

## Validation Method of Flame-AAS with Microwave Digestion for Mineral Analysis in Carbohydrate-Rich Samples

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### ABSTRACT

One of the effective methods for mineral destruction is by using microwave digestion. However, some parameters in the microwave manual book are different with AOAC method such as the volume of acid, temperature, and the length of destruction time ( $\Delta t$ ). Thus, a validation for mineral analysis method of Fe, Zn, and Cu by using microwave digestion method is required. In this study, carbohydrate-rich samples such as rice and canned pineapple were being used for the validation of mineral analysis method of Fe, Zn, and Cu using microwave digestion. The parameters of this research include LOD, LOQ, LDM, linearity, precision, accuracy, and reproducibility. The accuracy result for Fe, Zn, and Cu mineral was found in the range of 75-120% with good precision value (% RSD < % 2/3RSD Horwitz). Based on the results, accuracy for mineral Fe, Zn and Cu found in the range of 75-120% with good precision value (% RSD < % 2/3RSD Horwitz). The regression curves of the Fe, Zn, and Cu minerals gave the value of  $R^2 > 0.990$ . For LDM values in the rice samples consecutively for Fe, Zn, and Cu minerals were 0,06; 0,07; 0,04 mg/L, while for the canned pineapple were 0,07; 0,10; 0,01 mg/L, respectively. The results of a modified method for minerals Fe, Zn and Cu analysis in carbohydrate-rich samples was not significantly different from AOAC (2012). Thus the method was acceptable to be used in laboratory analysis.

**Keywords:** F-AAS, microwave digestion, an essential mineral, method validation

### INTRODUCTION

The quality of foods product can be seen from the existence of essential minerals such as Fe, Zn, and Cu, etc. There are several analytical methods that can be used for the measurement of those mineral content includes *Flame-AAS* (Atomic Absorption Spectrophotometry with Flame). F-AAS is a quantitative method for mineral analysis based on the atomic absorption when the sample solution is aspirated and transforms into an aerosol (a small form of granules that has a larger surface area). The flame formed an evaporation process and produce crystal salts, and atoms in the ground state absorb electromagnetic waves from the light sources (cathode lamps) and move into excited state, the reduction amount of light intensity that reaches the detector is considered as a measure for the sample concentration (Khalid, Alaama, & Abbas, 2016). One of a critical phase in this analysis is the reshuffled of an organic form on to solved inorganic and ready to be analyzed by the F-AAS.

There are two methods of sample destruction for food mineral analysis; those are dry digestion and wet digestion methods. Dry

digestion method is a reshuffle from of organic minerals in the sample into inorganic minerals by sampling paths in a muffle furnace and requires a certain heating temperature. Generally in this dry destruction requires a heating temperature between 400-800 °C (Bragg & Xue, 2011). The flaw in this method is time-consuming it takes 15 minutes to 8 hours (Taylor, Sneddon, Hardaway, Bobbadi, & Avanthi, 2006). It also might cause some loss of mineral components due to evaporation or adsorption in the furnace wall. Wet digestion is the reshuffling of the sample using acid or acid mixture and heating (Korn, Morte, Santos, & Castro, 2008). It is not so environmental friendly besides some minerals might be lost due to evaporation. Microwave digestion method is the process of destruction in a sealed vessel using a mixture of the oxidizing solution at 300 °C and 199 bar (No, 2012). It is an enclosed system which is fast, easy and gives accurate analysis result (Belay & Tadesse, 2014).

Microwave method which is used for mineral analysis usually comes with its manual which contains the digestion working instruction. Mineral digestion described in the

manual book is not always the same with those of AOAC standard method AOAC 999.10 (2012). The differences might comprise the volume of reagents used ( $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$ ), temperature, and the duration of the digestion. Different food matrix might also cause different results of the digestion process, which will result in different mineral analysis results. For the above-mentioned reasons, we conducted validation of microwave digestion method for mineral analysis of carbohydrate-rich samples such as rice and pineapple. In addition to containing carbohydrate components, rice and pineapple can also serve as a source of essential minerals. This study aims to (1) validate the mineral analysis of Fe, Zn and Cu using F-AAS with microwave digestion; (2) obtaining mineral content data of Fe, Zn, and Cu on rice and canned pineapple samples using the validated method.

