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# Radical Scavenger and Antioxidant Potency Of Kaopi and Kasoami Made By Yellow Cassava: A Traditional Functional Food from Southeast Sulawesi

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**ABSTRACT**

*Yellow cassava (Manihot esculenta crantz) is an antioxidant source due to the phenolic compound and flavonoid in Yellow cassava. The purpose of this study is to examine the phytochemical constituent of processed Yellow cassava, which were Kaopi and Kasoami qualitative and quantitatively, following by assaying its antioxidant capacity. Kaopi and Kasoami from yellow cassava were qualitatively screening their chemical constituent and quantitatively measured their total phenolic and flavonoid content. They were conducted using colorimetric methods. After that, the samples were assayed their antioxidant capacity, according to ABTS and DPPH methods. The results showed that both samples contain phenolic, flavonoids, and alkaloids. Continued by measured the total phenolic content which were 11.34 ± 0.61 41 mgGAE / g for Kaopi and 14.03 ± 1.82 mgGAE / g ex for Kasoami, respectively and the total flavonoid content were 0.49 ± 0.34 mgQE / g for Kaopi and 0.26 ± 0.24 mgQE / g for Kasoami. According to antioxidant capacity assay, Kaopi had $IC_{50}$ of 193.76 ± 1.50 mg / L for ABTS and 196.67 ± 1.04 mg / mL for DPPH, while Kasoami had $IC_{50}$ of 179,83±2,11 mg/L for ABTS and 182,59±1,52 mg/L for DPPH. It concluded that processed yellow cassava still has the antioxidant capacity and can be beneficial for health.*

**Keywords:** *Yellow cassava (Manihot esculenta Crantz); Kaopi; Kasoami; Antioxidant Capacity*

## I. INTRODUCTION

Oxidative stress is a normal phenomenon that occurs in the body. Typically, ROS (reactive oxygen species) is maintained at a low level. ROS, including free radicals such as superoxide anion, singlet oxygen, lipid peroxides, and hydroxyl radical, play a role in body functions such as cellular energy production and cell signaling apoptosis, gene expression, and ion transport. However, these ROS in large amounts can damage the body's molecules such as DNA, RNA, cells, proteins, and lipids, since they are highly reactive. In overcoming this, the body needs antioxidants. The antioxidant is a substance that can get rid of various free radicals and reactive species of oxygen and nitrogen (ROS and RNS). Oxidant / antioxidant balance is required in preventing this damage [1-3].

Plants are a source of natural antioxidants. Plants contain various kinds of phytochemicals that can act as antioxidants. Antioxidants that come from food can have beneficial effects on the body's health through various mechanisms such as antioxidant defenses, longevity, and maintaining cell integrity and repairing DNA [1,4]. One of the plants that contain antioxidants is cassava.

Cassava, with the Latin name *Manihot esculenta* Crantz, is a plant that is often used by the community as a staple food because of its carbohydrate content. However, cassava contains compounds that are antioxidants. Based on previous research, cassava from white and yellow varieties has the antioxidant capacity with phenolic content [5]. Cassava, as a staple food by the Butonese, was converted into Kasoami. Kasoami, which comes from cassava, is turned into Kaopi first. Foods that are rich in antioxidant activity are recognized by nutritionists and food science

alike. Therefore, this study aims to assay the phytochemical constituent of processed yellow cassava (*Manihot esculenta* Crantz), Kaopi and Kasoami, and their antioxidant capacity.

## II. MATERIAL AND METHODS

Sample Preparation:

The sample preparation process began with yellow cassava peeling, removing the brownish and white outer skin of the cassava root on the inner layer. The stripping of cassava tubers is carried out using a knife, cutting it lengthwise, and then pulling the skin out. Washing cassava tubers using clean and running water to remove dirt and mucus on the peeled cassava tubers' surface and then draining them. After being drained, the cassava tubers are shredded with a grater machine.

It was continued by pressing the grated cassava tubers hydraulic press until the juice does not flow anymore. The pressing of grated cassava tubers aims to reduce the water content, HCN and odor, leads to the speeds up drying. The result of pressing cassava tubers is called Kaopi. The Kaopi obtained is **1023 g**. Then, the Kaopi is loosened and aerated to reduce the moisture, distinctive aroma of cassava, and cassava's HCN content. After that, the treatment result was steamed with pot for 30 minutes and obtained a total of **1074 g** Kasoami.

