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Comparison of Wet and Dry Digestions in the Analysis of Fe in Spinach by Atomic Absorption Spectrophotometry

Irdhawati Irdhawati,[1*] I Gusti Ayu Putu Yunita Riyastini,[1] Manuntun Manurung[1]

[1]Program Study of Magister of Chemistry, Faculty of Mathematic and Natural Sciences, Udayana University, Kampus Bukit Jimbaran Bali 80361 Indonesia.

[*]Corresponding e-mail: irdhawati@unud.ac.id

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ABSTRACT

The method of digestion as a part of sample preparation is very important to determine the accuracy of the analysis result. In this study, the methods of wet and dry digestions were applied to determine of Fe content in several kinds of spinach obtained from the traditional market in Denpasar Bali. This research aimed to compare the result of Fe analysis by AAS method using both of digestion methods. This research was divided into several steps starting from sampling, determination of the samples species, sample preparation, digestion by wet digestion using aqua regia and dry digestion in the furnace, and Fe analysis by AAS. The result showed that the concentrations of Fe in root spinach, red spinach, cut spinach, and tricolor spinach through wet digestion method varied between 68.08–105.45 mg/kg, while the concentrations of Fe by dry digestion obtained between 27.52–42.03 mg/kg, which was over the accepted value. Based on the one-way ANOVA statistical test with a significance level of 5%, there was a significant differences of Fe concentration in spinaches by wet and dry digestions.

Keywords: AAS, iron (Fe), spinach, dry digestion, wet digestion

INTRODUCTION

Digestion is one of pretreatment samples before analyzing its substantial content. This process aims to convert the sample into a measurable material. Digestion can be divided into two types, namely wet and dry digestion. Wet digestion oxidizes samples using oxidizing agents of single or mixture strong acids then heated. Dry digestion does not use solvents, solid samples directly oxidize in a furnace at high temperature.

In the wet digestion process, temperature and heating duration, the type of acid and the oxidizing agent are very important factor. Several previous studies showed that the use of a good oxidizing agent can maximize the extraction of metal content in a sample. Usually this process takes 10 minutes to several hours at an ambient temperature of 120°C, leaving various elements in the acid solution in the form of inorganic compounds suitable for analysis [1]. Several things need to be considered in wet digestion, including the composition of the sample, acid strength, oxidizing strength and boiling point, solubility of the resulting salt, safety and purity of reagents. In general, strong acid such as HNO_3, HCl, H_2SO_4, H_3PO_4, $HClO_4$, HF and H_2O_2 can be used to oxidize organometallic bond in samples, alloys, minerals, soils, rocks and silicates The advantage of wet digestion method is during the digestion process only a few minerals will be lost, because the temperature is lower than boiling point of analyte, and the

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process is faster than dry digestion method. However, wet method requires a special fume hood if the reagent used is perchloric acid because of its dangerous, and often high contamination potential, because conducted in an open system under heating, there are risks of atmospheric contamination and a significant threat to the health of the laboratory technician due to releasing toxic gases. Wet digestion method have been widely applied to prepare samples for spectral analysis [2, 3].

Dry digestion is a method of breaking the organometallic bonds in the sample into inorganic metal form by high temperature heating in a furnace. In general, dry digestion requires heating temperatures ranging from 400-800ºC, but this temperature depends on the type of samples [4]. Water and volatile materials in the sample will evaporate and organic substances are burned and converted into CO_2, H_2O and N_2 gases. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility, some can be partially lost if burned at high temperatures, for example, iron, lead and mercury. The temperature used in ashing must be considered because many elements in the ash can evaporate at high temperatures. In addition, the ashing process can also cause the decomposition of certain compounds. Therefore, the ashing temperature for each sample varies depend on the components present in the material [1]. The advantages of the dry digestion method are safer, can analyze many samples simultaneously, and the ash resulting from combustion can be analyzed for certain mineral content. Disadvantages of the dry digestion method are that it takes a long time, ranging from 12-24 hours, is quite expensive because muffle furnaces run on electricity, and the loss of volatile minerals at high temperatures [2].

The previous data published by Akinyele and Shokunbi [5] showed that the level of iron in the wet digested tubers were significantly higher than those of the dry digested food samples. A comparison between two digestion methods of hot plate Hossner (total-total) and USEPA method 3051 (total-recoverable) for determining heavy metals content of urban soil affected by mining or industrial activities was conducted by Alsaleh et al. The result showed that the level of Fe by hot plate Hossner method was higher than USEPA 3051, although there were no significant differences between two digestion methods [3]. Hseu [6] was carried out evaluating heavy metals content using four digestion methods, i.e. nitric acid, dry ashing, nitric-perchloric acid, and sulfuric acid methods. The result indicated that the nitric acid method was the most efficient for recovering of heavy metals, and recommended as the method for digesting, based on recovery analysis, cost, and time taken. Dry ashing was recommended as a flexible method. The sulfuric acid yielded the lowest recovery of heavy metals. Nitric-perchloric acid was not recommended due to potentially hazardous during digestion.

