Nutrition Measurement of “Grombyang” – unique dishes of Pemalang, Central Java Province, Indonesia

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Abstract. Grombyang is one of the typical food from Pemalang Central Java which sold freshly in restaurant or food stall. To marketed commercially outside Pemalang, several parts of grombyang seasoning was made in instant form. The study aimed to analyze the content of nutrients (protein, and fat), and rancidity test contained in each grombyang seasoning which is dry galangal, fried onion, coconut srundeng, a mix of tauco, kluwek and palm sugar. Another aim was to analyze the number of bacteria in grombyang seasoning. The protein test used Kjeldahl method, and fat test used Soxhletasi method. While the bacteria test using Total Plate Number (ALT) method of bacteria and rancidity test using Thio Barbiturate Acid (TBA) method. The results showed that the protein content of each spice component such as srundeng coconut grombyang was 10.8%, petis 12.4%, fried onion 25.9%, palm sugar (aren) 12.3%. Fat content in spice component of grombyang for srundeng kelapa grombyang 41.2%, 50.7%, petis 12.3%, fried onion 24.9%, aren 51.5%. Total Plate Count Test (ALT) of the bacteria showed that total bacteria found in spice grombyang was 12.1 x 10^5 CFU/ml. It can be concluded that the highest protein content found in fried onions and the highest fat content found in palm sugar. Also, spice Grombyang had the number of microbes that were in the standard range of BPOM No. 16 of 2016 (10 x 10^8 - 10 x 10^9 CFU/ml).

1. Introduction

Food choices are primarily influenced by several aspects such as taste, cost, convenience, and nutritional value of the food [1]. Several conveniences could be obtained from preparation until consumption. In many societies, a woman plays an important role in food production, selection, purchase, and processing [2]. Nowadays, more women are having more job, which influences the duration of doing house chores. Other women who become a housewife are also quite busy organize its children. Data from Basic Health Research 2013 showed that there is an increasing number in mother having a job from 2007 (18.3%) to 2013 (23.6%) [3]. Therefore they tend to choose food that easy, practical, but still, have abundant nutritional value, not only for modern food but also traditional food which is familiar in Indonesia due to its diversity.
One of the traditional foods known in Pemalang is grombyang rice soup. This food name was derived from the present form, where the rice and sliced buffalo meat as main ingredients were fulfilled and covered with the gravy. Consequently, the meat looks like having sway (Javanese: grombyang) [4]. This dish was served in a small bowl with buffalo satay and emping crackers made from melinjo.

One of the challenges of traditional food in the modern era is the complexity of the seasoning and cooking process. Therefore many traditional foods are made in instant form especially the seasoning to eliminate several processes, needless skill to cook, and eventually sell it commercially [5]. Similar like other traditional food, grombyang instant seasoning started to made in the household kitchen on a larger scale. It was marketed in Pemalang and has P-IRT, where P-IRT is a food licensing for home scale industry. This license is only given to processed food products with a low level of risk and act as a guaranteed license for home-based food/beverage businesses that are sold and circulated in the community to meet food safety standards or distribution permits for food products [6].

Safe food consumption is necessary to be considered by producers and consumers. Under the Food Law No. 7 of 1996, food security is a condition and effort needed to prevent food from the possibility of biological, chemical and other contaminants that can disrupt, harm and endanger human health. Safe food is food that does not contain biological and microbiological hazards, chemical hazards and physical hazards [7].

Based on these provisions, the customer still needs information about the products even though no consumers are complaining about anything. Since grombyang instant seasonings primarily were made from tauco and srundeng, it needs nutrition analysis such as protein and fat content. Other ingredients to build the depth of flavor, such as kluwek, palm sugar, fried onions, and kitchen spices were also being analyzed. Tauco is a food seasoning made from soybean seeds (Glycine max) which has been boiled, mashed and stirred with wheat flour and left until fermented, while srundeng is grated coconut which is roasted until became fragrant and dry. Both of these ingredients become one of the spice components that are easy to experience rancidity and cause reduced shelf life [8,9]. Therefore, it is necessary to test its total bacteria count and rancidity analysis.