Extraction:

Kaopi (1023 g) and Kasoami (1074 g) from yellow cassava were macerated with 96% ethanol for 3 days in a sealed jar (1: 1 w / v). In the maceration process, every 24 hrs, the solvent is taken and replaced (re-maceration) to obtain the filtrate. The filtrate was then thickened using a rotary evaporator (40℃), and a thick extract was obtained, respectively, 14.6 g for Kaopi (1.34%) and 2.57 g (0.25%) for Kasoami.

Phytochemical Screening:

Phytochemical screening is conducted using a colorimetric method to detect secondary metabolites' presence in the extract.

a. Phenol and Tannin Test
   The extract solution was mixed with 2 ml of 2% FeCl3 solution. A turquoise or black color indicates the presence of phenols and tannins.

b. Flavonoid Test
   The extract solution was mixed with 2 ml of 2% NaOH solution. A thick yellow color turns colorless with the addition of a few drops of dilute acid, which indicates the presence of flavonoids.

c. Steroid Test
   The extract solution was mixed with 2 ml of chloroform, and $H_2SO_4$ was added. Red or greenish color produced in the lower chloroform layer indicates the presence of steroids.

d. Terpenoid Test
   The extract solution was added with 2 ml of $H_2SO_4$ and heated for about 2 minutes. The grayish color indicates the presence of terpenoids.

e. Alkaloid Test
   The crude extract was mixed with 2 ml of 1% HCl and heated slowly. The Mayer reagent is then added to the mixture. The turbidity of the resulting sediment is evidence of the presence of alkaloids.

Total Phenolic Content:

Determination of total phenol levels using a colorimetric method that refers to the procedure of Chun et al. (2003) with gallic acid (GAE) as a standard [7].

a. Preparation of 7% Na2CO3 reagent
   Weighed 3.5 g Na2CO3 then dissolved in distilled water up to 50 ml.

b. Determination of Total Phenolic Content
   1. Preparation of Standard Solution Gallic Acid Standard
      The solution of 1000 ppm gallic acid is prepared by weighing 10 mg of gallic acid dissolved with ethanol up to 10 mL, from the stock solution, pipetted as much as 2.5 mL diluted with ethanol up to 25 mL resulting in a concentration of 100 ppm. From this solution, pipettes 1, 2, 3, 4, 5 mL, and added with methanol up to 10 mL, resulting in concentrations of 10, 20, 30, 40, and 50 ppm.

   2. Measurement of Gallic Acid Standard Solution
      For each concentration of 10, 20, 30, 40 and 50 ppm added with 0.4 mL of Folin-Ciocalteau reagent, shaken and left for 4-8 min, continued by adding 4.0 mL of 7% Na2CO3 solution, shake until homogeneous and added with distilled water up to 10 mL and left for 2 hrs at room temperature. The absorbance was measured at a wavelength of 750 nm. A calibration curve was made for the relationship between the concentration of gallic acid (mg / L) and the absorbance.

3. Preparation of Extract Solution
   The extract solution was prepared by weighing 10 mg of extract and then dissolving it with 10 mL ethanol.

4. Determination of Total Phenol for Extract
   The extract solution was pipette (1 mL) and added with 0.4 mL of Folin Ciocalteau reagent, shaken and left for 4-8 min, continued by adding 4.0 mL of 7% Na2CO3 solution, shake until homogeneous. Furthermore, the solution was added with distilled water up to 10 mL and let stand for 2 hrs at room temperature. The absorption was measured at a wavelength of 750 nm. The measurement was performed in triplicates; thus, phenol content obtained is equivalent to gallic acid/g extract.