## EXPERIMENTAL SECTION

The research was conducted in five stages: food sampling; instrument performance test; analysis of mineral content by standard AOAC method and modification method (described in the manual book microwave digestion); while for validation parameters of Fe, Zn and Cu mineral analysis including linearity, precision, reproducibility, precision-accuracy, LOD (Limit of detection) and LOQ (Limit of quantitation). The validated method was then used to analyze the content of Fe, Zn and, Cu in rice and canned pineapple.

### Materials and Instrumentation

The materials in this study used standard pure analysis, Aquadest, Nitric Acid ( $\text{HNO}_3$ ) (Merck KgaA), Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ) 30% (Merck KgaA), standard solution Zn 1000 ppm, standard solution Fe 1000 ppm, standard solution of Cu 1000 ppm (CertiPUR Merck KgaA).

Close Microwave Digestion type MDS - 6G SINEO, Flame-AAS type Shimadzu AA 2000 and hollow cathode lamp Fe, Zn, Cu. Glass tools are washed with soap and brushed, then cleaned. Microwave vessels were also regularly cleaned by 50% nitric acid solution for 10 min at 175 °C (Adams, Chaudri, Rousseau, & Mcgrath, 2010).

### Samples

Samples of rice and canned pineapple obtained from local supermarkets in Bogor.

The canned pineapple has the same brand, expiration date, and production batch. Fat content in the sample should be low (<40%) (Cheng & Capar, 2014). The rice sample was washed with demineralized water and put in the oven at 80 °C for 6 hours, then mashed by mortar up to 60 mesh size (Kariyawasam, Godakumbura, Prashantha, & Premakumara, 2016). The rice was stored in the airtight package at room temperature while the blender canned pineapple sample stored in the freezer. Sampling for rice was done by quartering method and canned pineapple was by homogenization method.

## Procedure

### Sample Characteristics

#### Homogeneity Test

Homogeneity tests were carried out by taking samples from a different package, homogenized then divided and incorporated into several polyethylene containers. A randomly packed ( $n = 10$ ) sample then drawn from each other (subsample), homogenized and then two parts were taken for the analysis of ash content in duplicate (Fiamegos et al., 2016). The value of sampling variance and variance of repeatability analysis calculated as MSB (mean square between or differences between samples) and MSW (mean square within or differences= in the sample) (Sunanti, Samin, & Supriyanto, 2013).

#### Analysis of Ash and Water Content (AOAC, 2012)

Determination of ash content in the sample was done by AOAC 900.02A method while for water content test using AOAC 934.06 and 925.45-gravimetry vacuum oven method.

### Mineral Analysis

#### Standard Method (Microwave Digestion AOAC 999.10-2012)

Samples weighed 0.5 grams (dry weight). The weighed sample placed in a vessel and then added five mL of  $\text{HNO}_3$  65% and 2 mL of  $\text{H}_2\text{O}_2$  30%. Microwave program settings consist of 4 stages: 300W/3min/30 °C; 600W/5min/150 °C; 500W/22 min/180 °C; 300W/15min/200 °C. After the digestion process completed, the solution is cooled and diluted with demineralized water at a 25 mL flask. The above working steps are also carried

out for blanks (nitric acid and hydrogen peroxide).

#### **Modification Method**

The sample weighed as much as 0.5 grams (dry weight) then seven mL of HNO<sub>3</sub> (p) was added and allowed to stand for 20 min, added two mL HNO<sub>3</sub> (p) and last one mL H<sub>2</sub>O<sub>2</sub> 30%. The process of digestion with microwave finish with three stages: 300 W/10 min/30 °C; 600 W/5 min/150 °C; 500 W/15 min/180 °C for rice and 300 W/10 min/130 °C; 600 W/5 min/150 °C; 500 W/15 min/190 °C for canned pineapple. After the digestion process was complete, the solution was allowed to reach room temperature then transferred into a 25 mL flask and homogenized with demineralized water. The preparation of the blank solution (nitric acid and hydrogen peroxide) is carried out by the same procedure as the sample.

