Effect of Soaking Treatment on Anthocyanin, Flavonoid, Phenolic Content and Antioxidant Activities of *Dioscorea alata* Flour

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ABSTRACT

Purple Dioscorea alata *is one kind of tuber crops that rich in polyphenolic compounds. In Indonesia, the utilization of this yam is still very limited because it is easily damaged on the fresh form. Due to its high perishability, then the fresh tuber have to be processed into dried materials to expand its utilization. This study investigated the effect of browning inhibition treatments (immersion in water, Na-bisulfite, and ascorbic acid) on moisture, color, anthocyanin, flavonoid and phenolic content, and determined the antioxidant activities of* D. alata *flour. The moisture content of* D. alata *flour ranged between 6.89 to 7.71% db, which still in the range of wheat flour moisture content standards using Codex Standard. Browning inhibition treatment improved the color appearances and provided better values on anthocyanin, phenolic, and flavonoid content of* D. alata *flour. The color and antioxidant activities of* D. alata *flour were significantly correlated with its functional properties.*

Keywords: Dioscorea alata *flour; anthocyanin; flavonoid; phenolic; antioxidant activity*

ABSTRAK

Dioscorea alata *ungu merupakan salah satu jenis umbi-umbian yang kaya senyawa polifenol. Di Indonesia, pemanfaatannya masih terbatas karena mudah rusaknya umbi segar. Tingginya tingkat kerusakan ini mengharuskan umbi segar segera diproses menjadi produk kering untuk memperluas pemanfaatannya. Penelitian ini bertujuan untuk mengetahui pengaruh perlakuan penghambatan pencoklatan (perendaman dalam air, Na-bisulfit dan asam askorbat) terhadap kadar air, warna, kandungan antosianin, flavonoid dan fenolik, serta menentukan aktivitas antioksidan dari tepung* D. alata. *Kadar air tepung berkisar 6,89–7,71% bk, masih dalam kisaran kadar air standar untuk tepung terigu menggunakan Standar Codex. Perlakuan penghambatan pencoklatan meningkatkan tampilan warna dan memberikan nilai lebih baik pada antosianin, flavonoid, dan fenolik tepung. Warna dan aktivitas antioksidan tepung secara signifikan berkorelasi dengan sifat fungsionalnya.*

Kata Kunci: tepung Dioscorea alata*; antosianin; flavonoid; fenolik; aktivitas antioksidan*

INTRODUCTION

Purple *Dioscorea alata*, indigenous tuber crops in Indonesia, is a potential source of natural colorant and carbohydrate. It was reported to contain a small of protein, lipids, ash, crude fiber and vitamins [1-3]. Some research reported it contains saponins, inulin [4], phenolic compounds, such as cinnamic acid [5], sinapic acid, ferulic acid, quercetin [6], and catechin [7]. It was also reported to contain up to 93.3 mg cyanidin-3-glucoside equivalent (CGE) of anthocyanin per 100 g dry material, which was the highest compared to other yams [8]. Anthocyanins are chemically phenolic compounds belonging to the flavonoid family that responsible for violet, purple, blue, red, and pink color of a great variety of plants [9].

Related to its phenolic compounds, some researchers reported that flesh tuber of *D. alata* showed antioxidant activities. Chung et al. [2] reported, it has 0.58–0.86 mg g^{-1} gallic acid equivalent (GAE) of reducing power activity, and 0.22–0.73 mg g^{-1} Trolox equivalent (TE) of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Five cultivars of Phillippine *D. alata* were reported to have higher antioxidant capacity, with a value of IC$_{50}$ were 3.3–14.8 mg mL^{-1} for DPPH scavenging activity, 9.5–31.7 mg mL^{-1} for reducing power activity, 21.9–34.0 mg mL^{-1} for iron chelating capacity, and provided 92.4 to 95.6 at 50 mg

sample per mL methanol of total antioxidant activity [10]. It was also reported providing microbial inhibitory activity [11-12]; reducing blood sugar [13] and blood lipid content [14].

