

## Validation of Mineral (Fe, Zn, and Cu) Analysis Methods in Carbohydrate, Protein and Fat-Rich Samples Using Microwave Digestion Method

Didah Nur Faridah<sup>1,\*</sup>, Imas Solihat<sup>1,2</sup>, and Nancy Dewi Yuliana<sup>1</sup>

<sup>1</sup>Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Jl. Tanjung, Kampus IPB Dramaga, Bogor 16680, Indonesia

<sup>2</sup>AKA Bogor Polytechnic, Jl. Pangeran Sogiri No. 283, Tanah Baru, Bogor 16154, West Java, Indonesia

\* **Corresponding author:**

email: didah\_nf@apps.ipb.ac.id

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**Abstract:** Destruction process using both dry and wet conditions serves as a significant step in mineral quantification. Wet destruction using microwave-assisted digestion refers to the standard method of AOAC (2012); in this work, we modified the destruction procedures provided in the microwave digestion manual book, including temperature, reagent volume (HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>), and length of destruction. The experiment works aimed (1) to validate the procedures in destruction process using microwave digestion; (2) to quantify Fe, Zn and Cu in various food matrices (canned peas, canned fish, full cream powdered milk) using a validated method. The method was evaluated according to linearity, accuracy, precision, absolute and relative LOD, LOQ, and intra-reproducibility. Measurement of Fe, Zn and Cu were considered to have a satisfying accuracy at a range of 80–115%, with a good precision value (% RSD < 2/3 CV Horwitz), while regression curves R<sup>2</sup> > 0.995. The results showed that data collected from the modified method was not significantly different compared to those from the AOAC method. The currently developed method also fulfilled the acceptability requirements for laboratory analysis.

**Keywords:** minerals; F-AAS; method validation; microwave digestion

### ■ INTRODUCTION

The constituents of food, including water, carbohydrates, proteins, vitamins, fats, and minerals, may affect the properties of the food. Specifically, essential minerals (Fe, Zn, and Cu) [1-5] are closely related to some disadvantageous effects on food such as rancidity, changes in color, taste, and aroma. Flame-AAS is commonly used for measuring mineral levels [6-10], in which the destruction process is considered as one of the most important steps [11], often carried out in dry and wet conditions [12-14]. Dry destruction accounts for the loss of some mineral components in the furnace, while it is also a time-consuming process [15-16]. Meanwhile, open system wet destruction contributes to contamination towards the environment, and certain minerals can be lost due to evaporation. On the other hand, wet destruction with the closed system using microwave-assisted digestion has some advantages, such

as being rapid, easy, and produce accurate assessment [17-19], while also using less reagent.

In our experiment, the destruction method using microwave-assisted digestion is found to be more efficient in regards to the processing time, amount of solvent, and temperature, and regarded as a more renewable technique in comparison with the method prescribed by AOAC 2012. Based on the SOP for microwave digestion, destruction procedures may vary depending on the types of food matrices. In contrast, the procedure method of AOAC 2012 is applied for all types of sample, regardless of food matrices. The results of the analysis between the AOAC method and the modified method were not significantly different, resulting in a positive analysis gap.

The method for mineral destruction in this study which was derived from the manual of a microwave digestion device is different from the standard AOAC method 999.10 (2012) [20]. The differences included

reagent volume, temperature, and length of destruction. Different food matrices should require a specific treatment during the destruction process. For this reason, the food samples were selected according to the food matrix triangle (AOAC 2012), resulting in three main groups of food, i.e. protein-rich food (canned fish), fat-rich food (full cream milk powder) and carbohydrate-rich food (canned peas). The selection of canned food samples aimed to reduce factors that can cause instability in the method validation. The modified method was acceptable as long as it met the requirements after compared with the standard method [21].

The objective of this study was (1) to validate the quantification method of Fe, Zn and Cu using F-AAS with microwave digestion, and (2) to obtain data on the mineral content of Fe, Zn and Cu on several different food matrices using the validated method.

## ■ EXPERIMENTAL SECTION

### Materials

The chemicals used were analytical grade, including aquademineral, HNO<sub>3</sub> (p) (Merck KgaA), H<sub>2</sub>O<sub>2</sub> 30% (Merck KgaA), standard Zn solution of 1000 ppm (CertiPUR Merck KgaA, no 1.19806), standard Fe solution of 1000 ppm (CertiPUR merge KgaA, no.1. 19781), standard Cu solution of 1000 ppm (CertiPUR merge KgaA, no.1.1986).