#### **Validation of Analysis Methods**

##### **Precision Test**

This test is measuring the concentration of samples as much as seven times replication for the measurement of 3 types of minerals (Fe, Cu and, Zn). The value determined by calculating% RSD analysis. Precision is good if the RSD value analysis is <2/3 CV Horwitz.

##### **Reproducibility**

Reproducibility determined by measuring the concentration of samples in the same laboratory, the same operator at different times. Furthermore, the RSD value of the analysis compared with the RSD Horwitz value. Reproducibility is accepted if it has an RSD value worth smaller than the RSD Horwitz value. Test results at two different weeks tested by using t-test.

##### **Accusations**

This section done by using a sample that spiked with a standard solution. The experimental concentration of spiking is the percentage of the sample concentration (80% is low), 100% is medium, and 120% is high) which is between the linearity ranges. Added standard performed seven repetitions. Spiking was done at the beginning of the preparation before the microwave digestion. Accuracy determined by calculating percent recovery. Good accuracy is within the range of 75-120%.

##### **Linearity**

Instrument linearity performed by measuring the absorbance of standard solutions

of minerals (Fe, Zn and, Cu) at various concentrations. The linearity test of Fe and Cu minerals used 0 - 0,5 mg/L while Zn mineral concentration was 0 - 1,0 mg/L. The absorbance of each standard solution was measured and performed repetition three times.

#### **Limit Detection (LOD) and Quantitation Limit (LOQ)**

The values of LDI (Limit detection of instrument) and LOQ can obtained from linearity analysis data by determining the standard of calibration error. Theoretical LDI obtained from the formula  $[3 (sy/x)]/b$ . As confirmation was made by seven replicates measurement of the solution according to LDI concentration for each minerals, the LDI value was accepted if the analysis result did not give precision value and accuracy.

The theoretical LOQ derived from  $[10 (sy/x)]/b$ . Confirmation of LOQ values done by making a solution that has a concentration of theoretical LOQ value and measured with seven replicates for each mineral. LOQ is accepted if the data provides an acceptable precision and accuracy value. The LDM (Limit detection of the method) value determined by measuring seven replications of the mineral concentration in the sample prepared according to the theoretical LDI values. The LDM value is accepted if the analysis results do not provide acceptable precision and accuracy value (Riyanto, 2014).

## **RESULTS AND DISCUSSION**

Before to the measurement, the feasibility of F-AAS test equipment must be done to produce valid data such as determination of flow rate that should not be too big or too small because it will affect the magnitude of flame and atomization process. The sensitivity of the tool is done by using copper 2 ppm solution. The results obtained are 0.0352 ppm below the maximum response requirement of 0.04 ppm (Supriyanto, 2007) so the F-AAS can be using for analysis. Copper 2 ppm solution is used for sensitivity test because it has the ionization potential which is not too small or too big, so it is not easily ionized in flame and is stable, besides Copper, the lead solution also proper for the test.

#### **Test of Sample Characteristics**

The Water content in rice obtained at 9.14%. This is an acceptance with the

standards of SNI 6128-2015 (BSN, 2015) with a maximum water content of 14%. The water content in canned pineapple obtained 83.41% according to the requirements of USDA 09269 (USDA, 2016) with a maximum water content of 85.73%.

#### Sample Homogeneity Test

The sample homogeneity test was conducted to ensure that the mineral content in each part of the sample was the same and did not contain an interrupt matrix. The values of F test for rice and canned pineapple each is 1.13 and 0.72. From the analysis results F test is  $<F$  table  $\alpha = 5\%$  of 3.02. From both, the results can be stated that the sample has been homogeneous.

#### Mineral Analysis

Digestion is process destruction of organic forms into inorganic forms (Taufiq, Sabarudin, & Mulyasuryani, 2016). Elements such as C, H, O, N, S and, P will turn into water-soluble oxides such as  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{NO}_2$ . Close digestion with microwave has several advantages such as requires fewer time, less reactive reagents, more accurate analysis results (Altundag & Tuzen, 2011) reduces contamination in the sample and reduces the loss of volatile elements (Korn et al., 2008). The digestion process finished marked by the production of a clear solution, which indicates that the organic compound reform is going well. The sample solution is ready to be analyzed using F-AAS. The F-AAS is recommended for use in measuring minerals because of good sensitivity with a detection limit of about 1 ppm and low operational costs (Khalid et al., 2016) (Demirel, Tuzen, Saracoglu, & Soyak, 2008).

