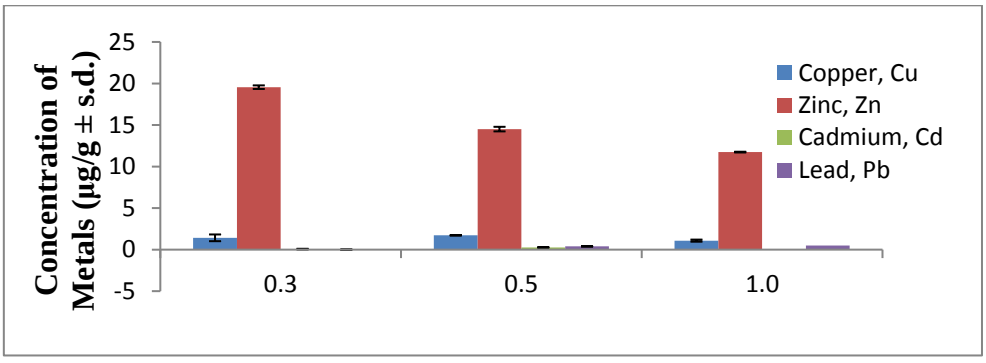

Figure 3: Concentration of metals in varied weight of sample.

3.4 Analysis of heavy metals under optimized conditions

In this study, three parameters of wet digestion for determination of the concentration of heavy metals in chicken liver were being studied. 0.5 g of chicken liver sample was digested in optimized condition by using 2.0 M sulphuric acid, H_2SO_4 98%. The four heavy metals namely copper, cadmium, lead, and zinc were analyzed by using F-AAS. The concentration of metals can be seen in the Figure 4.

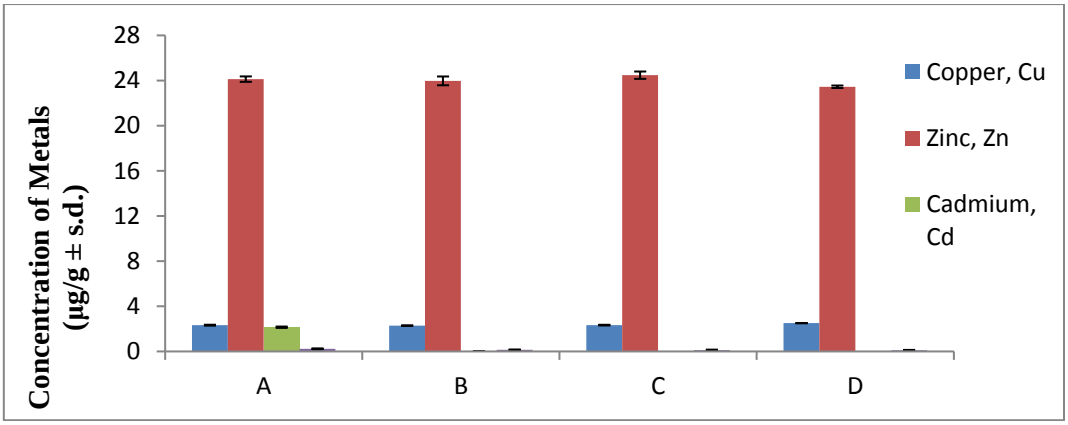

Figure 4: Concentration of heavy metals in chicken liver samples.

3.5 Method Validation

Reproducibility

In this experiment reproducibility was done in order to indentify the precision of the method by comparing the two measurements. Both results obtained in intraday and interday were compared to determine the closeness of the measurements with each other. By referring on both results obtained in Tables 5 and 6, there is slight different in concentration of heavy metals in the chicken liver samples. The result for both methods can be considered as close to each other. This result shows that both methods gave slightly different result for all heavy metals and can be considered as good precision.

Table 5: Concentration of metals obtained in intraday experimental

Metals	Concentration of Metals (µg/g ± s.d.)	%RSD
Copper	0.880 ± 0.010	1.136
Zinc	25.935 ± 1.285	4.955
Cadmium	0.370 ± 0.060	16.216
Lead	0.305 ± 0.020	6.557

Table 6: Concentration of metals obtained in interday experimental

Metals	Day 1		Day 2		Day 3	
	Concentration of Metals (µg/g ± s.d.)	%RSD	Concentration of Metals (µg/g ± s.d.)	%RSD	Concentration of Metals (µg/g ± s.d.)	%RSD
Copper	0.88 ± 0.01	1.14	1.41 ± 0.51	36.17	0.89 ± 0.02	2.34
Zinc	25.94 ± 1.29	4.95	24.51 ± 0.09	0.367	24.96 ± 0.38	1.50
Cadmium	0.37 ± 0.06	16.22	0.24 ± 0.06	25.00	0.38 ± 0.02	3.79
Lead	0.31 ± 0.02	6.56	0.29 ± 0.03	10.53	0.23 ± 0.03	10.87

Limit of Detection

Limit of detection is defined as three times of the standard deviation of 10 measurements of a reagent blank. In the study, the reagent blank was prepared by dilution of 7 mL of digesting solution, 2.0 M sulphuric acid, H_2SO_4 to 10 mL with deionized water. The limit of detection of Cu, Cd, Pb, and Zn are 0.011, 0.038, 0.028 and 0.023 µg/g respectively as shown in the Table 7 below.

Table 7: Limit of detection of the heavy metals

Metals	L.O.D (µg/g)
Copper	0.011
Zinc	0.038
Cadmium	0.028
Lead	0.023

Percentage of Recovery

Data obtained in Table 8 shows that the experimental has very poor recovery as percentage recoveries achieved were below than 85% for copper, cadmium, zinc and lead. The recovery is low because there will be some experimental loss and human error. During digestion, the temperature, time and acidity were critical factors to digest the samples. The loss of analytes during increasing temperature can lead to the reaction inefficiency. Also error can be occurring when the digestion was prolonged for any length of time beyond the minimum time required for ions to oxidize. Although, error may occur during recording the weight of the samples like improper handling of the balance.

Table 8: Percentage recovery for each desired heavy metals

Metals	Percentage Recovery (%)
Copper, Cu	28.03
Zinc, Zn	70.20
Cadmium, Cd	14.75
Lead, Pb	10.44

CONCLUSION

Wet digestion of the solid sample with oxidizing acid was successfully investigated. This method has the advantage of being effective on solid sample. It often destroys or removes the sample matrix, thus helping to reduce or eliminate some types of interference. In addition, this digestion does not require any special equipment, easily available and relatively low cost.

The heavy metal analysis in chicken liver was carried out using F-AAS. 7mL of 2.0 M sulphuric acid, H2SO4 was suitable extraction solution. The best weight that gives good absorbance of the digested heavy metals was 0.5 g. the proposed procedure can be applied for the determination of heavy metals in chicken liver with low detection limit and good precision but very poor recovery. Therefore, the proposed method should be re-optimized and re-validated in future study.

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