The food samples (canned peas, full cream powdered milk, and canned fish) were collected from similar brands, expired dates, and production batches. The powdered milk was stored in airtight packaging at room temperature (quartering sampling method), while canned peas and canned fish were stored in the freezer (homogenization sampling method) (FDA 2014) [22]. Homogenized samples were placed in 10 polyethylene bags, and their ash content was analyzed in duplicate [23]. The homogeneity of these samples was checked using different test criteria (F test) with the provision of  $F_{\text{count}} < F_{\text{table}}$ . Ash content test was achieved using the standard method of AOAC 900.02A.

### Instrumentation

The instruments used were Close Microwave Digestion type SINEO MDS-6G, Flame-AAS type Shimadzu AA 2000 and hollow cathode lamp of Fe, Zn, and Cu. The F-AAS setting is presented in Table 1.

### Procedure

#### Sample digestion procedure

Sample (0.5 g dry basis) was added with HNO<sub>3</sub> (p) and H<sub>2</sub>O<sub>2</sub> 30%. The standard method setting of destruction and modification is presented in Table 2. After completion of digestion, the sample was homogenized in demineralized water in a 25 mL flask.

**Table 1.** Setting of Flame-AAS

Mineral	Wavelength (nm)	Fuel	Burner height (mm)	Fuel flow (L/min)	Lamp current (mA)	Slit bandwidth (nm)	Sensitivity Cu 2 ppm
Fe	248.3	Air-C <sub>2</sub> H <sub>2</sub>	9.0	2.2	4.0	0.2	0.0352
Zn	213.9	Air-C <sub>2</sub> H <sub>2</sub>	7.0	2.0	5.0	0.7	
Cu	324.8	Air-C <sub>2</sub> H <sub>2</sub>	7.0	1.8	8.0	0.7	

**Table 2.** The setting of microwave digestion

Sample	Standard method	Modified method
Full cream milk powder		130 °C/10 min/300 W
		150 °C/5 min/600 W
		180 °C/5 min/500 W
	30 °C/3 min/300 W	200 °C/10 min/300 W
Canned peas	150 °C/5 min/600 W	130 °C /10 min/300 W
	180 °C/22 min/500 W	150 °C/5 min/600 W
	200 °C/15 min/300 W	180 °C/15 min/500 W
Canned fish		130 °C/10 min/300 W
		150 °C/5 min/600 W
		180 °C/15 min/500 W

Control solution (comprising of nitric acid and hydrogen peroxide) was made according to the same procedure as the sample.

#### **Validation of analysis methods**

The developed method was evaluated for its acceptability according to the following indicators, i.e., linearity, relative and absolute LOD, LOQ, precision, accuracy, and intra-lab reproducibility.

**Precision test.** This test is done by measuring the concentration of samples as much as 7 times replication for the measurement of 3 types of minerals (Fe, Cu, and Zn).

**Intra-lab reproducibility.** Intra-lab reproducibility is determined by measuring the concentration of samples in the same laboratory, by the same operator at different times. Each test was performed with three replications. Furthermore, the RSD value of the analysis is compared with the RSD Horwitz value. Intra-lab reproducibility is satisfactory if it has an RSD value smaller than the RSD Horwitz value. Test results at two different weeks were tested using the t-test.

**Accuracy.** The test is performed using a sample spiked with a standard solution. The experimental concentration of spiking is the percentage of the sample concentration that is 80% (low concentration), 100% (medium), and 120% (high). Spiking is done at the beginning of the preparation before the microwave digestion. Accuracy is determined by calculating percent recovery.