Microwaves are radiation of non-ionic electromagnetic waves, exposed molecules by radiation of microwave rotation of moment dipole without any changes in molecular structure (Belay & Tadesse, 2014). Microwaves have frequencies of 300 GHz-300 MHz with a wavelength of 1mm-1meter. The

efficiency of the destruction process by using microwave digestion effected by the volume of acids ( $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$ ), time and temperature (Momen, Zachariadis, Anthemidis, & Stratis, 2007).

The process in the destruction with microwave digestion occurs with the mixed sample with acid that inserted within the closed vessel, and then the resulted microwave radiation causes an increase of temperature and pressure. Temperature rise between 30-190 °C and rising of pressure between (1,1-2,2 Mpa in a low pH increase the speed of sample decomposition and solubility in the form of organic matter and convert the sample from the solid to the solution (Yuyun, Peuru, & Ibrahim, 2017). The high pressure on the vessel will increase the boiling point of the nitric acid thus increasing the oxidation power and accelerating the reaction. The oxidizing agents using  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  oxidize minerals into their salt form. The use of mixed acid is better than that of a single acid for mineral destruction processes (Idera, Omotola, Adedayo, & Paul, 2015). Nitric acid is being used by the digestion process will be an incomplete result in a cloudy solution (Yang, Yan, Cao, & Zhang, 2012). The gradient heating system aims to improve the reaction between NO and  $\text{O}_2$  to  $\text{NO}_2$  (Tiago et al., 2015). The resulting  $\text{NO}_2$  is reabsorbed in acid solution and regenerates  $\text{HNO}_3$ . The addition of  $\text{H}_2\text{O}_2$  will increase the presence of  $\text{O}_2$  in the vessel (No, 2012).

Carbohydrates in rice formed as a complex starch polymer (amylose and amylopectin). It will swell when contact with water so that the destruction process starts from 30 °C to 180 °C gradually. The canned pineapple contains no starch polymer but has a simple sugar content composed of glucose, fructose and, sucrose which has a simpler structure compared to polysaccharides making it easier to crush. Therefore, the digestion process starts at 130 °C to 190 °C gradually.

**Table 1.** The precision test result of mineral analysis

Sample	Mineral Concentration (mg/kg)					
	Rice			Canned Pineapple		
	Cu	Zn	Fe	Cu	Zn	Fe
RSD (%)	4.18	2.17	6.44	6.44	5.64	3.54
2/3CV Horwitz	9.97	6.99	7.22	7.22	11.33	6.62

**Table 2.** Measurement result for LOD and LOQ

Mineral	LDI (mg/L)	LOQ (mg/L)	LDM (mg/L)	
			Rice	Canned Pineapple
Cu	0.01	0.1	0.04	0.01
Zn	0.02	0.2	0.07	0.10
Fe	0.05	0.1	0.06	0.07

According to (Roha, 2012) the content of glucose and fructose in pineapple fruit is 0.51% and 0.83%. Before the destruction process, both the sample of rice and the canned pineapple are done "pre-destruction" stage (given time lag 20 minutes before the digestion) so that the sample reacts with the oxidizing acid. At high temperatures, mineral ions will activate oxygen atoms from starch which will remove hydrogen from carbon atoms in the glucose unit (Costa et al., 2011). The longer the microwave heating will increase the temperature inside the vessel, lowering the condensation process and eventually increasing the concentration of minerals in solution (Bakkali, Ramos, Souhail, & Ballesteros, 2009)(Bakkali et al., 2009).

The presence of minerals can affect the quality of foodstuffs such as copper and iron play a role in oxidation-reduction reactions because their oxidative potentials can cause undesirable changes in processed foods associated with browning reactions and fat oxidation resulting in a decreased nutritional value (Damodaran, Parkin, & Fennema, 2007). Besides iron plays a role in the color changes that occur in meat ( $\text{Fe}^{2+}$  red,  $\text{Fe}^{3+}$  brown). Also, zinc can stabilize the color on canned green beans.

