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Ultrasonic-assisted Acid Digestion Prior to Determination of Iron, Zinc and Nickel in Chicken Gizzard

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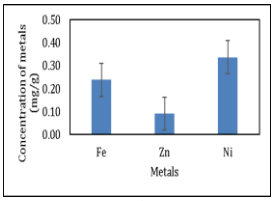
GRAPHICAL ABSTRACT



Concentration of trace metals in chicken gizzard

ABSTRACT

Trace metals are widely determined in various type of samples such as in industrial wastewater, sewage, soil sediment, poultry or in plants. Many analytical methods are focusing on sample preparation procedure to increase the efficiency of the extraction. Sample preparation is the most time-consuming part of analysis where the samples must be homogeneous. Most conventional techniques such as hot plate and dry ashing are aided with high temperature to aid in the solid sample's dissolution. Hence, the use of ultrasonic-assisted acid digestion has been recently developed as an efficient method for digestion of trace metals in biological sample. It is an alternative technique to conventional sample pre-treatment methods for shortened and simplified procedure prior to the measurement. Trace metals (Fe, Ni, and Zn) extracted from chicken gizzard samples using ultrasonic bath were determined by flame atomic absorption spectroscopy (FAAS). Three parameters which are type of solvents, time of sonication and weight of sample were studied using ultrasonic bath to obtain the optimum digestion condition. The optimized parameters (HNO3 - HCl solvent mixtures, 40 minutes sonication time and 0.2 g of sample weight were utilized to further analyzed trace metals in chicken gizzard. The concentration of Fe, Zn and Ni were successfully determined with values of (238.107 ± 0.019) mg/kg, (91.754 ± 0.061) mg/kg, and (335.813 ± 0.011) mg/kg respectively. For repeatability study, both intraday and interday measurement were 1.15% and 1.66% respectively. Optimization of ultrasonic-assisted acid digestion can be useful to increase the efficiency of the metal's digestion in biological samples prior to the determination by FAAS.

Keywords: Trace metals, ultrasonic-assisted acid digestion, ultrasonic bath, FAAS

1. INTRODUCTION

Trace metal refers to metal elements with relatively high density that are very known with toxicity that can affect the environment and organisms at higher level [1]. However, some heavy metals are essentials to organisms as it provides nutrient to maintain cells metabolism but only at lower concentration as they can cause toxic effects when the metal intake is overly consumed. The distributions of heavy metals in poultry animals may be influenced by their eating habits, growth, age, moulting, and they can also be affected from the contamination of poultry feeds, drinking water and processing [2]. However, trace metals such as iron, zinc calcium and magnesium have a very important role to play in the mechanism of nutrient circulation in the animal organs and they have been reported to be of bio-importance to human.

Atomic absorption spectroscopy (AAS) is a technique used in this study to determine metals and metalloids quantitatively by conversion of a sample to atomic vapor and measurement of absorption at a wavelength specific to the element of interest. AAS is one of the most known techniques for analysis of trace metals that covers all types of samples (environmental, biological and industrial).

A study conducted in Southern Nigeria showed that the level of Fe varied between 22.07 to 97.72 mg/kg for chicken meat; 19.28 to 45.72 mg./kg for gizzards and 14.14 to 35.03 mg/.kg for turkey meat [3]. The high levels of Pb in poultry products may results mainly from contamination of feeds and water sources. According to a study conducted by Okoye et al., the concentrations in ug/g of the heavy metals in poultry meat were in the range of 1.78 to 15.32, 9.70 to 147.07, 15.82 to 47.79 and 0.03 to 2.29 of Cd, Pb, Cu and Zn respectively [4]. Another study reported by Elsharawy in the evalutation of heavy metals residue in chicken meat in New valley, the mean concentrations of Fe in breast, thigh, liver and gizzard samples were 6.77± 0.24 µg/g, 7.49± 0.18 µg/g, 9.36± 2.96 µg/g and 5.85± 1.85 µg/g respectively while the mean concentrations of Cu were 0.15± 0.012 µg/g, 0.26± 0.008 µg/g, 1.16± 0.008 µg/g and 0.35± 0.003 µg/g respectively [5].

Sample preparation or sample pre-treatment is the crucial part of any analytical procedure because it is the most time-consuming step. Commonly, sample preparation involves the transfer of target analyte from solid to a liquid phase, and consequently preventing any interferences in further measurement of analyte. Ultrasonic-assisted sample digestion is one of the approaches that often used for accelerating and simplifying the sample treatment [6]. It can be a possible approach to other classical and non-classical digestion techniques [7]. Ultrasound energy produces acoustic cavitation which is the situation where the microbubbles are formed and collapse under an ultrasonic field. The collapse of the

microbubbles induces a high temperature within it in a short time when the microbubbles nearly having a constant pressure which it is called as adiabatic [8]. The schematic diagram of ultrasonication is well illustrated in in Figure 1. The transfer of ultrasound energy occurs when the sound impinges directly on the bottom of the flask and produces acoustic cavitation. The ultrasonic bath is one of most common instrumentation that is easily available for sonochemistry apart from ultrasonic probe.