**Linearity.** Instrument linearity was performed by measuring the absorbance of standard mineral solutions (Fe, Zn, and Cu) at various concentrations. The linearity test of Fe and Cu minerals used concentration 0–0.5 mg/L while Zn mineral concentration was 0–1.0 mg/L. Absorbance of each standard solution was measured and repetition was performed three times.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ).** The values of LDI (Limit detection of instrument) and LOQ can be obtained from linearity analysis data by determining the standard value of calibration error ( $sy/x$ ). Theoretical LDI is obtained from the formula  $[3 (sy/x)]/b$ . Confirmation was conducted by  $7\times$  repeated measurement of the solution according to the theoretical LDI concentration for 3 types of minerals (Fe, Zn and

Cu), The theoretical LOQ is derived from  $[10 (sy/x)]/b$ . Confirmation of LOQ values was performed by making a solution with a concentration of the theoretical LOQ value and measured with seven replicates for each mineral (Fe, Zn, and Cu). The LDM (Limit detection of method) value is determined by measuring seven replications of the mineral concentration in the sample prepared according to the theoretical LDI values.

#### **Data analysis**

Data were expressed as mean, followed with deviation standard and relative standard deviation (RSD). Furthermore, statistical analysis was used to determine the p-value (independent t-test) in intra-lab reproducibility. Results obtained from the modified method were compared to the standard method.

## ■ RESULTS AND DISCUSSION

### **Preparation of Sample**

Prior to destruction (by microwave digestion), the sample size needs to be reduced, allowing to produce a larger surface area and enhance the reaction rate. Samples containing organic solvents, such as wine, must be evaporated up to a fifth of the initial volume since the solvent can induce an explosion.

The selection of food matrix is based on major components such as carbohydrate, protein, and fat, arranged into the food matrix triangle (Fig. 1). The proximate analysis was in accordance with the placements on literature studies (USDA 2006) [24], exhibited in Table 3.

Furthermore, the water content in all samples (Table 4) was in the range of SNI and USDA standard. Moisture level in canned fish was found to be higher compared to literature USDA 15089, which might be ascribed to the difference in the type of sample. The determination of water content was conducted using a vacuum oven method. Vacuum oven drying was conducted at low temperature and pressure, in order to retain volatile components present in the samples tested [25].

The homogeneity test is performed by measuring ash content, enabling to ensure that the sample tested to contain a comparable amount of mineral and does not

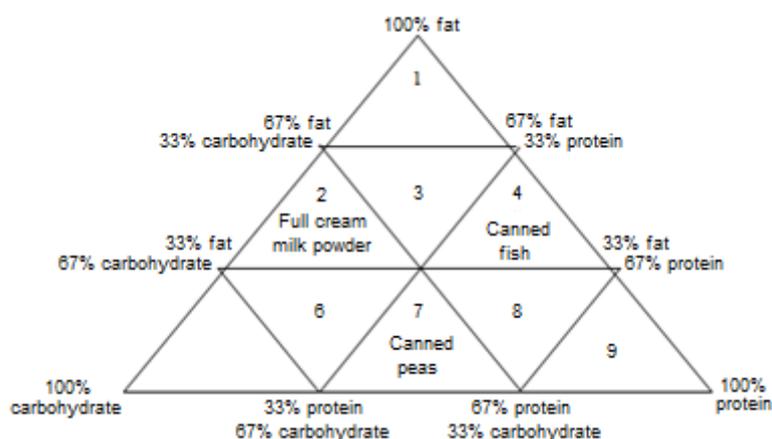


Fig 1. Food triangle

Table 3. Proximate composition based reference

Sample	Content (g/100g)					
	Water	Protein	Fat	Carbohydrate	Fiber	Total sugar
Canned peas	85.13	3.01	0.48	10.60	3.3	3.20
Canned fish	66.86	20.86	10.45	0.54	0.1	0.43
Full cream powdered milk	2.47	26.32	26.71	38.42	0	38.42

Source: USDA 2016

Table 4. Water content

Sample	Water content (%)	
	Analysis result	Reference
Full cream powdered milk	4.26	max 5 (SNI 2970-2015)
Canned peas	80.16	85.13 (USDA 11306)
Canned fish	76.54	66.86 (USDA 15089)

Table 5. Sample homogeneity test

Subject	Ash content (%)					
	Full cream milk powder		Canned peas		Canned fish	
	1 (ai)	(bi)	1 (ai)	2 (bi)	1 (ai)	2 (bi)
average	6.11	5.99	1.25	1.23	1.98	1.99
MSB	0.01		0.001		0.001	
MSW	0.01		0.001		0.001	
F <sub>count</sub>	0.96		0.87		1.38	

Term of acceptance:  $F_{\text{count}} < F_{\text{table}} (3.02)$ 

contain disturbing matrix (Table 5). Ash content can be used as an illustration of the minerals contained in the sample [26]. The results demonstrated that the F value was 3.02, being lower than F table 5%, indicating the acceptable homogeneity.