2. Methods
2.1. Material
Component of grombyang seasoning (srundeng, tauco, fried onion, and palm sugar), aquades, alcohol, 0.1 N hydrochloric acid, sulfuric acid, ethyl acetate, toshiro indicator, catalyst mixture (selenium mixture) methanol, 30% sodium hydroxide and n-hexsane (hexsane solvent) PCA medium, 50 ml NaCl and plastic.

2.2. Tools
A set of distillation tools, Burette, Beaker glass, test tubes, measuring cups, measuring flasks, Erlenmeyer, volume pipettes, micro pipettes, autoclaves, test tube shelves, analytic scales, funnels, watch glass, evaporator, petri dish, incubator, freezer, colony counter, methylated lamp, stirrer, analytical balance, tissue, cotton, aluminum foil, incubator 37C, Soxhlet extraction, Kjeldahl flask, oven, desiccator, UV-visible spectrophotometer, mortar, glass preparation, batch, microscope, stove, pan

2.3. Procedures
2.3.1 Protein Analysis
Determination of protein content was carried out using the Kjeldahl method. The Kjeldahl method consists of 3 stages: destruction stage, distillation stage, and titration stage. Destruction stage started with weighed 1 gram of blended sample and put it in a 100 mL Kjeldahl flask, then pipette 10 mL of concentrated sulfuric acid into the Kjeldahl flask. Add a catalyst (selenium mixture) to accelerate destruction. Kjeldahl pumpkin was heated started with a small flame for a while and raised the temperature slowly. Destruction can be stopped when a clear greenish solution was obtained.

Next process was the solution then cooled and diluted with distilled water up to 100 mL. After homogeneous and cold, the solution was pipetted as much as 5 mL and put it in a distillation flask. Add 10 mL of 30% sodium hydroxide solution through the wall in the distillation flask to form a lower layer.
The distillate flask was installed and disposed of with a condenser, then the tip of the condenser is immersed in a collecting liquid. Steam from boiling liquid will flow through the condenser to the Erlenmeyer reservoir. The container Erlenmeyer is removed with 10 mL of 0.1 N hydrochloric acid solution which has been dripped with methyl red indicator. Look at the distillation results with litmus paper; if the results are no longer alkaline, then the distillation is stopped.

After the distillation process, the next stage was titration. The result of distillation which is placed in Erlenmeyer containing 0.1 N hydrochloric acids was dripped with five drops of methyl red indicator directly titrated using 0.1 N sodium hydroxide solution. The end point of the titration was marked pink to yellow. This treatment is carried out three times for each sample.

2.3.2. Fat Analysis
Soxhletation tools were installed, then a sample of 4 grams was wrapped in filter paper, tied with thread, put into a soklet with 50ml hexane solvent in a soklet flask. Socletation was done at a temperature of 105°C until the droplets were clear or approximately 30 minutes. The liquid extract obtained was then cooled for 15 minutes in a desiccator.

2.3.3. Total Bacteria Count
Calculation of the number of bacteria was performed according to TPC (Total Plate Counts). First, sterilize the cup which had been planted with PCA media using autoclave at 121°C for 15 minutes with a pressure of 1 atm. Weighing 5 grams of sample and then pureeing it and put into 45 ml NaCl solution then the sample was homogenized. Then obtained a 10\(^{-1}\) dilution sample suspend.

The sample was diluted 10\(^{-1}\) using a 0.01ml micro pipette into the test tube 1 (10\(^{-2}\) dilution sample suspension). Then the solution in the test tube one was taken with a 0.05 ml micro pipette and then put into a test tube 2 (suspend of the 10\(^{-3}\) dilution sample) and taken again using a 0.05 ml pipette and put into the test tube 3 (suspend of the 10\(^{-4}\) dilution sample).