Mineral content of Fe, Zn and, Cu with the standard method and modification obtained  $p\text{-value} > \alpha = 5\%$  so stated both methods produce data that is not significantly different.

The results of the analysis according to, the mineral content of Fe, Zn and Cu in pineapples respectively are 0,5-30 mg/kg; 0,05-22 mg/kg; 0,04-10 mg/kg.

### Validation of Analysis Methods

#### Precision

Precision test results can be seen in **Table 1**. From the table, it can be seen that the precision for Fe, Zn and Cu for rice and pineapple samples each yield a value of % RSD  $< 2/3$  CV Horwitz.

#### LOD and LOQ

The detection limit divided into the instrument detection limit (LDI) and the detection limit method (LDM). LDI is the smallest concentration of the analyte that can still be detected by the instrument while the LDM is the smallest concentration of an analyte in the sample still be detected. Detection limits testing using a calibration curve. Quantitative limits are the lowest concentrations of an analyte that can determined with an acceptable level of precision and accuracy (quantity can be determined), the LOQ value is the lower limit of the calibration curve. The measurement results for LDI and LOQ can be seen in **Table 2**. The LDI values for the three minerals are smaller than those of Silvestre MD et al. (2000) whereas LDI for Cu, Fe and Zn are 0,07 mg/mL; 0,07 mg/mL; 0,11 mg/mL.

**Table 3.** The test result for the determination of reproducibility from 1<sup>st</sup> week and 2<sup>nd</sup> week and.

Repeat	Content (mg/kg)					
	Cu		Zn		Fe	
	1	2	1	2	1	2
<b>Rice</b>						
Average	0.94	0.87	24.34	24.48	24.72	25.01
P-value	0.122		0.712		0.546	
<b>Canned Pineapple</b>						
Average	0.43	0.41	0.67	0.69	23.82	24.11
P-value	0.167		0.299		0.375	

Acceptance requirement:  $p\text{-value} > \alpha 95\%$

**Table 4.** Accuracy of mineral from rice and canned pineapple

mineral	spiking 80%	Recovery (%)			Canned Pineapple		
		Rice 100%	120%	80%	100%	120%	
Fe	92.16	89.78	82.05	92.28	85.53	84.14	
Cu	82.87	82.06	81.97	89.18	84.95	99.91	
Zn	84.44	85.89	86.85	93.56	103.74	90.88	

Acceptance requirement: 75-120%

### Linearity

The regression equation produced for Fe :  $y = 0.084x - 0.0002$  with  $R^2 = 0.9970$ ; Cu :  $y = 0.1335x - 0.0004$  with  $R^2 = 0.9969$ ; Zn :  $y = 0.4125x + 0.0086$  with  $R^2 = 0.9977$ . The test shows good linearity, there is an increase of absorbance response to the increase of mineral concentration in standard solution. The linearity curve obtained has a value  $R^2$  greater than 0.990. This value corresponds to the acceptability limit.

### Reproducibility

Test results for reproducibility can seen in **Table 3**. The test results show the  $p$ value >  $\alpha$  5% for all minerals in rice and canned pineapple with no conclusion significantly different. The sample yields a mineral content that is not different if measured at different times.

### Accuracy

Accuracy aims to see the efficiency of methods based on recovery of standard solutions added to the sample (spiking) (Ajai, Ochigbo, Abdullahi, & Anigboro, 2014). The results of the test for the mineral accuracy of Fe, Zn and Cu are shown in **Table 4**. Range value from the three minerals are from 80-100%, the results are still within the acceptance limit of AOAC about 75-120%. Indicates that analytical procedures with modification methods for all mineral are acceptable. % RSD analysis results in less than 2/3 CV Horwitz.

### CONCLUSION

The method of modification in the process of digestion for the analysis of minerals Fe, Zn and Cu in high carbohydrate samples (rice and canned pineapple) was not significantly different from AOAC (2012) and has acceptably qualified for use in laboratory analysis.

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