## Validation of the Methods

### Precision

Table 6 presents the satisfying precision for the determination of Fe, Zn, and Cu in all samples, which was

indicated by % RSD < 2/3 CV Horwitz. This result displays that all random errors can be controlled. The random errors include instability of instrument performance, changes in temperature, the difference in techniques, and analysts.

### LOD and LOQ

The indicators for detection limit are absolute LOD (instrument LOD) and relative LOD (method LOD). Absolute LOD represents the smallest concentration of

**Table 6.** Precision test results

Sample repeat	Mineral concentration (mg/kg)								
	Full cream powdered milk			Canned peas			Canned fish		
	Cu	Zn	Fe	Cu	Zn	Fe	Cu	Zn	Fe
average	0.87	24.34	24.72	1.35	7.01	2.82	0.95	9.42	23.33
SD	0.05	0.50	0.53	0.02	0.06	0.06	0.05	0.07	0.18
RSD (%)	6.79	2.07	2.14	1.63	1.63	0.89	4.89	0.76	0.77
2/3CV Horwitz	10.77	6.60	6.58	10.19	10.20	7.96	10.75	7.61	6.64

Term of acceptance: %RSD < 2/3 CV Horwitz

analyte that can still be detected by the instrument, while relative LOD is the smallest concentration of an analyte in the sample that can still be detected and the results are presented in Table 7. Limit detection testing was carried out by using a calibration curve with the formula  $3[sy/x/b]$ . LOQ value constitutes the lowest limit of the calibration curve.

The results for relative LOD can be seen in Table 8. The absolute LOD values for the three minerals examined were smaller than those reported by a previous study [12],

reaching 0.07 mg/mL for Cu, 0.07 mg/mL for Fe, and 0.11 mg/mL for Zn. It means the methods have good sensitivity. The relative LOD is essential in dealing with constraints due to different signals/responses for each food matrix.

#### Linearity

The regression equation for Fe, Cu, and Zn was presented as follows:  $y = 0.084x - 0.0002$  with  $R^2 = 0.9970$ ;

**Table 7.** Absolute LOD and LOQ

Mineral		Absolute LOD (mg/L)	LOQ (mg/L)
Cu	average	0.01	0.1
	SD	0.0021	0.0032
	RSD (%)	16.60	3.28
	2/3CV Horwitz	5.14	15.18
Zn	average	0.02	0.2
	SD	0.0012	0.0047
	RSD (%)	17.52	2.38
	2/3CV Horwitz	22.70	13.64
Fe	average	0.05	0.1
	SD	0.0236	0.0081
	RSD (%)	52.10	7.61
	2/3CV Horwitz	17.00	22.40

**Table 8.** Relative LOD

Mineral		Relative LOD (mg/L)		
		Full cream powdered milk	Canned peas	Canned fish
Cu	average	0.03	0.07	0.01
	SD	0.0026	0.1578	0.0306
	RSD (%)	8.77	212.18	307.43
	2/3CV Horwitz	18.12	15.77	21.35
Zn	average	0.1	0.04	0.06
	SD	0.0853	0.0180	0.0042
	RSD (%)	70.67	44.12	6.78
	2/3CV Horwitz	14.66	17.26	16.23
Fe	average	0.06	0.09	0.06
	SD	0.0140	0.0173	0.0210
	RSD (%)	23.65	18.88	35.31
	2/3CV Horwitz	16.32	15.29	16.31

$y = 0.1335x - 0.0004$  with  $R^2 = 0.9969$ ;  $y = 0.4125x + 0.0086$  with  $R^2 = 0.9977$ , respectively. As exhibited in Table 9, the experimental result showed satisfying linearity, in which the higher concentration of tested mineral corresponded to the rise of absorbance.

The intercept for Fe, Zn, and Cu reached -0.0002, 0.0086 and -0.0004, respectively, suggesting the occurrence of systematic errors in F-AAS instrument. Furthermore, the  $R^2$  value was  $>0.990$ , which is in accordance with the acceptance limit, as depicted in Fig. 2.