Total Flavonoid Content:
Determination of total flavonoid levels using the colorimetric method, which refers to the procedure of Chang et al. (2002) with quercetin (QE) as the standard.

a. Preparation of Quercetin Standard Solution
   Quercetin standard (10 mg) was weighed and dissolved in 10 ml of ethanol for 1000 ppm. From a standard solution of 1000 ppm quercetin, 1 mL of pipetted and dissolved in 10 mL of ethanol to obtain 100 ppm. Then, several concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm are made. From each concentration of the standard quercetin solution, 3 mL of methanol, 0.2 mL of 10% AlCl3, 0.2 mL of 1 M potassium acetate were added to it, and distilled water was added up to 10 mL. After that, it was incubated for 30 minutes at room temperature and measured the absorbance on UV-Vis spectrophotometry with a wavelength of 431 nm.

b. Preparation of Extract Solution
   The extract solution was prepared by weighing 10 mg and then dissolving it with 10 mL ethanol.

c. Determination of Total Flavonoid Levels
   The sample (1 mL) was pipetted and added with 3 mL of methanol, 0.2 mL of 10% $AlCl_3$, add 0.2 mL of potassium acetate, and distilled water up to 10 mL. The solution was stored for 30 minutes in a dark place at room temperature, and the absorbance was measured on UV-Vis spectrophotometry with a wavelength of 431 nm. The sample solution was performed in triplicates; thus, the contents of flavonoids obtained were equivalent to quercetin.

Antioxidant Assay:

a. DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Free Radical Inhibitor Activity Test

The test was conducted according to the modified Blois (1958) method [9]. DPPH was prepared in a concentration of 0.1 mM in methanol solvent. Simultaneously the extract was prepared in different concentrations (10, 20, 30, 40, and 50 mg / L). A total of 1 mL of DPPH solution was mixed with 2 mL of extract solution at each concentration. The mixture was then incubated in the dark at room temperature for 30 min. Control was made by mixing 1 mL of DPPH solution with methanol as solvent. The absorbance was measured against the blank at 517 nm using a spectrophotometer (Jenway). The lower absorbance of the reaction mixture indicates a higher DPPH free radical inhibitory activity. The standard used was Ascorbic acid (Sigma-Aldrich). Samples were prepared and measured in triplicates. The percentage of inhibitory activity of each extract on the DPPH radical was calculated as% DPPH inhibition (I%) using the following equation:

I% = [(Ao-As) / Ao] × 100
Ao is the absorbance of the control, and As is the absorbance of the extract solution test.

b. ABTS [2,2'-Azinobis (3-Ethylbenzothiazoline-6- Sulphonic Acid) Free Radical Inhibitor Activity Test]

The test was conducted by referring to the modified Re et al. (1999) method [10]. The ABTS ● + stock solution is prepared by mixing 7 mM of ABTS (Sigma Aldrich) solution with an equal amount of 2.45 mM potassium per sulfate (Merck) solution. The mixture is left in the dark at room temperature for 12-16 hrs prior to usage. The ABTS ● + working solution was obtained by diluting the methanol stock solution to provide an absorbance of 0.70 ± 0.02 at 734 nm. Extract solutions were prepared in different concentrations (10, 20, 30, 40 and 50 mg / L). Next, 2.0 mL of the ABTS ● + solution was mixed with 1 mL of each extract. The mixture was then incubated in the dark at room temperature for 10 min. Control was made by mixing 2.0 mL of ABTS ● + solution with 1 mL of methanol. The absorbance was measured against the blank at 734 nm using a spectrophotometer (Jenway). The standard used was Ascorbic acid. Samples were prepared and measured in triplicates. The percentage of inhibitory activity of each extract on ABTS radicals was calculated as% inhibition of ABTS (I%) using the following equation:

I% = [(Ao-As) / Ao] × 100
Ao is the absorbance of the control, and As is the absorbance of the extract solution test.

c. Determination The $IC_{50}$

The $IC_{50}$ value of each sample is calculated based on the percentage of inhibition to radicals from each concentration of the sample solution. After obtaining the percentage of inhibition (y) of each concentration (x), the points (x and y) are plotted on the coordinate plane, then the line equation y = ax + b is determined by calculating by

linear regression where a and b are constants, x is the sample concentration (mg / L), and y is the percentage of inhibition (%). Antioxidant activity is expressed by Inhibition Concentration 50 (IC50), namely the concentration of the sample (x), which can reduce 50% of radicals (y = 50).

## III. RESULTS AND DISCUSSION

Phytochemical screening is the initial stage of determining the compound content of a sample qualitatively [6]. The results of the colorimetric Kaopi and Kasoami phytochemical screening of Yellow Cassava demonstrated that both samples contain phenolic compounds, flavonoids, and alkaloids (**Table 1**).