Spinach contains nutritionals such as protein, Ca, Fe, vitamins (A, C, K), riboflavin (B2), niacin (B3), B6, and folate (B9) [7]. The mineral content of spinach is quite high, especially iron which can be used to prevent fatigue due to anemia. Iron in small amounts needed to form red blood cells. The body requires Fe as much as 7-35 mg/day which is partly obtained from water. Absorption of Fe that exceeds the dose required by the body can cause health problems. This is because the human body cannot excrete Fe, so for those who frequently receive blood transfusions, their skin color becomes black due to the accumulation of Fe. Drinking water containing iron tends to cause nausea when consumed. In addition, in large doses can damage the intestinal wall. Death is often caused by damage to the intestinal wall [8]. The result of the research by Naser et al. (2012) regarding to the identification of the elemental content of manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) by wet digestion method with HNO_3 and HCl in spinach (*Spinacia oleracea*), red spinach (*Amaranthus tricolor*), bottle gourd (*Lagenaria vulgaris*), and pumpkin (*Cucurbita moschata*) collected from three locations found

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that the highest concentrations of Mn, Fe, and Cu were found in spinach [9]. The maximum limit of Fe metal contamination in food, especially vegetables is 5.0 mg/kg [10].

Based on the description above, it is necessary to do a study on the comparison of wet and dry digestions in Fe analysis for several types of spinach by AAS. The results of Fe content by wet and dry digestions will be analyzed using statistical method of analysis of variance one way (anova one way). Dry ashing and wet digestion methods were applied in destruction the samples to give recommed the more appropriate method in determining Fe in several species of spinach. The reliability of this methods for estimation of Fe in spinach samples has been checked by spiking standard solution to determine the recovery of measurement.

EXPERIMENT

Chemicals and instrumentation

The materials used in this study were the stem and leaves of root spinach, red spinach, cut spinach, and tricolor spinach as samples, bought from traditional market in Denpasar Bali. Some of chemicals were used such as iron(III) chloride hexahydrate as standard solution; nitric acid and hydrochloric acid for wet digestion, and distilled water. All of chemicals were purchased from Merck without further purification.

The tools were used analytical balance (Ohaus Px224/E), oven (Memmert), blender (Turbo), metal block digester (ZX), porcelain crucible, furnace (WiseTherm F/FH), ball filler, glassware, and a set of Atomic Absorption Spectrophotometer (Shimadzu/AA-7000 series).

Methodology

Sample preparation

The fresh spinach samples were washed and dried at room temperature during 24 hours and cut into smaller sizes. The water content in samples were determine by weighing 2 grams of dry samples and heated in the oven at 105ºC for 1 hour to evaporate the water content. The sample was cooled in a desiccator at room temperature and weighed. After weighing, the samples were reheated again until constant mass obtained. The same treatment was carried out three times for each spinach sample. Around 30 g dry samples were grinded using blender.

Wet digestion

In the wet digestion process, two grams of dried sample was transferred into a digestion tube and added 16 mL the mixture of nitric acid and hydrochloric acid (1:3) as oxidizing agent and heated at (120 ± 2)°C during 60 minutes in a metal block digester. Samples and blank solution were digested at the same time, with three replicates [11]. The digested solutions were cooled and filtered into 50,0 mL flask and volume adjusted using distilled water. The total content of Fe was measured by AAS using standard addition method.

Dry digestion

Dry digestion was carried out by weighing 15 grams of the sample in a porcelain crucible and heated in a furnace at temperature 450ºC for 15 hours. Then, 2 grams of the ash was weighed and dissolved with HNO_3 0.1 M, filtered and the volume was adjusted to 50 mL. The total concentration of Fe was determine using standard addition method using AAS.

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Calibration curve

The mixture of Fe standard and samples as tested solutions was measured by AAS. The result of absorbance measurement was plotted with the concentration of Fe standard, to find linear regression equation.

$$y = a + bx$$

y = absorbance, a = intercept, b = slope, and x = concentration of Fe

In additional standard method, the concentration of analyte in the samples are determined by extrapolated to the line across y = 0. Therefore, the total concentration of Fe in spinach samples (x) can be determined, with x = -a/b.