### Intra-lab reproducibility

Table 10 presents the intra-lab reproducibility, showing  $p$ -value  $> \alpha$  5% for all minerals in canned fish, canned peas and full cream powdered milk, suggesting that the value did not significantly differ [27].

### Accuracy

Accuracy represents the method efficiency based on recovery from standard solutions added to the sample (spiking). The results showed that the accuracy of Fe, Zn, and Cu levels varied from 80 to 100%, which is still in the range of acceptability limit of AOAC (75–120%). This indicates that the procedure in the modified method is acceptable for all minerals. Result of the recovery test can see in Table 11.

### Mineral Analysis

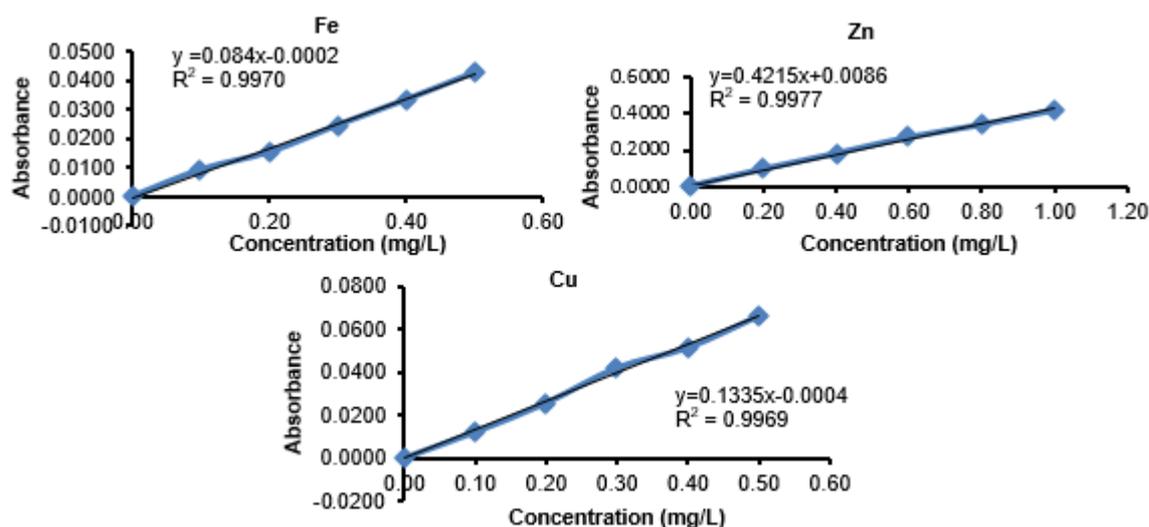
To optimize the results, some conditions during the destruction phase in microwave digestion need to be

controlled, including the amount of nitric acid and hydrogen peroxide, temperature, sample weight, pre-digestion, and destruction length. The optimization can be achieved using various techniques, such as the factorial design of Plackett-Burman. However, this step was excluded, considering that all procedures in this work are based on the standard of microwave digestion instrument for each food matrix. The efficiency of the destruction process using microwave digestion is influenced by the volume of acid substances ( $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$ ), time, and temperature [28].

Power setting in both methods (standard and modified) was adopted from the manual book, while

**Table 9.** Linearity test results

Mineral	Concentration (ppm)	Average (abs)
Fe	0.1	0.0092
	0.2	0.0152
	0.3	0.0245
	0.4	0.0332
	0.5	0.0425
Zn	0.2	0.0954
	0.4	0.1817
	0.6	0.2730
	0.8	0.3421
	1.0	0.4237
Cu	0.1	0.0121
	0.2	0.0257
	0.3	0.0422
	0.4	0.0516
	0.5	0.0665