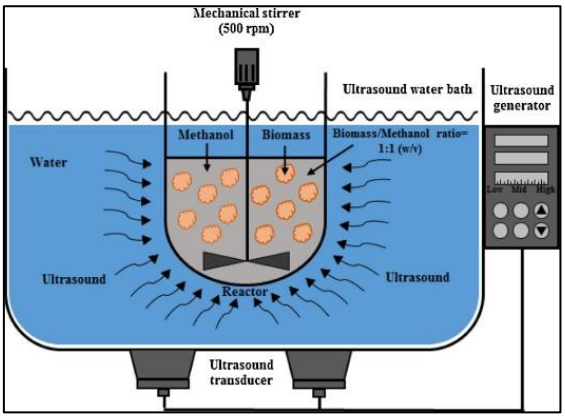


Figure 1. Schematic diagram of ultrasonication method

2. EXPERIMENTAL

The chicken gizzard samples were collected from local market and were cut into small pieces prior to the drying of the samples for 24 hours in an oven at 90°C. The samples were grounded into smaller particles size (≤ 140 µm) and were stored in a container at refrigerator until further use. The samples were digested using ultrasonic digestion method with optimized parameters which are the type of solvent (HNO_3, HNO_3 – HCl (1:3), HNO_3 – H_2SO_4 (1:1)), time of sonication (20, 40, 60, 80, 100 min) and weight of sample (0.2, 0.4, 0.6, 0.8, 1.0). The powdered samples were digested with 7 mL of 65% HNO_3; mixture of 65% HNO_3 and 37% HCl or mixture of 65% HNO_3 and 98% H_2SO_4 in an ultrasonic bath for 30 min (fixed condition for optimization of type of solvents) with temperature sample solution before it was sonicated for another 30 min. The optimum conditions for each parameter were obtained after analyzing the metal using flame atomic absorption spectroscopy and were utilized to further analyzed trace metals in chicken gizzard (Fe, Zn and Ni). In this study, a total of three types of method validation were used which include precision, limit of detection (LOD) and limit of quantification (LOQ), and percentage recovery. The precision method was done in intraday and interday. The LOD and LOQ were quantitively determined by measuring seven blank solutions. Table 1 shows the optimized parameters and the fixed variables.

Table 1. Parameters for optimization of ultrasonic-assisted acid extraction

Optimized parameters	Studied conditions	Fixed conditions
Type of solvents	HNO_3	HNO_3
	HNO_3 – HCl (1:3)	
	HNO_3 – H_2SO_4 (1:1)	
Sonication Time (min)	20, 40, 60, 80, 100	60
Weight of sample (g)	0.2, 0.4, 0.6, 0.8, 1.0	0.6

3. RESULTS AND DISCUSSION

3.1. Optimization of Solvent Types

In this study, the best solvent was selected based on the extraction that can give the highest absorbance by FAAS. Table 2 shows the concentration of Fe with different type of solvent. The best solvent to extract Fe in chicken gizzard was HNO_3 – HCl mixture as it shows the highest absorbance and concentration value compared to both HNO_3 and HNO_3 – H_2SO_4. Nitric acid is a strong oxidizing agent and it is widely used to extract trace metals from any biological sample. HNO_3 at high temperature can decompose most organic molecules and converts hydrocarbons into carbon dioxide and water [9]. However, according to a study reported by Doner and Ege, the uses of HNO_3 alone was not as effective enough to liberate trace metals in the sample due to its poor percentage recovery [10].

It was reported that oxidation of biological samples with high organic matter is usually incomplete with only HNO_3 and therefore, a combination of mixtures of acids such as with HCl or H_2SO_4 are necessary [11]. HNO_3 – HCl acid mixtures formed an aqua regia solution which is highly oxidative.

Table 2. Concentration of Fe at different type of solvents

Type of solvents	Concentration of Fe ($\bar{x}$ ± s.d) mg/kg
HNO_3	110.367 ± 0.009
HNO_3 – HCl	252.010 ± 0.019
HNO_3 – H_2SO_4	168.453 ± 0.028

3.2. Optimization of Sonication Time

For this study, the chicken gizzard sample was digested in mixture of HNO_3 – HCl at different sonication time for sample extraction. Fe shows the highest reading at sonication time of 40 min with temperature that was kept constant about 60°C. Table 3 shows the concentration of Fe at each respective time of sonication. In order to obtain good quantitative recoveries, the time of digestion must be at optimum. When using an ultrasonic bath, the temperature of digestion medium will slowly increase with the increasing of sonication time. As stated by Capote and Castro, the high temperature and pressure exerted within a collapsing cavitation bubble produced by ultrasound irradiation causes the formation of free radicals and other species [7]. However, a significant depletion of the recoveries might be obtained as the sonication time increases due to degradation of the compounds [12].