Utilization of *D. alata* was difficult to be expanded both for food or other purposes because of the high perishability on the fresh form. Akissoe et al. [15] stated that yam tubers could be processed into chips and flour using the established method. One of the major problems that usually arise in yam flour processing is a discoloration phenomenon. The existing of brown color on end–product associated with enzymatic oxidation, caused by polyphenol oxidase [16] and peroxidase [17]. This phenomenon largely has been tried to be minimized because it deals with nutritional, functional, and organoleptic losses, such as the formation of brown color and off–flavor on the flour [18-19].

Some treatment was reported can be used to minimize this browning enzymatic phenomenon, such as blanching treatment [15] and using an anti-browning agent [20-21]. Akissoe et al. [15] stated blanching could reduce the peroxidase activity, though this heat treatment also reduced the yield, lightness, and chemical compounds on yam flour. Another treatment is by using anti-browning agents, such as sulfite and ascorbic acid. Sulfite could inhibit the polyphenol oxidase, while ascorbic acid could reduce the o-quinones back to their phenolic substrates [22]. Ahmed et al. [20] reported that sulfites provided higher brightness index, swelling capacity and total phenolic on sweet potato flour.

The objective of this study was to identify the effect of browning inhibition treatment (immersion in water, Na-bisulfite, and ascorbic acid) on moisture, color, anthocyanin, flavonoid, and phenolic content and to determine the antioxidant activity of *D. alata* flour.

EXPERIMENTAL SECTION

Materials

The primary material used in this study were two local cultivars, indigenous Indonesian, of purple *D. alata* (Kulonprogo and Malang) tubers. Materials were used for the submersion solution, such as tap water, Na bisulfite, and ascorbic acid. Other materials were used the chemicals for proximate analysis, potassium chloride, sodium acetate, sodium carbonate, hydrochloric acid, Folin-Ciocalteu Reagent, sodium nitrite, aluminum carbonate, sodium hydroxide (Wako Pure Chemicals Industries Ltd.), Gallic acid Reagent (Nacalai Tesque, Inc., Japan), Catechin standard, methanol, 1,1-diphenyl-2-picrylhydrazyl free radicals scavenging (Tokyo Chem. Industries), distilled water, Whatman filter paper no.1.

Instrumentation

The equipment used in this research were scales, slicer, cabinet dryer, oven, grinder, chromameter (Minolta CR-210), pH-meter (F52 Horiba), spectrophotometer (UV 1300 Shimadzu), disposable cuvettes for the spectrophotometer, incubator shaker, and equipment for proximate analysis.

Procedure

First, the tubers of *D. alata* were harvested and washed. Then, the fresh tubers were proximate analyzed using standard methods of the AOAC [23]. Then the flour producing, the tubers were washed, hand peeled, sliced, and soaked on three submersion solution treatments (tap water, Na-bisulfite 0.2% w/v, and ascorbic acid 0.1% w/v) for 20 min. Then samples were dried at 55 °C for 10 h and dish milled on 60-degree mesh. Samples were packaged using PP plastic and stored in the refrigerator until used. The color and moisture content of the samples were analyzed. The functional properties, such as anthocyanin, flavonoid, and phenolic content, and antioxidant activities were also analyzed as well.

Samples extraction

For anthocyanin, flavonoid, phenolic and anti-oxidant activities determination, the samples were extracted using maceration method. Approximately 0.5 g of *D. alata* flours were extracted with 10 mL of 5% v v^{-1} acetic acid and kept overnight with intermittent shaking. The supernatant was filtered through Whatman no.1 filter paper, and the filtrate was collected as sample extracts. Sample extracts were protected from light and kept in the refrigerator until analysis.

Proximate analysis

The moisture content, ash, crude fat, and crude protein were determined by the standard methods of the AOAC [23]. Moisture contents were analyzed using a convection oven, 0.5 g of *D. alata* flours were dried at 105 °C for at least 12 h until it reached a constant weight. Ash content was determined by measurement of residues left after combustion in a furnace at 550 °C for 8 h. Crude fat was determined by exhaustively extracting samples in Soxhlet apparatus using anhydrous diethyl ether as the solvent. Crude protein determination involved the use of routine Kjeldahl Nitrogen assay (N × 6.25). Carbohydrate was determined as the difference between moisture, ash, protein, and fat contents. The proximate composition is recorded on the basis of edible portion fresh weight of the unprocessed sample as g/100 g fresh weight.