**Fig 2.** Linear regression curve for quantification of Fe, Zn, and Cu

**Table 10.** Intra-lab reproducibility

Repeat	Content (mg/kg)					
	Cu		Zn		Fe	
	1	2	1	2	1	2
<b>Full cream milk powder</b>						
average	0.94	0.87	24.34	24.48	24.72	25.01
SD	0.06	0.05	0.50	0.73	0.53	1.18
% RSD	6.78	5.78	2.07	2.99	2.14	4.70
2/3 CV Horwitz	16.15	16.33	9.89	9.88	9.87	9.86
p-value	0.110		0.712		0.545	
<b>Canned peas</b>						
average	1.36	1.34	7.01	6.88	2.82	2.80
SD	0.02	0.02	0.06	0.12	0.09	0.10
% RSD	1.51	1.52	0.89	1.73	3.45	3.59
2/3 CV Horwitz	15.28	15.31	11.94	11.97	13.69	13.70
p-value	0.120		0.089		0.668	
<b>Canned fish</b>						
average	0.95	0.95	9.42	9.48	23.33	23.24
SD	0.05	0.009	0.07	0.07	0.18	0.10
% RSD	4.89	1.02	0.76	0.77	0.77	0.43
2/3 CV Horwitz	16.12	16.12	11.41	11.40	9.96	9.96
p-value	0.821		0.210		0.355	

Term of acceptance: p-value >  $\alpha$  95%**Table 11.** Results of the recovery test

Spiking minerals	Recovery (%)								
	Full cream powdered milk powder			Canned peas			Canned fish		
	80%	100%	120%	80%	100%	120%	80%	100%	120%
Fe	94.52	85.19	85.23	92.16	89.78	82.05	92.28	85.53	84.14
Zn	101.21	91.23	100.76	91.66	81.79	86.93	90.45	97.28	99.43
Cu	84.15	94.64	83.18	87.48	85.46	82.05	92.68	82.11	89.52

Term of acceptance: 80–115%

destruction temperature was carried out according to the standard of AOAC (2012). The sample weight (0.5 g, dry weight) was also based on the arrangement of AOAC. Destruction process allows changing the form of inorganic constituents to organic constituents, in which it involves the conversion of organic elements such as C, H, O, N, S and P into CO<sub>2</sub>, H<sub>2</sub>O, NO<sub>2</sub>, while inorganic elements still exist in their original form. Open destruction using microwave digestion promotes several advantages, including faster time operation, fewer reagents used, higher accuracy, thereby reducing contamination in samples and loss of volatile elements.

During the microwave-assisted process, molecules are

exposed to non-ionic electromagnetic radiation, which induces rotational dipole moments without changing their molecular structure [29]. The effect of the wave in microwave appears magnetically [30] and dielectrically [31]. Dielectric material comprised of molecules obtaining a dipole moment or a neutral charge (having a balanced positive and negative charge). Additionally, the dielectric wave in the microwave is due to the capability of storing electric charges at different potentials. Water is an electrical molecule, possessing hydrogen atoms (positively charged) and oxygen atoms (negatively charged). The exposure of the electrical field to dielectric materials led to changes in the direction of

the current because of repulsion and attraction within positive charges or negative charges; thus, the wave occurs magnetically. More heat was produced in samples containing more water than in those with less water. The frequency in the microwave ranged from 300 to 300.000 MHz with a wavelength of 1 mm–1 m.

Samples in microwave-assisted destruction are mixed with strong acids in a closed vessel. In that system, pressure (1.1–2.2 Mpa) and temperature (30–190 °C) increased due to microwave radiation, allowing to convert the solid form into a liquid. High pressure and temperature (around 200 °C) [32] on the vessel is able to raise the boiling point of nitric acid. Thus, it strengthens oxidation and accelerates the reaction. HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> are used as oxidizing agents because they are capable to oxidize minerals into their salt form. Nitric acid at high temperature is decomposed into water-soluble oxides such as NO and NO<sub>2</sub>. The use of mixed acids is better than a single acid for the mineral destruction process; the destruction process is incomplete if nitric acid is the only used compound, resulting in a cloudy solution [33]. The addition of H<sub>2</sub>O<sub>2</sub> leads to intensifying the presence of O<sub>2</sub> in the vessel [34]. The oxidizing mixture consisting of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> produces a greater % recovery than a mixture of HNO<sub>3</sub> and HCl, and a mixture of HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> [35].

The gradient or multilevel heating system aims to intensify the reaction between NO and O<sub>2</sub> to form NO<sub>2</sub> [36]. The NO<sub>2</sub> is reabsorbed in acidic solution, and it regenerates HNO<sub>3</sub> [37]. The reaction process in the vessel is described in Fig. 3 [38].

