

## Determine of Copper Metal in Cooking Oil by Saponification Method with Atomic Absorption Spectrophotometer (AAS)

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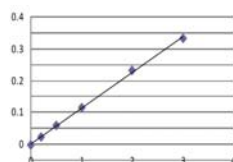
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### GRAPHICAL ABSTRACT



### ABSTRACT

A research on the analysis of copper metal in the cooking oil saponification by AAS method. This study aimed to validate analytical methods, including precision and accuracy in the determination of copper metal in cooking oils through saponification and knowing destructed Cu content in cooking oils on the market. This research was done by creating a simulation of cooking oils and then added with a solution with various concentrations of  $\text{Cu}^{2+}$ , reacted with KOH for saponification, then destructed dried, and absorbance was measured using an atomic absorption spectrometer. Results of accuracy (recovery) be obtained average of 96.88, 97.35, and 97.36% and precision values of 0.8233, 0.3154, and 0.4803%. It can be concluded that this method is very well used to determine the concentration of copper metal contained in cooking oils, and Cu content in cooking oils on the market for bulk cooking oil 1 at 11.5 mg/kg, bulk cooking oil 2 at 10.9 mg/kg, and for branded cooking oil at 7.4 mg/kg.

*Keywords:* cooking oil, saponification, Cu, destruction

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## 1. INTRODUCTION

Cooking oil is one of the nine basic commodities in Indonesia. In 1993, public spending Indonesia for cooking oil consumption per capita per month is 4.5% of the total for food [1]. Consumption of cooking oil in Indonesia is expected to continue to increase in line with the increase in the number and income of the population.

In the cooking oil processing industry is possible oil contamination by metals derived from the means of production, especially the manufacture of cooking oil is done traditionally. With the continuous heating process during the production process, can cause the metal ions are released. One of the metals that are commonly used in the manufacture of production equipment is the metal copper (Cu). Factors that played a role in the process of lipid peroxidation is the presence of transition metals such as Fe or Cu which is a powerful catalyst even in small quantities. Copper ions are in free form can bind back to the molecule or ion that has a lone pair of electrons to form a new complex ion. New complex ions formed can cause rancidity [7]. In this case the copper ions will form complexes with fatty acids that are in the cooking oil. Each type of food has a nutritional content and contamination are diverse. Metal contaminants typically act as heavy metals, which to some extent are present at very detrimental. Similarly, Cu contamination in the oil, there is a function as needed minerals and some are acting as contamination.

Metallic copper (Cu) is one of the dangerous heavy metals. Copper metal can cause negative effects or toxic to

humans at certain concentration limits. The symptoms that appear due to the toxicity of Cu metal on the weather man is halitosis, dry throat and stomach, feeling like vomiting and diarrhea continued for days, there is blood in the stool (faeces), dizziness and fever [5]. Toxicity owned by new copper will work and show the influence of metal when it has been entered into the body of the organism in large quantities or exceed the tolerance value related organisms [9]. Given the influence of Cu heavy metal toxicity, then Cu examination in cooking oil becomes very important.

A research on heavy metal content in clove oil have been carried out by using a wet destruction of acid (HCl), after it was extracted [12]. The use of this method can actually be done, but this method requires a long time. This is because the destruction used wet-acid and concentrated strong acid (HCl,  $\text{HNO}_3$ , and  $\text{H}_2\text{SO}_4$ ) which is a polar compound. The compound will not be mixed with cooking oil that is non polar, so they are very difficult to react. Other problems also frequently occur in dry destruction, which will take several days of analysis. It is caused when used in temperatures that are too hot will cause burning cooking oil and endangering safety. It is therefore necessary to find a method that is safe, fast and gives good results. It is known that cooking oil saponifiable with the addition of a base, such as NaOH or KOH. In this study will be carried out sample preparation cooking oil through the formation of soap that is not flammable. Dry so that destruction can be done safely. Principles of analysis of heavy metals in cooking oil by saponification method is to saponificated cooking oil by using a base to break down triglycerides into

glycerol and soap (fatty acids). Soaps that have formed dry destructed in high temperature. The results of destruction dissolved in HNO<sub>3</sub>, then Cu (II) is checked by Atomic Absorption Spectrometry (AAS). This study aimed to validate analytical methods, including precision and accuracy in the determination of copper metal in cooking oil through saponification and knowing destructed Cu content in cooking oil on the market.