Color analysis

The color of the *D. alata* flour was measured using Minolta CR-210 portable chromameter. The hunter lab color coordinate system L*, a*, b* values were recorded.

Total anthocyanin content (TAC) analysis

TAC was analyzed as cyanidin-3-glucoside using the pH differential method [24-25]. The extract, 1 mL (50 mg mL^{-1}), was diluted with two buffer solutions: potassium chloride (0.025 M, pH 1.0) and sodium acetate (0.4 M, pH 4.5). The absorbance was measured at 520 and 700 nm, and the differential absorbance between the pH 1.0 and 4.5 samples was calculated using equation 1.

$$A = \left[\left(A_{520} - A_{700} \right)_{pH1.0} - \left(A_{520} - A_{700} \right)_{pH4.5} \right] \qquad (1)$$

TAC (cyanidin-3-glucoside equivalents (CGE), mg L^{-1}) were calculated using equation 2.

$$TAC = \left(A \times MW \times DF \times 1000 \right) / \left(\varepsilon \times l \right) \qquad (2)$$

where: A = absorbance on equation (1), MW (molecular weight) = 449.2 g per mole for cyanidin-3-glucoside (cyd-3-glu), DF = dilution factor, ε = 26900 molar extinction coefficient, in L×mol×cm, for cyd-3-glu, l = path length in cm, 1000 = factor for conversion from g to mg.

Total phenolic content (TPC) analysis

The TPC was measured using Folin-Ciocalteu reagent based on procedures described by Singleton et al. [26] and Jothy et al. [27], with some modifications. Briefly, 0.5 mL extract (5 mg mL^{-1}) was mixed with 1.5 mL (1:10 v v^{-1} diluted with distilled water) Folin-Ciocalteau's reagent and kept at 22 °C for 5 min. Then 2 mL of sodium carbonate (Na$_2$CO$_3$, 7.5% w/v) was added, mixed, and kept for another 90 min in the dark with intermittent shaking. The absorbance of the blue color that developed was measured at 725 nm using a spectrophotometer.

Gallic acid was used for constructing the standard curve. The total phenolic compounds concentration in the extract was expressed as milligrams of gallic acid equivalent per gram (mg GAE g^{-1}) of extract.

Total flavonoid content (TFC) analysis

The TFC were determined according to the colorimetric method described by Zhinshen et al. [28] and Jothy et al. [27], with some modifications. Briefly, 0.5 mL extracts (50 mg mL^{-1}) was added in three Bijoux bottles and mixed with 2 mL of distilled water. Then 0.15 mL of sodium nitrite (NaNO$_2$, 5% w/v) was added to each bottle, and the reaction mixture was allowed to stand for 6 min. After that 0.15 mL aluminum trichloride (AlCl$_3$, 10% w/v) was added, and allowed to stand for 6 min, followed by addition of 2 mL of sodium hydroxide (NaOH, 4% w/v) to the reaction mixture. Then distilled water was added to bring the final volume up to 5 mL. The reaction mixture was mixed thoroughly and allowed to stand for another 15 min. Then absorbance of pink color that developed was measured at 510 nm using a spectrophotometer. Distilled water is used as a blank.

The final absorbance of each sample was compared with standard curve plotted from catechin. The flavonoid contents were expressed in milligrams of catechin equivalent per gram (mg CE g^{-1}) of extract.

Antioxidant activity analysis

The scavenging activity of *D. alata* extract on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenger was analyzed using Hsu et al. [29] method. Aliquots of 1 mL extract at a concentration ranging from 0–10 mg mL^{-1} of freshly prepared 0.1 mM DPPH in 80% methanolic solutions were thoroughly mixed, and kept for 50 min in the dark. The dilution was carried out in the concentration range until the sample solution was colorless, so there was no bias in the measurement result. The absorbance of the reaction mixture at 517 nm was measured on a spectrophotometer. Methanol solution 80% was used as a blank. The DPPH scavenging effect was calculated using equation 3.

$$\text{DPPH scavenging effect (\%)} = \left[\left(A_0 - A_1 \right) / A_0 \right] \times 100\% \qquad (3)$$

where: A_0 is the absorbance of the blank, and A_1 is the absorbance in the presence of the extract.