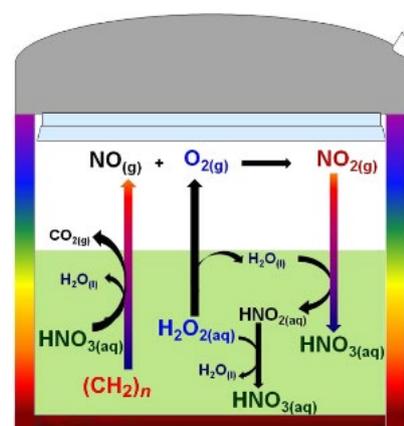
The longer heating process in the microwave would lead to a higher temperature in the vessel, responsible for attenuation of the condensation process and eventually increase the mineral level in the solution. A complete destruction process is marked by the formation of a clear solution, which indicates the reformation of an organic compound. Sample solutions are then ready to be analyzed using F-AAS. F-AAS is recommended for use in measuring minerals because of its good sensitivity with a detection limit of more or less 1 ppm and also its low operational cost.

Soluble dietary fiber is a carbohydrate-based polymer widely found in nuts, fruits, and vegetables [39].

The dominant fiber contents in peas are stachyose (19.83 mg/g) and verbascose (16.10 mg/g) [40]. In addition, canned peas contain simple sugars, including glucose, fructose, sucrose, and galactose; hence, the destruction process starts from 130 °C to 180 °C. Addition of nitric acid (6 mL) is in accordance with a previous work of Bakkali et al. on vegetable samples, yielding the highest accuracy volume [6].

Full cream powdered milk is rich in fat. The covalent bond between fatty acids is difficult to break down, while fat has a low relative potential of oxidation, which means that there is a need to perform destruction at temperature up to 200 °C with a greater amount of oxidizing acid (10 mL). Lactose (D-galactose and D-glucose) constitutes a major simple sugar in milk [41]. Therefore, the initial temperature of digestion begins at 130 °C. Such destruction process was previously carried out by Belete et al. [42] in cow milk samples, using the destruction temperature of 165–190 °C following a 10 min pre-destruction phase.

Canned fish represents a food sample with high protein content. The main protein content in fish, i.e., actin and myosin, is susceptible to denaturation when exposed to microwaves (Fig. 4) [43]. Although these myofibrillar proteins start to denature at about 85 °C, a higher temperature was needed to induce denaturation when they exist in canned fish, reaching up to 130 °C. Protein molecules contain peptide bonds with a large molecular weight; thus, the amount of oxidizing acid needed is 7 mL.



**Fig 3.** The reaction process in the microwave digestion vessel

The presence of minerals can affect the quality of food. Copper (Cu) and iron (Fe) play a role in oxidation-reduction reactions because the oxidation potential can cause unwanted changes in processed foods related to fat oxidation which promotes a decrease in the nutritional value of food [44]. In the process of oxidation of fat/oil, Fe and Cu are able to accelerate the process of initiation, propagation, and termination (acting as a catalyst). In addition, the valence of Fe dictates the color pigment of myoglobin and hemoglobin in fresh meat; ferric (+3 valence state) accounts for brown color, while ferrous (+2 valence state) accounts for red color. The reaction of Fe with sulfur in canned food also induces the formation of iron sulfide (FeS) complex, which is a black solid.

Enzymatic browning is also linked to the presence of Cu. This mineral serves as a cofactor of the enzyme polyphenol oxidase (PPO), as a catalyst for non-enzymatic browning reactions and oxidation of ascorbic acid. Zn can

stabilize the color of canned green beans. ZnO can be used as a coating agent on canned foods capable of preventing the formation of FeS during the heating process. The levels of Fe, Zn, and Cu obtained through the standard and modified methods can be seen in Table 12.

Statistically, we found that data obtained from both protocols did not differ significantly, as evidenced by p-value  $> \alpha = 5\%$  for all samples examined.

In terms of time and cost, the modified method seemed to be more preferable than the standard method. The hydrogen peroxide was less needed in the currently developed method (1 mL for each sample) than the standard method (2 mL for each sample). It is noteworthy that hydrogen peroxide is three times more expensive than nitric acid. Additionally, the modified method required a shorter time of the process (30 min) than the standard method (45 min).