## 2. EXPERIMENTAL

### 2.1 Materials and instruments

The samples are simulated cooking oil and cooking oil in the market. Other materials used are kupro sulphate hydrate (CuSO<sub>4</sub>.5H<sub>2</sub>O), nitric acid (HNO<sub>3</sub>), hydrochloric acid (HCl), potassium hydroxide (KOH), sodium tetraborate (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>.10H<sub>2</sub>O), methyl red (MM), phenolphthalein (PP), and filter paper.

The tools used in this study consisted of Atomic Absorption Spectrometry (AAS) Shimadzu AA 6200, milk strainer, analytical balance, furnace, burette, turning cooler, porcelain crucible, coconut milk container micrometer and tools that ordinary glass used in the laboratory.

### 2.2 Preparation of standard curve

Weigh 0.3929 g CuSO<sub>4</sub>.5H<sub>2</sub>O and diluted to 100 mL with distilled water to obtain stock solution. Prepare working solution by diluting stock solution. Prepare solution containing 0.2, 0.5, 1.0, 2.0, 3.0 ppm by diluting 100 ppm working solution to 100 mL with distilled water. Absorbance of 0.2, 0.5, 1.0, 2.0, 3.0 ppm standard measured by AAS. Wavelength used for analysis of copper is 324.7 nm. The measurements obtained from linear curve expressing relation between absorbance of solution concentration, so that regression equations and correlation coefficients can be determined [10].

### 2.3 Preparation of simulated cooking oils

Weigh 400 g coconut meat peeled with smoothing steel, then shredded until smooth. Dissolve in water with 5:3 ratio. From 5 grated coconut added 3 L water. Grated coconut is squeezed to remove oil contained in granular coconut meat that has been refined. Coconut milk that formed was filtered using filter cloth to separate it with the waste. Coconut milk is allowed to stand until formed in two layers. Discarded water layer, and a layer of pure milk is heated to temperatures of approximately 120°C until there is an oil layer, then decanted and oil obtained. Simulated cooking oil obtained used in experiment 2.4., 2.5., and 2.6.

### 2.4 Determination of saponification numbers

Weigh 2 g simulated cooking oil, then slowly added 50 mL KOH 0.7M and a few grains of stone boiling. Associated with cooling behind and carefully boiled for

about half an hour, so oil and KOH mixed homogeneous and formed soap. Once cool, add a few drops of PP and excess KOH is titrated with 0.4772N HCl until it became colorless, then do the same thing against the blank (without using the sample titration). Replication is done 3 times.

### 2.5 Addition of copper metal

Weigh 1 g simulated cooking oil as much as four times, each of which is poured into the crucible. Crucible A filled without addition of Cu solution that serves as a control, crucible B filled with addition of 0.5 mL of 50 ppm Cu, crucible C filled with addition of 1 mL of 50 ppm Cu, and crucible D filled with addition of 2 mL of 50 ppm Cu. All mixed with rocking, while occasionally heated beaker glass. It is intended that Cu<sub>2+</sub> metal can form complexes with fatty acids (approximately up not formed 2 layers). Replication is done 3 times.

### 2.6 Saponification by dry destruction

From results above (2.5.), each coupled with a rate of 5 mL KOH 1M was heated on a hot plate. After the soap formed was transferred into the furnace crucible and heated at 500°C for 5 h to form gray and black authoring has been lost. Gained added ash 4N HNO<sub>3</sub> to dissolve copper metal, put into 10 mL volumetric flask, and add distilled water up to the mark boundaries, concentration measured by AAS.

### 2.7 Determination of copper metal

Weigh 1 g sample of cooking oils on the market, which is bulk cooking oil and branded cooking oil, then saponifiable using 5 mL KOH 1M. After it, dried at a temperature of 500°C destructed for ± 5 h. Ash obtained, then added 4N HNO<sub>3</sub> to dissolve copper metal. Subsequently, the samples that have been prepared are put into 25 mL volumetric flask and add distilled water to mark boundaries. The absorbance measured by AAS.

