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Antioxidant, total phenolic content and physicochemical properties of modified cassava flour

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Abstract. This study aimed to evaluate the antioxidant activity, phenolic content, and nutritional properties of the yellow cassava variety called Adira. Sample was freeze dried cassava (cassava); oven dried cassava (cassava flour) and modified cassava flour (mocaf). Antioxidant properties ofthe extracts were evaluated using a DPPH *(2,2-diphenyl-1-picryl-hydrazyl)* assay and phenolic content using the *Folin-Ciocalteu* method. Nutritional properties such as water, ash, protein, lipid, and carbohydrate were analyzed. Antioxidant activity was 25.03%, 30,37%, and 31.23% for cassava, cassava flour, and mocaf respectively and in line with phenolic content mocaf had the highest total phenolic content of 3.46 mg/g GAE. Carbohydrate was the major content in Adira mocaf of > 85%, while the other content was protein of 1.44 % - 2.41 %, ash of 2.21 % - 2.85 %, and lipid of 0.04 % - 0.10 %. Analysis by the FTIR (Fourier Transform Infrared Spectroscopy) analysis presented the OH group which may have been generated from phenolic and flavonoids compounds in the mocaf.

1. Introduction

Cassava (*Manihot esculenta*, Crantz) is a popular plant that is widely grown and utilized as a carbohydrate in Indonesia. However, the fresh tuber cannot be stored for a long time, it will change to dark blue color due to the formation of cyanide which is a toxic substance. Thus, mocaf is commonly manufactured to prolong the shelf-life of cassava [1][2], and for variation of further food products such as gluten-free biscuits [2].

Cassava can be grown on land with low fertility, and thus can provide food in famine or war conditions [3]. A previous study also revealed that cassava is more adaptable to environmental changes which also makes cassava more sustainable [4]. Cassava farming is important to increase its quality, yield, and sustainability to meet the supply of public consumption needs. However, cassava farming needs to be properly regulated so that it does not harm the environment.

The main components of cassava are carbohydrates (about 80% – 90% dry weight or 32% – 35% wet weight) and a small portion is a protein (1% - 3% dry weight or 0.4% - 1.5% wet weight); and fat about 0.1% - 0.3% wet weight [5][6]. As the main source of calories, cassava is used as a staple food in some developing countries. Natural antioxidants in cassava act as a secondary metabolite [7][8]. Phenolic, flavonoid, anthocyanin, and carotenoid as natural antioxidants or phytochemical antioxidants possess many functions such as extending their shelf-life by preventing deterioration of the tubers. In addition, antioxidants have the ability in reducing the risk of diseases [9].

The fermentation process of cassava with lactic acid bacteria increases the protein content [10].

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Another study also confirmed that modification of cassava flour processing using microbial agents through fermentation can increase protein content [11]. However, there are still a few studies that comprehensively studied the physicochemical properties and biological activities of mocaf. Thus, this research proposed to investigate the antioxidant, phenolic content, and physicochemical properties of the yellow cassava variety called Adira. It is hoped that the study of modified cassava flour will also be part of efforts to improve the economy of farming communities, the sustainability of cassava agriculture, and the protection of the environment.

2. Materials and methods

The material used for this research was yellow cassava (Adira varieties) from Boyolali, Jawa Tengah, Indonesia. Another material used for characterization was 2,2-diphenylpicrylhydrazyl (DPPH, Sigma), methanol (Merck), gallic acid (Merck), Na_2CO_3 (Merck), *folin-ciocalteu* (Merck), aquadest, Boricacid (Merck) Sulfuric acid (Merck), Sodium hydroxide (Merck 06498), Kjeldahl tablets (Merck), hydrochloric acid (Merck), and petroleum benzene (Merck).

2.1. Sample preparation

The tubers were peeled, washed, and cleaned. For the fresh sample, cassava was grated, freeze dried. Cassava flour is made from peeled roots, grated, and dried for 24 hours at 50°C [12]. Mocaf was made from cleaned cassava, sliced into chips with a thickness of 2 mm, soaked until all parts of the chips are perfectly fermented in starter solution (1 gram of starter per liter of water) for 24 hours, and followed by soaking in $Na_2S_2O_5$ (0.15%) for 30 minutes. The remaining water from fermented chips was then discarded before the drying process in the oven at 50° C for 24 hours [13]. All the samples were ground into powder form, placed in a zip lock, and stored at 4°C. The samples (freeze dried, flour, and mocaf) were macerated using methanol (1: 10 w/v) at 30° C for 24 hours. The resulting mixture was filtered, evaporated, and the crude extracts of methanol were obtained. All resultant extracts were properly collected and kept in refrigeration for further analysis.