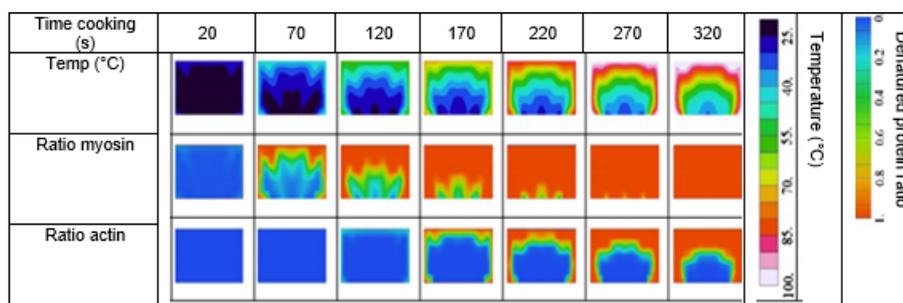


Fig 4. The process of actin and myosin denaturation [42]

Table 12. The result of analysis mineral with standard and modification method

Samples	Standard method (mg/kg)			Modified method (mg/kg)		
	Fe	Zn	Cu	Fe	Zn	Cu
<b>Full cream powdered milk</b>	24.99	24.62	0.85	24.60	24.54	0.84
SD	0.80	0.13	0.01	0.20	0.19	0.02
% RSD	3.21	0.51	1.69	0.81	0.76	2.95
2/3 CV Horwitz	6.57	6.59	10.92	6.59	6.59	10.95
p-value	0.575	0.714	0.561			
<b>Canned peas</b>	2.88	7.11	1.31	2.87	7.02	1.36
SD	0.06	0.03	0.02	0.04	0.04	0.01
% RSD	2.11	0.44	1.35	1.24	0.57	0.99
2/3 CV Horwitz	9.10	7.94	10.23	9.10	7.95	10.18
p-value	0.827	0.222	0.09			
<b>Canned fish</b>	23.44	9.78	1.04	23.44	9.47	0.99
SD	0.23	0.24	0.03	0.23	0.05	0.03
% RSD	0.98	2.43	2.50	0.98	0.49	2.70
2/3 CV Horwitz	6.63	7.57	10.60	6.63	7.60	10.67
p-value	0.245	0.211	0.262			

**Table 13.** Solvent alternative test

Parameters	Mineral content (mg/kg)					
	Cu		Zn		Fe	
	Demineralized water	HNO <sub>3</sub> 0.05M	Demineralized water	HNO <sub>3</sub> 0.05M	Demineralized water	HNO <sub>3</sub> 0.05M
average (mg/kg)	0.94	1.25	24.34	25.88	24.72	28.86
SD	0.06	0.08	0.50	1.34	0.53	0.43
% RSD	6.78	6.10	2.07	5.18	2.14	1.50
CV Horwitz	16.15	15.48	9.89	9.80	9.87	9.646
p-value	0.000		0.028		0.000	

The suitability of solvent alternative was also observed, in which this experiment was only tested for full cream powdered milk. Toughness test was carried out to evaluate the effects of the solvents on analytical results. In this case, 0.05M HNO<sub>3</sub> was used instead of demineralized water; thus the sample condition was similar to the standard solution. Furthermore, the addition of acid to the solution can contribute towards igniting and preventing precipitation and absorption on the glass wall. The solubility of mineral seemed to possess a positive relation to the length of exposure to nitric acid [45]. The results showed that the type of solvent significantly altered the test results ( $p < 0.05$ ) (Table 13). This suggests that replacement with 0.05 M HNO<sub>3</sub> was unacceptable.

The F-AAS performance test aims to ensure that the instrument is feasibly used in the analysis process to produce valid data. The number of atoms distributed in the flame is different, so it is necessary to determine the different burner height for each mineral.

## ■ CONCLUSION

Modification methods in the process of destruction for analysis of Fe, Zn, and Cu in various food matrices (canned peas, full cream powdered milk, and canned fish) were not significantly different from AOAC (2012) and has fulfilled the acceptability requirements for use in laboratory analysis. Mineral analysis result obtained by the modified method in the process of destruction for Fe, Zn and Cu minerals for canned peas: 2.87; 7.02; 1.36 mg/kg, full cream powdered milk 24.60; 24.54; 0.86 mg/kg, canned fish: 23.44; 9.47; 0.99 mg/kg.

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