### 2.8 Analysis method validation

#### 2.8.1 Precision

Accuracy is a degree of repeatability of an analytical method. Stated accuracy results obtained with repeated measurements under the same conditions. Accuracy can be determined by calculating the standard deviation and coefficient of variation absorbance values of each standard solution with a concentration of equations (1) and (2):

$$SD = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}} \dots \dots \dots (1)$$

$$KV = \frac{SD}{x} \times 100\% \dots \dots \dots (2)$$

### 2.8.2 Determination of recovery

Recovery can be determined by comparing analysis and actual Cu<sup>2+</sup> concentration using saponification method. Recovery can be calculated by equation (3) below [13].

$$R = \frac{C_{sp}}{K_s} \times 100\% \dots\dots\dots (3)$$

## 3. RESULTS & DISCUSSION

### 3.1 Standard curve of Cu

Copper standard curve can be prepared by preparing standard solutions at different concentrations of Cu<sup>2+</sup>. The materials used to make a standard solution of Cu<sup>2+</sup> is CuSO<sub>4</sub>.5H<sub>2</sub>O, because it is readily soluble in water and has a high purity. Preparation of Cu<sup>2+</sup> standard with varying concentrations is due to the Cu concentration measured by AAS copper metal is linear in the range of concentrations. To determine Cu concentrations by AAS, use air-acetylene flame and measured at a wavelength of 342.7 nm [4]. Each standard solutions absorbance was measured by AAS, then made of the results obtained linear regression equation. The absorbance of samples showing ability to absorb electromagnetic radiation at maximum wavelength which is owned by a solution. Copper metal standard curve can be seen in Figure 1.

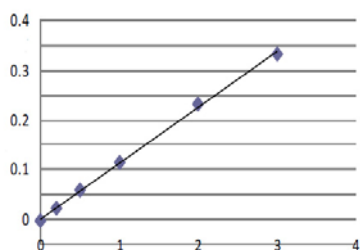


Fig. 1 Copper metal standard curve measured by AAS

The standard curve above shows that the greater Cu<sup>2+</sup> concentration, the greater the absorbance. This is in accordance with Beer-Lambert law that the absorbance of a compound is proportional to the concentration [3]. By measuring the absorbance of the sample solution, plotted next to the standard curve, can be determined so that the metal concentrations in sample solution. Linear regression equation obtained was  $y = 0.1127x + 0.0005$  with a correlation coefficient of 0.9991.

### 3.2 Determination of Saponification Numbers

Saponification numbers can be used to determine the amount of oil and fat molecules. Saponification numbers expressed as number (mg) of KOH needed to saponificated

1 g of oil or fat. This saponification number determination can be used to determine the needs of KOH used for oil saponification. The analysis done by weighing 2 g of simulated cooking oil that added 50 mL of 0.7M alcoholic KOH and refluxed for 30 minutes. The excess of KOH is titrated with 0.5N HCl with phenolphthalein indicator added (PP). Three result data can be seen in Table 1. It can be seen that average saponification number is 258.1346 mg/g. Therefore, 1M KOH used to saponificated 1 g oil needed by 5 mL.

Table 1 The determination of saponification numbers

No.	Oil Weight (g)	Vol. titration to sample (mL)	Vol. titration to blank (mL)	Saponification number (mg/g)
1.	2.0096	41.10	60.52	258.77
2.	2.0081	41.10	60.45	257.96
3.	2.0052	41.20	60.50	257.67
Average	2.0076	41.13	60.49	258.13

### 3.3 Destruction of Cooking Oils

Sample preparation is a very important step in analyzing the elements spectrometry. Saponification is a method by reacting oils (triglycerides) with a base to form a soap that aims to make sample not flammable when destructed. A metal content can be measured when the metals in the sample had been freed from organic material. The metal liberation of organic matter carried by the destruction. In the special section AAS instrument, the samples were prepared to experience the atomization [6]. Self destruction is one of the means used in sample preparation and aims to break down a compound into its elements so it can be analyzed further. Dry destruction is an overhaul by ashing sample and requires a certain heating temperature around 500°C, but the temperature is highly dependent on the type of sample to be analyzed [8].