2.2. Characterization

2.2.1. Antioxidant activity. Sample extracts (1000 ppm) was made using methanol as solvent as stock solution. DPPH (1.01 mM in MeOH) was added to sample and reacted for 30 min in dark at 30° C. Microplate reader (Thermo-scientific, US) was used to measuring the absorbances at 517 nm [14]. The antioxidant activity was measured as follows:

$$DPPH\ scavenging\ activity\ (\%)\ \frac{A0-A1}{A0}x100 \qquad (1)$$

where A0: control absorbance and A1: sample absorbance

2.2.2. Total phenolic content (TPC). Folin-Ciocalteu assay was used to measure the total phenolics content [10]. Samples (0-200 μg/ml) 100 μL was added 2.8 mL aquadest, followed adding with sodium carbonate (2%, 2mL). The mixture was added with *Folin-Ciocalteu* (100 μL) and incubated for 30 minutes [15]. Microplate reader (Thermo-scientific, US) was used to measuring the absorbances at 760 nm and mg gallic acid equivalent (mg GAE) used to expressed total phenolics.

2.2.3. Proximate. Thermogravimetry method used for moisture content and ash content, micro Kjeldahl for protein, soxhlet extraction for lipid [16], and carbohydrate using by difference methods [17].

2.2.4. FTIR analysis. Functional groups of mocaf was analyzed using FTIR- ATR method (attenuated total reflectance) (ATR) to study the functional group of components in the modified cassava flour. The wavenumber was in the range of 4000 cm-^{1} to 600 cm-^{1} using Vertex 80, Bruker, Germany.

3. Results and discussion

3.1 Antioxidant activity

The antioxidant capacity was evaluated based on their radical scavenging activity toward radicals of DPPH. DPPH was used because the assay was easy, rapid, and sensitive. Figure 1 show the result of the antioxidant activity of cassava, cassava flour, and mocaf extract. The scavenging activity of samples was 25.03%, 30,37%, and 31.23% for cassava, cassava flour, and mocaf respectively. A study for processing yam flour using acid bacteria fermentation increased antioxidant activity [18]. Phenolic, flavonoid, anthocyanin, and carotenoid as natural antioxidants or phytochemical antioxidants have numerous benefits on tubers that prevent the deterioration as well as prolong the lifespan [9].

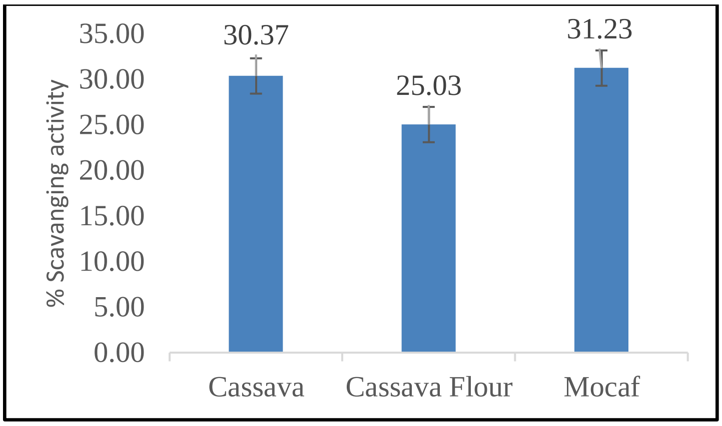

Figure 1. Scavenging activity of cassava, cassava flour, and modified cassava flour of Adira variety.

3.2 Total phenolic content (TPC)

Phenolic compound is the essential constituent contained in natural products which give functionalities for human health. Standard for the evaluation of the total phenolic content (TPC) ofcassava, cassava flour, and mocaf was Gallic acid. Figure 2 showed that mocaf had the highest TPC (3.46 mg GAE /g), followed by cassava and cassava flour (3.12 and 3.04 mg/g GAE).

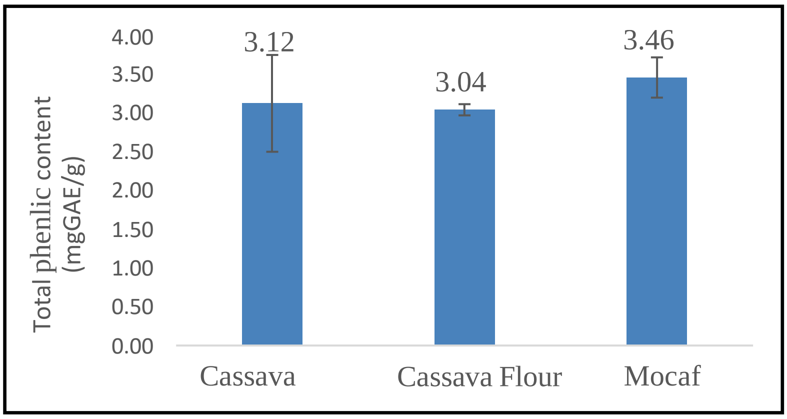

Figure 2. Total phenolic content of cassava, cassava flour, and modified cassava flour of Adira variety.