The extraction efficiency of Fe in the samples decreased slightly when sonication time was increased from 40 to 60 min and there was insignificantly decreased up to 100 min. This was perhaps due to the re-adsorption of Fe on the sample surfaces [13]. A study in the extraction of triazine herbicides observed a slight decrease in recovery values after increasing the sonication time more than 40 min, probably due to heating that might decompose the herbicides [14]. Therefore, in this study, 40 min was chosen as the best sonication time in ultrasonic digestion.

Table 3. Concentration of Fe at different sonication time

Time of Sonication (min)	Concentration of Fe ($\bar{x}$ ± s.d) mg/kg
20	250.223 ± 0.032
40	257.820 ± 0.008
60	89.812 ± 0.011
80	92.940 ± 0.074
100	96.068 ± 0.024

3.3. Optimization of Sample Weight

In this third parameter, the solvent used (HNO_3 – HCl mixture) with time of sonication (40 min) were kept constant throughout the experiment while five different weight (0.2, 0.4, 0.6, 0.8 and 1.0 g) of sample were studied. Table 4 shows the concentration of Fe at different weight of sample. In this study, 0.2000 g was chosen as the best sample weight as Fe was shown at the highest reading compared to the rest of the weight. There is a decreasing trend for concentration of Fe

when the sample weight was increased. This shows that increasing the sample weight did not help with the improvement of metal leaching from the sample.

There are few studies have reported that increasing the amount of sample while retaining a constant volume of acid solvent might diminish the extraction efficiency as the solvent is too low to extract the analytes efficiently [12]. Consequently, the weight of sample has to be increased along with the amount of extracting solvent in order to keep the ratio of sample to solvent constant.

Table 4. Concentration of Fe using different sample weight

Weight of Sample (g)	Concentration of Fe ($\bar{x}$ ± s.d) mg/kg
0.2000	465.215 ± 0.046
0.4000	231.160 ± 0.082
0.6000	194.686 ± 0.043
0.8000	131.884 ± 0.034
1.0000	120.000 ± 0.056

3.4. Digestion of Chicken Gizzard Sample Under Optimized Conditions

In this study, the optimum conditions that were studied previously was used for determination of Fe, Zn and Ni in chicken gizzard. The extraction of trace elements in chicken gizzard were done by dissolving 0.2000 g of powdered sample in mixture of HNO_3 – HCl and was sonicated for 40 min at temperature of 60°C. The concentration of studied metals is shown in Table 5. Ni has the highest concentration compared to Fe and Zn. Based on the results obtained, it shows that Fe has not exceeded the maximum permissible limit of elements whereas Ni and Zn concentration in chicken gizzard have exceeded the stated amount in Table 2. Hence, the consumption of chicken gizzard due to its abundant Fe content is good for blood production. This is because iron is found in the red blood cells that is called as haemoglobin where it helps to transport oxygen throughout our body. However, the consumption in excessive amount by human will lead to serious illness as the excessive amount of metals in chicken gizzard will affect human's biological system.

Table 5. Concentration of metals and the maximum permissible limit of elements

Metals	Concentration of Metals ($\bar{x}$ ± s.d) mg/kg	Maximum permissible limit of elements (mg/kg)
Fe	238.107 ± 0.019	425.500
Zn	91.754 ± 0.061	60.000
Ni	335.813 ± 0.011	67.900

3.5. Method Validation

Method of validation was studied for its precision, limit of detection, limit of quantification and percentage of recovery. Both intraday and interday measurement show a good relative standard deviation (%) as shown in table 3. The LOD and LOQ for Fe are 4.644 and 15.479 mg/kg respectively while the recovery value shows 89%. The higher recovery showed a very small bias that was affecting the method and hence increasing the trueness of the data. This shows that the method almost has a complete reaction during the digestion process where the reactants are almost completely converted to products with minimal losses of analyte when the sample was exposed to high temperature, light and solvent. Hence, due to the percent recovery falls in between the acceptable range, this method is suitable for low concentration of analyte in a sample.