**Table 1**. Phytochemical screening of Kaopi and Kasoami from Yellow Cassava

| Sample | Phenolic | Flavonoid | Steroid | Terpenoid | Alkaloid |
|---|---|---|---|---|---|
| Kaopi from yellow cassava | + | + | - | - | + |
| Kasoami from Yellow cassava | + | + | - | - | + |

*: + indicates positive, while - indicates negative

After the phytochemical screening, the total phenolic and flavonoids content was determined quantitatively. In determining the total phenolic content, a series of standard solutions of gallic acid was made to obtain a regression equation used to determine the sample's total phenolic content by measuring the absorbance at 750 nm. The concentrations used were 10, 20, 30, 40, and 50 mg / L. From the results of these measurements, linear regression was obtained, which is shown in **Figure 1.**

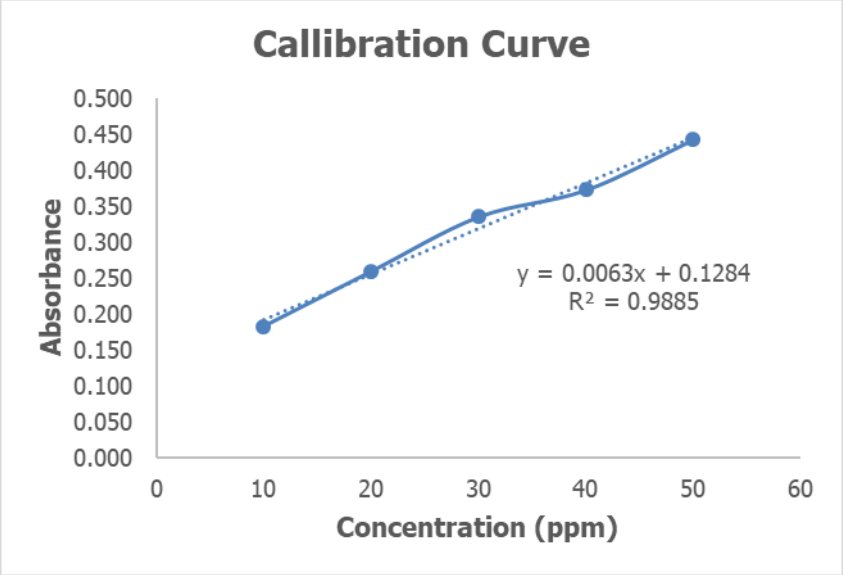


**Figure 1.** Calibration Curve of Gallic Acid Solution Standard

From the results of total phenolic content, it was found that all samples contained phenolic compounds. According to total phenolic content test, kaopi contained a total phenolic content of 11.34 ± 0.61 41 mgGAE / g ex and kasoami contained 14.03 ± 1.82 mgGAE / g ex (**Table 2**).

**Table 2.** Total Phenolic Content Measurement of Kaopi and Kasoami from Yellow Cassava

| Sample | Replication | Absorbance | Total Phenolic (mgGAE/g extract) | Total Phenolic Mean (mgGAE/g extract) |
|---|---|---|---|---|
| Kaopi from Yellow Cassava | 1 | 0,208 | 12,03 | 11,34±0,61 |
| | 2 | 0,198 | 11,05 | |
| | 3 | 0,2 | 10,93 | |
| Kasoami from Yellow Cassava | 1 | 0,231 | 15,51 | 14,03±1,82 |
| | 2 | 0,204 | 12,00 | |
| | 3 | 0,224 | 14,59 | |

In determining total flavonoid levels, the standard quercetin solution series with concentrations of 10, 20, 30, 40, and 50 mg / L to obtain the regression equation by measuring the absorbance at 431 nm. The linear regression obtained is presented in **Figure 2**.