IC$_{50}$ value is the sample extract concentration at 50% inhibition activity. The IC$_{50}$ value was determined by interpolation using plotting the extract concentration versus the DPPH scavenging effect.

Statistical analysis

The data were analyzed by the analysis of variance (ANOVA) and the significant differences among means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

RESULT AND DISCUSSION

Proximate Results of Fresh Tuber

The proximate analysis of *D. alata* tubers that include: moisture, ash, fat, protein, and carbohydrate content, could be seen in Table 1. The results showed that levels of yams moisture content were high enough, ranging from 71.76 to 84.75% of fresh weight. These results coincided with the tropical tubers moisture content which derived from the Southern Pacific region, Sri Lanka, and Nepal, as reported by Chen and Lin [30], Huang et al. [31], and Bhandari et al. [32]. The high moisture content on the fresh form of *D. alata* tuber caused the easily damaged of this yams.

The ash content of *D. alata* tubers was around 0.43–0.69%. This result was in line with Chen and Lin

[30], and Bhandari et al. [32]. Ash content of the agricultural product is usually influenced by genetic factors, soil conditions, and plant growing environment. In this case, the tubers originating from Kulonprogo and Malang came from cropping with different soil types and different altitude, but it had no influences on ash content.

The protein and fat content ranged from 1.06 to 1.54%; and 0.16 to 0.34%, respectively. These results were smaller than the findings reported by Chen and Lin [30] and Huang et al. [31]. It was expected because of a different age of tubers and variety or type of tubers. However, these results were consistent with the results of Bhandari et al. [32].

The carbohydrate content of *D. alata* tubers was around 12.93 to 26.29%. It was in line with the Bhandari et al. [32], which reported that the carbohydrate content of Nepal yams ranged from 17.4 to 25.9%, and the energy values of the yams ranged 78–119 kcal/100 g fresh weight. So, *D. alata* can be used as an alternative to carbohydrate sources other than rice.

The Moisture Content of *D. alata* Flour

Average moisture contents of two cultivars of *D. alata* flour are shown in Table 2, ranged from 6.89 to 7.71% db. These moisture contents were still in the range of safety moisture content on Codex Standard for wheat flour, < 15.5% (Codex Stan 152–1985), and cassava flour, < 13% (Codex Stan 176–1989). The moisture content of the flour becomes important because it is a critical point related to the shelf life, quality deterioration during storage, the activity of the enzyme and the growing possibility of insect, fungi, mold, microbes and another microorganism [39]

The Color Value of *D. alata* Flour

The L*, a*, b* values of *D. alata* flour were compared (Table 2). Brightness (L*) values of *D. alata* flour ranged from 69.5 to 79.2; redness (+a*) values ranged from +2.2 to +4.0; and yellowness (+b*) values ranged from +5.1 to +10.9. Na-bisulfite treatment significantly contributed to the higher brightness (L*) values of flour on both cultivars,

71.4 for Kulonprogo and 79.2 for Malang. While ascorbic acid treatment provided the lowest redness (+a*) index on both cultivar, 2.8 for Kulonprogo and 2.2 for Malang. For yellowness (+b*) index, Na-bisulfite treatment significantly produced the lowest value, 5.1, on both cultivars.

These results in line with Ahmed et al. [20] whose stated that sulfite treatment provided higher brightness values (L*) on sweet potato flour. In this study, Na-bisulfite and ascorbic acid contributed of inhibiting the formation of browning color on *D. alata* flour, Na-bisulfite by increasing the brightness level and reducing the yellowness level of flour, whereas ascorbic acid on reducing the redness level on the flour. The existing of browning color on the flour could be caused by enzymatic oxidation, polyphenol oxidase [18] and peroxidase [17]; and non-enzymatic browning produced on reducing sugars condense with amino groups [33]. In line with Ahmed et al. [20], Na-bisulfite showed better results compared to ascorbic acid on the flour browning inhibition, but in contrast with Arogundade and Mu [21]. In this case, sulfite was a good color preservative on flour, and also on fruit and vegetables, and it retards both enzymatic and nonenzymatic reaction [34].