Table 6. Measurement for intraday and interday

Day	Concentration of Fe ($\bar{x}$ ± s.d) mg/kg	Relative Standard Deviation (%)
Intraday	134.783 ± 0.006	1.15
Interday	138.619 ± 0.009	1.66

4. CONCLUSION

Ultrasonic-Assisted Acid Digestion (UAAD) using ultrasonic bath have been investigated for digestion of biological solid samples. This method enables operation at lower temperature which is safer compared to other conventional methods. Other than that, ultrasonic extraction is easier to use, easily available and relatively low cost. The variables affecting the performance of UAAD have been studied and discussed. The temperature of ultrasonic bath medium was kept at 60°C while the other parameters including type of solvents, time of sonication and weight of sample: were studied since they may influence the digestion efficiency. The optimized conditions that were obtained for the ultrasonic digestion of chicken gizzard sample were; HNO_3 – HCl mixture of solvents, 40 min of sonication time and 0.2000 g sample weight. The optimum conditions were applied to analyse the concentration of Fe, Zn and Ni in the chicken gizzard sample. The concentration of Fe, Zn and Ni were (238.107 ± 0.019) mg/kg, (91.754 ± 0.061) mg/kg, and (335.813 ± 0.0011) mg/kg respectively. Ni shows the highest metal concentration in chicken gizzard. The method validation was used to confirm if the analytical procedure employed for a specific test is suitable for its intended use. The precision for Fe metals in the sample were taken in the interval of 3 days. For precision, both intraday and interday measurement were (134.783 ± 0.006) mg/kg and (138.619 ± 0.009) mg/kg respectively. Both measurements show a minimal change with low relative standard deviation (%) which were 1.15% (intraday) and 1.66% (interday). The data also shows a good recovery percentage (89%) that makes the method more suitable to analyse low concentration of metals.

REFERENCES

[1] Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., and Sutton, D. J. (2012). 'Heavy metals toxicity and the environment', Molecular, Clinical and Environmental Toxicology, 101, 133-164

[2] Abduljaleel, S. A., Shuhaimi-Othman, M., and Babji, A. (2012). 'Assessment of trace metals contents in chicken (gallus gallus domesticus) and quail (coturnix coturnix japonica) tissues from selangor (malaysia)', Journal of environmental Science and Technology, 5(6), 441-451.

[3] Iwegbue, C. M. A., Nwajei, G. E., and Iyoha, E. H. (2008). 'Heavy metal residues of chicken meat and gizzard and turkey meat consumed in southern nigeria', Bulgarian Journal of Veterinary Medicine, 11(4), 275-280.

[4] Okoye, C.O.B., Ibeto, A. A. U., and Ihedioha, J. N. (2011). 'Heavy metals analysis of local and exotic poultry meat', International Journal of Applied Environmental Sciences, 5(1), 49-55.

[5] Elsharawy, N., and Elsharawy, M. (2015). Some heavy metals residues in chicken meat and their edible offal in new valley, 2nd Conference of Food Safety. August 2015. Suez Canal University, 53-60.

[6] Ashley, K., Andrews, R. N., Cavazos, L., and Demange, M. (2001). 'Ultrasonic extraction as a sample preparation technique for elemental analysis by atomic spectrometry', Journal of Analytical Atomic Spectrometry, 16(10), 1147-1153.

[7] Priego-Capote, F., and de Castro, L. (2007). 'Ultrasound-assisted digestion: A useful alternative in sample preparation', Journal of Biochemical and Biophysical Methods, 70(2), 299-310.

[8] Ashokkumar, M. (2011). 'The characterization of acoustic cavitation bubbles – an overview', Ultrasonics Sonochemistry, 18(4), 864-872.

[9] Priego-Capote, F., and Castro, D. L. (2007). ' Ultrasound-assisted sample digestion', Techniques and Instrumentation in Analytical Chemistry, 26, 69-97.

[10] Doner, G., and Ege, A. (2004). 'Evaluation of digestion procedures for the determination of iron and zinc in biscuits by flame atomic absorption spectrometry', Analytica Chimica Acta, 520(1), 217-222.

[11] Kazi, T. G., Jamali, M. K., Arain, M. B., Afridi, H. I., Jalbani, N., Sarfraz, R. A., and Ansari, R. (2009). 'Evaluation of an ultrasonic acid digestion procedure for total heavy metals determination in environmental and biological samples', Journal of Hazardous Materials, 161(2), 1391-1398.

[12] Gatidou, G., Thomaidis, N., Stasinakis, A., and Lekkas, T. (2007). 'Simultaneous determination of the endocrine disrupting compounds nonylphenol, nonylphenol ethoxylates, triclosan and bisphenol a in wastewater and sewage sludge by gas chromatography-mass spectrometry', Journal of Chromatography A, 1138(2), 32-41.

[13] Moreno-Cid, A., and Yebra, M. C. (2002). 'Flow injection determination of copper in mussels by flame atomic absorption spectrometry after on-line continuous ultrasound-assisted extraction', Spectrochimica Acta Part B: Atomic Spectroscopy, 57(5), 967-974.

[14] Rubio, M. G., Font, V. B., Díaz, A. M., and Cañada, M. J. A. (2006). 'Determination of triazine herbicides and diuron in mud from olive washing devices and soils using gas chromatography with selective detectors', Analytical Letters, 39(4), 835-850.