Sample solutions on the 1,2 and 3 test tubes were put into each plate which had been poured by PCA media approximately 50°C as much as 15-20ml. The pouring must be near the fire. Then the cup was shaken or rotated so that the sample solution was evenly distributed in the cup. Wait until the media solidifies and put into plastic and put into the incubator in an upside down position at 370°C for 24-48 hours. After a maximum of 48 hours then put in the freezer. Finally, the plate was observed and calculated using the colony counter.

2.3.4. Rancidity Test
Samples that have fried, weighed 10 grams, added 10 mL of TCA heated for approximately 2 minutes, cooled and then filtered. Taken 5 mL of filtrate and add 5 mL of Thiobarbituric Acid reagent. After the solution was heated ± 30 minutes above the water bath, the color would turn into pink; then the sample was cooled. Absorbance was measured at a wavelength of 528 nm. Determination of thiobarbiturate acid numbers using the following formula: \(^9\)

\[
(1) \quad \text{TBA} = \frac{3}{\text{sample weight (gram)}} \times A \times 7.8
\]

\[
A = \text{Absorbance in 528 nm wavelength}
\]

\[7.8 = \text{TBA number, mg malonaldehyde/Kg sample}\]

\[3 = \text{iod number, the degree of unsaturated fat}\]

3. Result and Discussion
3.1. Protein and Fat Analysis

| No | Sample                                                                 | Result | % Protein | % Fat |
|----|------------------------------------------------------------------------|--------|-----------|-------|
| 1  | Dry Galangal                                                           |        | 10.8      | 50.7  |
| 2  | Mix of tauco+kluwek+palm sugar (TKP)                                   |        | 12.4      | 12.3  |
| 3  | Fried onion (FO)                                                      |        | 25.9      | 24.9  |
| 4  | Coconut Srundeng (CS)                                                 |        | 12.3      | 51.5  |
The highest protein content was found in fried onions (25.9%) while the highest fat content was found in coconut srundeng (51.5%). Srundeng was made from shredded coconut with spice paste, palm sugar, and tamarind. The main ingredient, coconut, was a primary source of fat, even its fat content was made up of approximately 92% saturated fatty acids [10].

Protein is a polymer with amino acids as its monomer — protein functions in forming new cells in the body and replacing cells in damaged tissue. The specialty of the protein structure is the presence of nitrogen (N) atoms. Therefore one way to quantitatively analyze proteins is by determining N content in food ingredients or other ingredients which could be detected by using the Kjeldahl method. This method allows us to measure the nitrogen (N) content of proteins and ammonium ions by re-titrating after oxidation of proteins by sulfuric acid and heating. It is suitable for determining levels of a protein that is not dissolved or proteins that have undergone coagulation due to the heating process and the processing that is usually done on food [11].

Table 1 displays that the highest protein content was found in fried onions (25.9%) while the highest fat content was found in srundeng (51.5%) followed by dry galangal (50.7%). Fat content was analyzed by Soxhlet method which the principle was by extracting fat from a fat solvent such as petroleum benzene, petroleum ether, acetone, and others. Fat weight was determined by separating the fat from its solvent. Coconut as the main ingredient in srundeng was high in saturated fat content [10], while dry galangal was made from shredded old galangal which was roasted until became dry and had the ability in binding the water content so that with reduced water content can increase the fat content.

### 3.2. Total Bacterial Count

| Sample | Dilution Serial | Total Bacterial (CFU/ml) |
|--------|----------------|-------------------------|
| GS 1   | 314            | 3.1x10⁴                  |
| GS 2   | 224            | 360x10⁴                 |
| GS 3   | 157            | 0.1x10⁴                 |

GS: Grombyang Seasoning

Total bacterial in Grambyong seasoning has as much as 12,1 x 10⁵ CFU/ml, which is still considered to be safe according to the standards set by the Food and Drug Supervisory Agency (BPOM) No. 16 of 2016 concerning the categories of microbiological criteria in processed food which states that the standard number of colony/ml bacteria samples was 10 x 10⁸ - 10 x 10⁹ CFU/ml [11].