The final concentration of Fe in the samples were calculated by equation:

$$[Fe] = \frac{C.V.f}{b}$$

[Fe] = the final concentration of Fe in the samples (mg/kg)
C = the concentration of Fe (x) (mg/L)
V = volume of digested solution (L)
f = dilution factor
b = mass of sample (kg)

Limit of detection and limit of quantification

Limit of detection (LoD) and limit of quantification were determined based on the linear concentration data. The measurement of LoD was calculated by the following equation:

$\hat{y}i = a + bx$

$$S_{y/x} = \sqrt{\frac{\sum(y_i - \hat{y}i)^2}{n-2}}$$

$$Sb = \frac{S_{y/x}}{\sqrt{\sum(\bar{x}-x)^2}}$$

LoD = yb + 3 Sb
LoQ = yb + 10 Sb
Sy/x = standard deviation of the linier line
Sb = standard deviation blank
b = slope
yb = intercept
LoD = limit of detection
LoQ = limit of quantification

The $\hat{y}i$ value was obtained by substitution x value in linear regression equation with standard concentration [12].

Accuracy

The accuracy of measurement was determined by calculation of percentage of recovery using standard addition method. The concentration of the standard solution was measured in the presence of the sample solution as a matrix. The measurement result of the standard solution was compared with the theoretical standard concentration. Measurement was conducted in three replicates.

$$\text{Accuracy} = \frac{[standard+sample] - [sample]measurement}{[standard]theoritical} \text{ x100\%}$$

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Statistical calculation

Statistical tests were carried out to determine the significant difference of Fe content in the samples by wet and dry digestion method. The type of statistical test was performed with analysis of variance (ANOVA) oneway analysis to compare the significantly between two digestion methods, and within four species of spinach samples, with a significance level of 5%, using Microsoft Excel 2013 program. The criteria for hypothesis at a significant level of 5% were if $F_{calculated} \leq F_{table}$, there is no significance different, otherwise if $F_{calculated} > F_{table}$, two methods compared has significantly different [13].

RESULT AND DISCUSSION

Determination of Water Content in Samples

The water content in the four spinach samples can be seen in Table 1.

Table 1. The water content of spinach

No.	Sample	Percentage (%)
1.	Root Spinach	88,09 ± 0,35
2.	Red Spinach	82,87 ± 0,39
3.	Cut Spinach	87,16 ± 0,43
4.	Tricolor Spinach	84,20 ± 0,38

The highest water content was found in root spinach samples, which was (88.09±0.35)% and the lowest content was in red spinach, which was (82.87±0.39)%. According to Grubben et al., 1993, the normal water content in 100 g of spinach is 88.9 g (88.9%) [14]. The composition of nutrients and water content in *Amaranthus tricolor L.* species varies greatly. This can be caused by differences in plant age, ecological conditions, and fertilization.

Wet and Dry Digestion Methods

Wet digestion is the process of breaking the organo-metallic bonds by heating the sample using strong oxidizing agents, by single or mixed mineral acids. The calibration curves for samples of root spinach, red spinach, cut spinach, and tricolor spinach were shown in Figure 1.

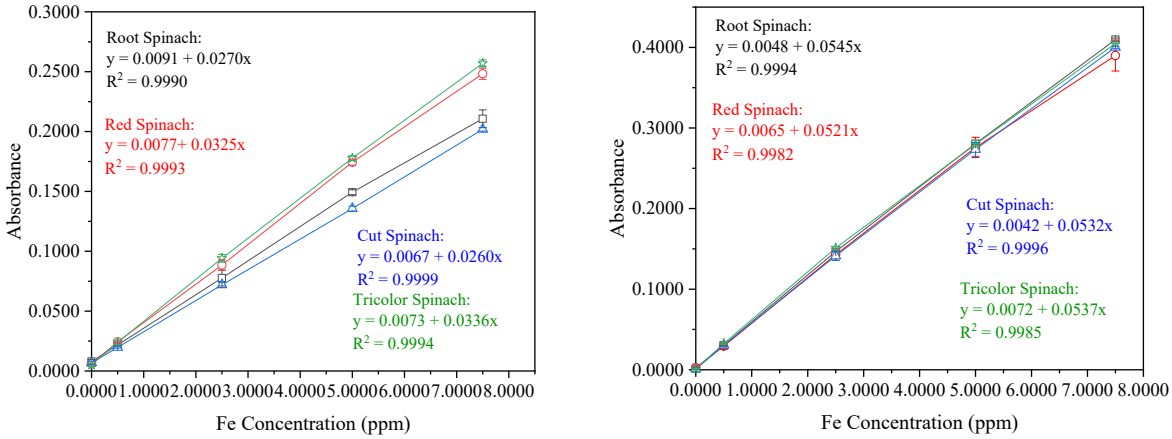

Figure 1. Calibration curve of Fe content in root spinach, red spinach, cut spinach, and tricolor spinach by wet digestion (a) and dry digestion (b) methods

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The linearity of Fe was in the range of 0 to 7.5 mg/L. The intercept values were not zero, but it was found at 0.0091, 0.0077, 0.0067, and 0.0073 for root spinach, red spinach, cut spinach, and tricolor spinach, respectively. The values indicated samples contain Fe, without added Fe standard. The slope was found in the similar value of four kind of spinach between 0.0260 to 0.0336. The coefficient correlation of equation was 0.999, showed a significant correlation between concentration and absorbance, as the response of instrument. The result of Fe analysis in the spinach samples were shown in Table 2.