Total Anthocyanin Content (TAC) of *D. alata* Flour

The TAC of *D. alata* flour is shown in Table 3, ranged from 12.6 to 74.3 mg CGE 100 g^{-1} samples, db. The lowest value was Malang with ascorbic acid treatment (12.6 mg CGE 100 g^{-1} samples, db), and the highest was Kulonprogo with Na-bisulfite treatment (74.3 mg CGE 100 g^{-1} samples, db). These values were

Table 1. The proximate results of *D. alata* fresh tubers

Parameters	Cultivars	
	Kulonprogo	Malang
Moisture	75.06±3.30	83.69±1.06
Ash	0.55±0.06	0.56±0.13
Protein	1.13±0.07	1.52±0.02
Fat	0.32±0.02	0.18±0.02
Carbohydrate*	22.93±3.36	14.04±1.11

*by different.

Table 2. Moisture content and color measurement of *D. alata* flour

Cultivars/Treatment	Moisture Content (% db)	Color measurement		
		L*	a*	b*
Kulonprogo				
Water	7.65 ± 0.39 bc	69.6 ± 0.91 a	+3.0 ± 0.13 c	+10.0 ± 0.22 c
Na-bisulfite	7.49 ± 0.50 bc	71.4 ± 0.41 b	+4.0 ± 0.14 d	+5.1 ± 0.08 a
Ascorbic acid	7.27 ± 0.15 bc	69.5 ± 0.19 a	+2.8 ± 0.17 bc	+10.3 ± 0.10 d
Malang				
Water	7.01 ± 0.28 a	73.2 ± 0.54 c	+2.8 ± 0.20 bc	+7.6 ± 0.09 b
Na-bisulfite	7.71 ± 0.32 c	79.2 ± 0.16 e	+2.6 ± 0.18 b	+5.1 ± 0.15 a
Ascorbic acid	6.89 ± 0.17 a	74.3 ± 0.54 d	+2.2 ± 0.18 a	+10.9 ± 0.17 e

a–e The same letter in the same column indicates no significant differences at 5% level by Duncan's multiple range test (n = 6)

Table 3. Total anthocyanin, phenolic, and flavonoid content of *D. alata* flour

Cultivars/Treatment	Content on a dry basis of samples		
	TAC (mg CGE 100 g⁻¹)	TPC (mg GAE g⁻¹)	TFC (mg CE g⁻¹)
Kulonprogo			
Water	46.7 ± 3.35 c	11.6 ± 0.31 c	7.0 ± 0.14 d
Na-bisulfite	74.3 ± 3.83 f	12.7 ± 0.67 d	7.4 ± 0.21 e
Ascorbic acid	50.0 ± 2.26 d	11.5 ± 0.13 c	7.3 ± 0.23 e
Malang			
Water	26.4 ± 2.65 b	7.3 ± 0.10 a	3.9 ± 0.18 a
Na-bisulfite	58.0 ± 1.90 e	9.6 ± 0.24 b	5.3 ± 0.25 b
Ascorbic acid	12.6 ± 1.05 a	9.6 ± 0.29 b	6.2 ± 0.30 c

[a–e] The same letter in the same column indicates no significant differences at 5% level by Duncan's multiple range test (n = 6)
TAC = Total anthocyanin content   CGE = cyanidin-3-glucoside equivalent
TPC = Total phenolic content   GAE = gallic acid equivalent
TFC = Total flavonoid content   CE   = catechin equivalent

lower than anthocyanin content of the fresh *D. alata* tuber, 31.0–93.3 mg CGE 100 g⁻¹ db [6,8], but higher compared to sweet potato flour,1.7–21.8 mg CGE 100 g⁻¹ samples, db.