Bacterial counted in Grombyang instant seasoning was 12,1 x 10⁵ CFU/ml, which is considered to be safe due to still under reference number according to the standards set by the Food and Drug Supervisory Agency (BPOM) No. 16 of 2016 [11]. However, still need further handling to avoid an escalation in bacterial number above the reference criteria. The need for good hygiene practices, storage, proper handling such as cooking method, and retail of Grombyang instant seasoning in a clean environment are the best means of controlling the growth of microorganisms in food [12].

### 3.3. Rancidity Test

Table 3 displays on the TBA number was increasing in each component of Grombyang instant seasoning. Storage time increases rancidity [13]. The increasing value of TBA determines that rancidity has occurred on the products. Indonesia National Standard number 2352-1991 about Determination of TBA validated that a product was considered as rancid when having TBA number above 3 mg malonaldehyde/kg sample [13]. Malonaldehyde is formed at the end of the oxidation process therefore
at the beginning of storage the TBA number was still relatively small and has increased in line with storage time [14]. This increase in TBA number is displayed in Figure 1.

### Table 3. Rancidity test of Grombang Seasoning

| Sample                                      | D0       | D1       | D2       | D3       |
|---------------------------------------------|----------|----------|----------|----------|
| Dry Galangal                                | 1.6536   | 3.0264   | 3.1122   | 5.655    |
| Mix of *tauco*+*kluwek*+palm sugar (TKP)  | 0.7644   | 3.0342   | 4.1106   | 7.098    |
| Fried onion (FO)                           | 2.73     | 2.6442   | 2.6754   | 7.098    |
| Coconut *Srundeng* (CSR)                   | 2.3244   | 2.3556   | 2.6208   | 3.9312   |

D = Day, mgMA/kg sample
D0 : Day 0, D1 : Day 5, D2 : Day 10, D3 : Day 15

**Figure 1. TBA number in a component of Grombyang Instant Seasoning**

Figure 1 illustrates the increase in rancidity from the day "0" to "15" and the highest increase occurred on the "15" day. This rancidity itself is a smell or taste change that we often encounter in foods that contain oil and fat [8]. The rancidity of this instant seasoning component is caused by seasoning which contains oil and fat which can be oxidized from the way of storage, processing and due to heat treatment [15]. Therefore TBA number could also determine Grombyang instant seasoning’s shelf life by using linear regression equation: y (absorbance) = ax (concentration) + b and y (absorbance) = TBA number /7.8. The result was shown in Table 4.

Coconut Srundeng has the highest shelf life among all component, and dry galangal and fried onion hast the lowest shelf life among all component.

The thiobarbituric acid test is a test used to determine rancidity. This test is based on the formation of a red pigment as a result of a condensation reaction between 2 molecules of thiobarbituric acid (TBA) and a malonaldehyde molecule. TBA is a specific test for the results of unsaturated fatty acid oxidation and can be applied to food fat tests that contain fatty acids at high levels of unsaturation. The value of
TBA is one index to determine the degree of lipid oxidation which is calculated based on the number of malondialdehyde. Data showed that the rate of rancidity increases concerning storage time. It was similar to data using sample cocoyam chips [16].

Table 4. The shelf life of Grombyang Instant Seasoning

| Sample                                | Shelf Life (x X 5) (day) |
|---------------------------------------|--------------------------|
| Dry Galangal (DG)                     | 1.5                      |
| A mix of tauco+kluwek+palm sugar (TKP)| 5                       |
| Fried onion (FO)                      | 1.5                      |
| Coconut Strundeng (CSR)               | 7                        |

Damage to food products can occur due to oxidation or hydrolysis of food components [17]. The National Standardization Agency in 1991 stated that the maximum limit of oil rancidity is 3 mg malonaldehyde / Kg sample. The quality of oil will decrease because rancid oils contain aldehydes and malonaldehyde [17].

4. Conclusion
The highest protein content found in fried onions and the highest fat content found in palm sugar. Each component of grombyang seasoning had its function and their nutrition value. Also, Grombyang seasoning had the number of microbes that were in the standard range of BPOM No. 16 of 2016 (10 x 10⁸ - 10 x 10⁹ CFU/ml).

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