Table2. The Total Fe Content in Spinach Samples by Wet Digestion Method

Samples	Fe content (mg/kg)	
	Wet method	Dry method
Root Spinach	105,45 ± 14,79	27.52 ± 4.00
Red Spinach	74,28 ± 26,44	39.65 ± 19.57
Cut Spinach	80,63 ± 14,59	24.40 ± 13.85
Tricolor Spinach	68,08 ± 16,02	42.03 ± 2.72

The highest Fe content in the root spinach was (105.45 ± 14,79) mg/kg, while the lowest was found in the tricolor spinach, which was (68.08 ± 16,02) mg/kg. From the four samples tested, all samples contained Fe with concentrations exceeding the permissible threshold of 5.0 mg/kg according to BSN (2009) [10]. This indicates that all spinach samples contained Fe more than acceptable value.

The accumulation of Fe in the spinach vegetable could be due to the presence of several compounds in the spinach that can bind with Fe. These compounds are carbohydrates and proteins. According to the USDA (2019), the highest nutrient content in 100 g of raw spinach is carbohydrates 4.02 g and protein 2.46 g [15].

Cellulose and pectin as derivatives of carbohydrates have very high absorption of heavy metals. Pectin provides a large porosity and absorption rate, while cellulose provides a large hydrophilicity and a large water absorption capacity as well [16]. With the ability to absorb pectin and cellulose, the cell structure can accumulate heavy metals, even in the growth process of agricultural production. Metals such as Fe will be absorbed by plants and concentrated in the roots. As the plant grows and matures, these toxic metals will accumulate in the stems and leaves of the plant. The accumulation and retention of heavy metal cations in samples containing cellulose and pectin occur mainly because of their cation exchange ability [17].

Proteins are large molecules consisting of one or more chains of amino acids. Through reactions with free carboxyl groups and free amino groups, proteins can bind to cations and anions. The precipitation of proteins by heavy metal ions depends on the arrangement of protein salts. A number of metal ions have the ability to form compounds by forming coordination covalent bonds and react immediately with the N-terminal, amine substitution in the peptide chain, and N-imidazole. Fe is a metal which is able to form complex compounds with proteins [18].

The Fe content in all of spinach samples was found between 24.40 and 42.03 mg/kg. This indicates that all samples of spinach by dry digestion method were contaminated with Fe. Based on BSN (2009) the maximum limit of Fe contamination in food, especially vegetable is 5.0 mg/kg. The Fe content in the spinach samples by dry digestion was smaller than wet digestion methods. It shows that Fe has good solubility and can be well oxidized in strong acid, compared

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with dry oxidation in high temperature. Fe was classified as volatile metal, so it was evaporated easily in the high-temperature of dry digestion.

The LoD, LoQ, and accuracy values were calculated from the absorbans average of the three replicates of measurement. The result calculation of LoD, LoQ, and accuracy for wet and dry digestion methods can be seen in the Table 3.

Table 3. The LoD, LoQ and Accuracy value

Samples	Fe content (mg/Kg)					
	Wet method			Dry Method		
	LoD (ppm)	LoQ (ppm)	Accuracy (%)	LoD (ppm)	LoQ (ppm)	Accuracy (%)
Root Spinach	0.0109	0.0165	95.80	0.0549	0.1748	99.52
Red Spinach	0.0342	0.0970	98.31	0.0520	0.1666	97.05
Cut Spinach	0.0182	0.0450	99.31	0.0383	0.1251	99.97
Tricolor Spinach	0.0329	0.0963	98.18	0.0486	0.1551	98.99

The LoD and LoQ values were found much lower than Fe concentration in the samples, indicated the result of measurement has a good confidence level, different significantly with blank solution. Furthermore, the accuracy between 95,80% in wet method, and 99,97% in dry method showed the measurement value closed to the true value, and the recovery of standard solution in the presence of sample as a matrix did not interference the measurement.

Statistical Test

The F value calculation was conducted by anova one way statistical method using Microsoft Excel 2013. The result of calculation was obtained F calculated: 11.47 and F table: 5.79. The calculated F value is more than F table indicated there is a significant difference in Fe content between the result of measurements by using wet and dry digestion method for the four kind of spinach samples.

CONCLUSION

The concentration of Fe in spinach can be determined by wet and dry digestions. The results showed the level of Fe obtained by wet digestion is more than dry digestion, due to the boiling point of Fe. Based on the one-way ANOVA statistical test with a significance level of 5%, there is a significant difference of Fe content by wet and dry digestion methods for the four spinach.

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