The literature also had a similar result, that the addition of mocaf in rice flour could increase the total phenolic content of rice noodles and cassava–based rice analogs [19][20]. The result of this total phenolic content can contribute more to an understanding of the antioxidant properties that arise from cassava tuber, cassava flour, and mocaf of Adira variety. The presence of phenolic constituents has been related with the antioxidant activity.

3.3 Proximate

Table 1. showed the proximate composition of cassava, cassava flour, and mocaf. Mocaf had the highest content of water, protein, and lipid, but was lower in carbohydrate. The result showed that the fermentation process of cassava using lactic acid bacteria increases the protein content [10].

Table 1. Nutrient value of cassava, cassava flour, and mocaf (%db).

Sample	Water (%)	Ash (%)	Protein (%)	Lipid (%)	Carbohydrate (%)
Cassava	4.83 ± 0.21	2.85 ± 0.10	1.44 ± 0.01	0.04 ± 0.00	90.83 ± 0.13
Cassava flour	5.85 ± 0.31	2.21 ± 0.51	2.40 ± 0.17	0.07 ± 0.00	89.47 ± 0.36
Mocaf	9.48 ± 0.50	2.72 ± 0.10	2.41 ± 0.20	0.10 ± 0.01	85.29 ± 0.27

3.4 FTIR analysis

The functional groups in the cassava tuber, cassava flour, and modified cassava flour (mocaf) were investigated using FTIR. It was confirmed that FTIR instruments are easy, fast, and practical to use in food detection and analysis [21]. The FTIR spectra of the samples was presented in Figure 3.

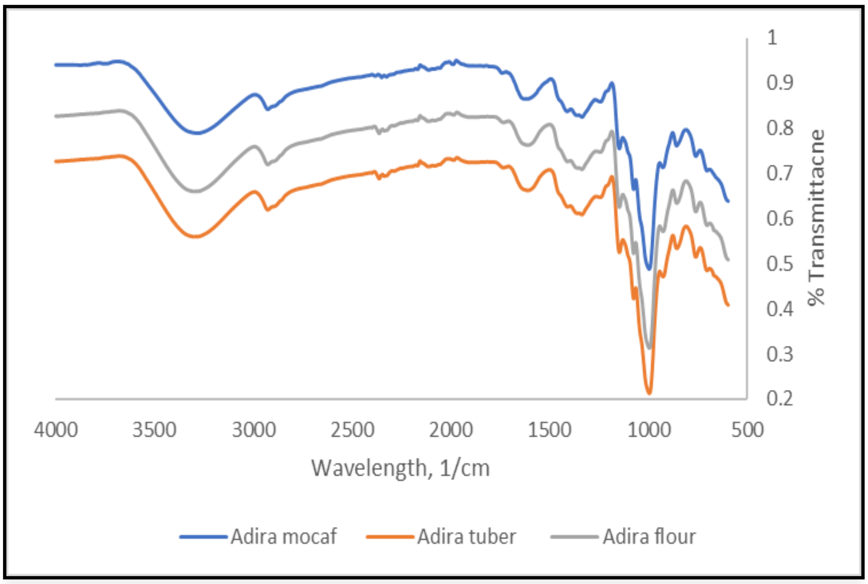

Figure 3. The FTIR spectra of cassava, cassava flour, and mocaf.

The FTIR spectra of Adira tuber, flour, and mocaf showed that the treatment of flour processing/fermentation did not change the functional groups significantly, since the peaks detected were similar. This was probably caused because all three samples were produced from cassava and thus consisted of similar major components of amylose and amylopectin. This phenomenon was also observed in the literature [22]. The broad absorbance peak around 3166-3456 cm^{-1} revealed the hydroxyl groups (OH). Wavenumber at 1626 cm^{-1} came from the C=C group while the C-H group from carbohydrates possibly came from 995 cm^{-1}. Hydroxyl group can arise from water, phenolic, flavonoid or alcohol content in the samples. Meanwhile, the peak around 2835-2950 cm^{-1} also showed the presence of C-H, NH_3, and O-H that possibly came from phenolics, amino acids, carbohydrates, and carboxylic acids [23][24][25]. A peak around 1728 cm^{-1} was probably caused by C=O groups.

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4. Conclusions and suggestions

Cassava, cassava flour, and mocaf contain antioxidant activity and phenolic compounds. Antioxidant activity was 25.03%, 30,37%, and 31.23% for cassava, cassava flour, and mocaf respectively. Total phenolic content of mocaf was 3.46 mg/g GAE. The major component in all samples were Carbohydrates (> 85%) and the protein content was 1.44% - 2.41 %), ash (2.21 % - 2.85 %) and lipid (0.04 % - 0.10 %). Functional groups showed the presence of an OH group that was possibly phenolic and flavonoid from several bioactive compounds.

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