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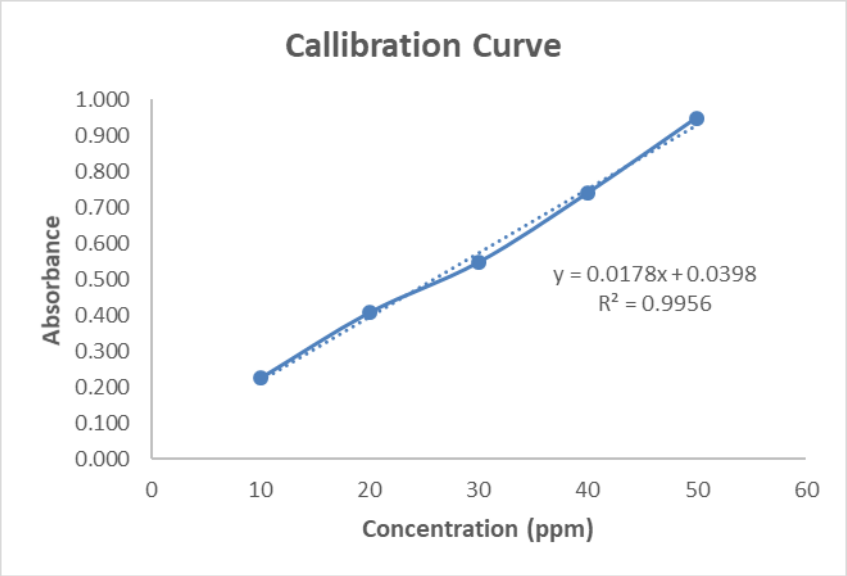


**Figure 2.** Calibration Curve of Quercetin Standard Solutions

From the total flavonoid content measurement results, both samples contain flavonoids. These results were determined, and it was found that the total flavonoid levels of Kaopi were 0.49 ± 0.34 mgQE / g ex, while for kasoami, it was 0.26 ± 0.24 mgQE / g ex (**Table 3**).

**Table 3.** Total Flavonoid Content Measurement of Kaopi and Kasoami from Yellow Cassava

| Sample | Replication | Absorbance | Total Flavonoid (mgQE/g extract) | Total Flavonoid Mean (mgQE/g extract) |
|---|---|---|---|---|
| Kaopi from Yellow Cassava | 1 | 0,042 | 0,12 | 0,49±0,34 |
| | 2 | 0,054 | 0,80 | |
| | 3 | 0,05 | 0,55 | |
| Kasoami from Yellow Cassava | 1 | 0,049 | 0,49 | 0,26±0,24 |
| | 2 | 0,04 | 0,01 | |
| | 3 | 0,045 | 0,28 | |

Phenolic compounds and flavonoids have potential as antioxidants. These two compounds capable of neutralizing radical compounds, chelated iron, and reduce oxidation activity. Various studies have stated a linear relationship between total phenolic and flavonoid content on antioxidant capacity [11,12]. According to results conducted (**Table 4**), Kaopi had $IC_{50}$ with 193.76 ± 1.50 mg / L for ABTS and 196.67 ± 1.04 mg / mL for DPPH, while Kasoami had $IC_{50}$ with 179,83±2,11 mg/L for ABTS and 182,59±1,52 mg/L for DPPH. In comparison with the standard used, the $IC_{50}$ of both samples was still higher. Thus conclude that the antioxidant capacity of both samples was lower than ascorbic acid.

**Table 4.** Antioxidant capacity of samples by DPPH and ABTS

| Sample | Concentration (mg/L) | % Inhibition | | $IC_{50}$ (mg/L) | |
|---|---|---|---|---|---|
| | | ABTS | DPPH | ABTS | DPPH |
| Kaopi from Yellow Cassava | 10 | 2,80 | 14,38 | 193,76±1,50 | 196,67±1,04 |
| | 20 | 5,01 | 16,02 | | |
| | 30 | 6,93 | 17,45 | | |
| | 40 | 10,58 | 20,16 | | |
| | 50 | 12,94 | 21,91 | | |
| Kasoami from Yellow Cassava | 10 | 4,87 | 15,91 | 179,83±2,11 | 182,59±1,52 |
| | 20 | 6,64 | 17,23 | | |
| | 30 | 9,15 | 19,10 | | |
| | 40 | 12,55 | 21,62 | | |
| | 50 | 15,35 | 23,71 | | |
| Ascorbic acid | 10 | 53,87 | 54,52 | 9,58±0,57 | 7,73±0,43 |
| | 20 | 58,21 | 57,75 | | |
| | 30 | 73,17 | 68,90 | | |
| | 40 | 86,95 | 79,17 | | |
| | 50 | 98,73 | 87,95 | | |

## IV. CONCLUSION

Kaopi and Kasoami from yellow cassava (*Manihot esculenta* crantz) are traditional food from Butonese that provide antioxidant capacity with chemical constituent such as phenolic compounds flavonoids might be playing a role in providing the effect.

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