In the present study using method of dry destruction. Before the destruction of the saponification process is done in advance. The first thing to do is to prepare in advance of four pieces, each crucible crucible filled with oil up to 1 g. A crucible filled with 1 g of oil without the addition of Cu aimed as a control. B crucible filled with 1 g of oil was added 0.5 mL of 50 ppm Cu, C crucible filled with 1 g of oil was added 1 mL of 50 ppm Cu, and D crucible filled with 1 g of oil was added 2 mL of 50 ppm Cu. During the process of adding metal ions, the heating is done in order that the metal ions are added to and form a complex blend perfectly with oil. Here Cu have unpaired electrons that are highly reactive in catalyzing the oxidation and reduction reactions in the oil. Presence of transition metal ions such as Cu excess in the oil is potentially in the formation of ROS (reactive oxygen species), ROS is what will interact with Cu forming lipid peroxidation. In this case Cu forms a complex with fatty acids. Cu will be able to form complexes with fatty acid that has a number of atoms C14 to C20 [2], which can serve as a chelating agent. If a metal has dikhelat it will form a stable complex. Component fatty acids can function

as a metal chelating agent because of the presence of one or two groups carboxyl adjacent hydroxyl groups react with metal ions to form a stable complex. Copper in the form of ions have oxidation number +1 and +2, but it temodinamika stable as copper (II). Copper (II) with d9 structures have different stereochemistry, usually rectangular planar or distorted octahedral. Compounds used as CuSO4.5H2O can form complexes Cu rectangular shaped planar [13].

After that, each of the oil in the crucible reacted with alcoholic KOH 1M in 5 mL KOH where the amount and concentration obtained from the determination of the saponification number. Used in this study due to alcohol alcoholic KOH to dissolve the oil phase and can also be mixed with the water phase. Once it is done again until the mixture is heating oil and soap with KOH to form perfectly. The process of destruction done to the soap that has been formed by means of authoring the furnace with a temperature of 500°C for approximately 5 h. It aims to improve the process of destruction. Destruction is performed until the sample formed white ash and no residual carbon in the crucible. Ash formed was dissolved in 4N HNO3, because the solubility properties of Cu soluble in nitric acid. Subsequently, the samples that have been prepared put into 10 mL volumetric flask and add distilled water to mark boundaries. In this method, the replication procedure is done three times to test the effectiveness of methods of lathering which is a new method in the analysis of metal content in cooking oil.

### 3.4 Determination of copper metal in simulated cooking oils

After destruction, absorbance of sample solutions and actual Cu concentration added in samples were measured. At the Cu measurement system uses flame. Atomization flame using air-acetylene fuel gas measured at a wavelength 342.7 nm Cu. Measurements were performed three times and the average value was taken for each sample solution. Cu concentration measurement results are added to the simulated cooking oil can be seen in Table 4.2.

**Table 2** The actual Cu content added to the simulated oil

Initial Cu conc. addition (ppm)	Cu conc. (ppm)	Average Cu conc. (ppm)
Cu 50 ppm	48.705	48.728
Cu 50 ppm	48.764	
Cu 50 ppm	48.715	

While the results of determination of Cu with various concentrations were added in the simulated cooking oil can be seen in Table 3. After determining the percent recovery was carried out by comparing the levels of Cu<sub>2+</sub> obtained from the saponification method by measuring levels of Cu<sub>2+</sub> solution of 50 ppm (Table 2) prior to the saponification method (at first). Determination of percent recovery was conducted to determine how effective the method is

performed in the analysis lathering metal in the oil. Expected percent recovery ranged from 95-105% [13]. Initial assay results obtained from measurements of initial assay measurement results.

**Table 3** Cu metal concentration in simulated cooking oil

Cu <sup>2+</sup> addition (mg/kg)	Cu <sup>2+</sup> conc. (mg/kg)	Cu <sup>2+</sup> conc. (mg/kg)	Recovery (%)
Cu <sup>2+</sup> 25 ppm	23.427	24.364	96.15%
	23.811		97.73%
	23.604		96.75%
Cu <sup>2+</sup> 50 ppm	47.541	48.728	97.56%
	47.288		97.04%
	47.329		97.13%
Cu <sup>2+</sup> 100 ppm	94.828	97.456	97.30%
	94.452		96.92%
	95.362		97.85%

The results of accuracy (recovery) of the above table shows that the first, second, and third simulations oil with addition of various Cu concentrations obtained good results as entered in the expected range (95-105%).

In the first treatment with the addition of 25 ppm Cu results accuracy (recovery) of 96.15, 97.73, and 96.75%. It is also found in the second and third treatment with the addition of 50 and 100 ppm Cu gives results accuracy (recovery) of 97.56, 97.04, 97.13, 97.30, 96.92, and 97.85% respectively, these values into the expected range, while to measure the accuracy of the saponification method we use analytical accuracy (precision).

Accuracy is the result obtained with the same measurements on the way over and under the same conditions. And the value obtained for the addition of 25 ppm Cu KV = 0.8233%, for the addition of 50 ppm Cu KV = 0.3154% and the addition of 100 ppm Cu KV = 0.4803% accuracy where the limits should not be more than 3%. By looking at the values of accuracy and precision in the above, we can conclude this method is best used to analyze heavy metals in cooking oils.

### 3.5 ANOVA Test

One-way analysis of variance classification used in the experiment by using a single factor. ANOVA was used to compare three or more things "the state" as well. Analysis of variance procedure was introduced by R.A. Fisher, trying to analyze the diversity of the responses and divide into sections dealing with the diversity of known sources and the remainder attributed to random error (random error). Known source of variation is associated with the independent variables, namely the factors tested. In this case compare whether there were significant differences between the additions of Cu with varying levels of Cu initially used



to test the F-test, using the table F. Anova test shown in Table 4.

**Tabel 4** Determination of ANOVA Test

Replication	A	B	C	D	
1	96.15	97.56	97.30	97.41	TOTAL
2	97.73	97.73	96.92	97.53	
3	96.75	96.75	97.85	97.43	
Total results	290.63	292.04	292.08	292.37	1167.12
Replication	3	3	3	3	12
Average results	96.88	97.35	97.36	97.46	
$(\sum x)^2$	28156.538	28429.669	28437.345	28493.414	113516.966
$\frac{(\sum x)^2}{n}$	28155.266	28429.121	28436.909	28493.406	113514.702

Note:

- A = %recovery of Cu 25 ppm addition
- B = %recovery of Cu 50 ppm addition
- C = %recovery of Cu 100 ppm addition
- D = %recovery actual Cu

From the calculation can be seen that the F-count value 0.717, while the F table value = 4.07. We can conclude that there is no difference between the treatment effect. Means lathering using this variation does not affect the concentration of the final product concentration.

### 3.6 Determination of copper metal in cooking oil on the market

In the determination of Cu metal in oil used 3 samples, respectively, are 2 kinds of bulk cooking oil and 1 kind of branded cooking oil. The sample of bulk cooking oil on the market is a palm cooking oil, while the simulated cooking oil using coconut cooking oil. It can be allowed due to palm and coconut cooking oil are the main content of triglycerides and saponifiable. Analysis of this cooking oil sample is the same as the simulated cooking oil processing, the samples were diluted in 25 mL volumetric flask and replicate measurements performed three times.

**Table 5** Determination of Cu in cooking oils in the market

Sample	Absorbance	Conc. (ppm)	Conc. (mg/kg)
Bulk cooking oil 1	0.0723	0.4593	11.5
Bulk cooking oil 2	0.0687	0.4367	10.9
Branded cooking oil	0.0469	0.2979	7.4

It can be seen that Cu metal content in bulk cooking oil is greater than in branded cooking oil. This is because there are possible additional processes contaminated by metals in bulk cooking oil. Given the quality requirements of Cu metal content in the oil is determined by the Indonesian national standard [11] is equal to 0.1 mg/kg, Cu

content in the measured samples of cooking oil exceeds the allowed threshold making it less suitable for consumption.

## 4. CONCLUSION

The research methods of Cu metal content in cooking oils through saponification gives good results. In the first treatment, with addition of 25 ppm Cu results accuracy (recovery) average of 96.88% with precision values of 0.7922%. In the second treatment, with addition of 50 ppm Cu results accuracy (recovery) average of 97.35% with precision values of 0.2575%, and with addition of 100 ppm Cu results accuracy (recovery) of 97.36% with precision values of 0.3162%. The F-test result showed no difference between the various treatments.

The copper metal content in cooking oils in the market for bulk cooking oil 1 had higher concentration at 1.04 mg/100 g, bulk cooking oil 2 at 1.9 mg/100 g, and branded cooking oil at 0.74 mg/100 g, this shows that bulk cooking oil has higher Cu content than branded cooking oil.

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