Na-bisulfite treatment significantly contributed to providing the highest TAC on both varieties, 74.3 mg CGE 100 g⁻¹ samples, db for Kulonprogo and 58.3 mg CGE 100 g⁻¹ samples, db for Malang. Sulfite was reported as a good color preservative [34], through maintaining the anthocyanin, purple pigment inside *D. alata* tuber and during drying and milling processes. Sulfites also capable of retards both enzymatic and nonenzymatic reaction, and provided the higher total anthocyanin values compared to other treatments.

Total Phenolic Content (TPC) of *D. alata* Flour

The TPC of *D. alata* flour ranged from 7.3 to 12.7 mg GAE g⁻¹ samples, db; Table 3. The lowest TPC was Malang with water treatment (7.3 mg GAE g⁻¹ samples, db), and the highest was Kulonprogo with Na-bisulfite treatment (12.7 mg GAE g⁻¹ samples, db). These values were higher than previous reports, 0.24–4.78 mg GAE g⁻¹ samples, db [2,6,10], and comparable with total phenolic content on sweet potato flour, 2.06–13.9 mg GAE g⁻¹ samples, db [20-21,35].

Na-bisulfite treatment provided the highest TPC significantly on Kulonprogo, 12.7 mg GAE g⁻¹ samples. Na-bisulfite and ascorbic acid treatment had no significant influence. Whereas on Malang, Na-bisulfite and ascorbic acid had no significantly different, 9.6 and 9.5 mg GAE g⁻¹ samples, respectively. The lowest TPC value was *D. alata* flour of Malang with water soaking treatment, 7.3 mg GAE g⁻¹ samples. Major losses of phenolic content could be through the action of an oxidative enzyme, so inactivation of polyphenol oxidase could provide the higher total phenolic content [20]. The phenomenon suggested that Na-bisulfite could minimize the losses of the phenolic compound during enzymatic browning reaction, better than ascorbic acid and water.
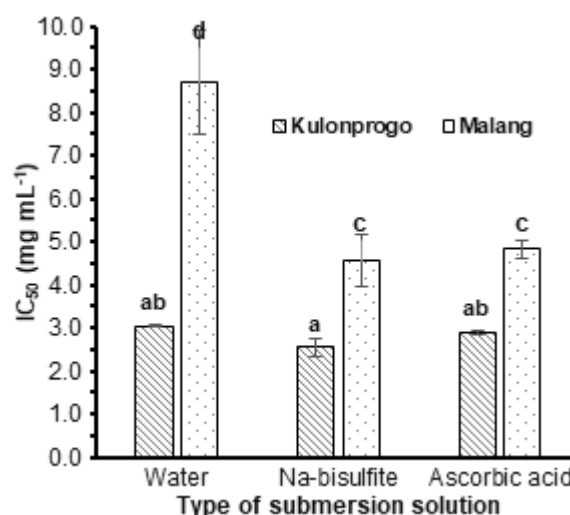

Fig 1. IC₅₀ of antioxidant activities (mg mL⁻¹) for *D. alata* flour

Total Flavonoid Content (TFC) of *D. alata* Flour

TFC of *D. alata* flour ranged from 3.9 to 7.4 mg CE g⁻¹ samples, db, Table 3. The lowest flavonoid content was Malang with water treatment (3.9 mg CE g⁻¹ samples, db), and the highest was Kulonprogo with Na-bisulfite treatment (7.4 mg CE g⁻¹ samples, db), but had no significantly different with Kulonprogo using ascorbic acid treatment. On Kulonprogo, Na-bisulfite and ascorbic acid treatment provided the same level of flavonoid content, whereas, on Malang, ascorbic acid provided the highest flavonoid content. In this case, ascorbic acid provided better flavonoid content compared with Na-bisulfite and water. This is due to the antioxidant capacity of ascorbic acid which prevents the degradation of flavonoid compounds with its radical scavenging capacities. Besides, ascorbic acid could reduce the o-quinones back to their phenolic substrates before producing brown pigments [22].

Table 4. The correlation coefficient (*r*) between parameters of *D. alata* flour

Parameters	TAC (mg CGE 100 g^{-1})	TPC (mg GAE g^{-1})	TFC (mg CE g^{-1})
Brightness (L*)	ns	-0.509**	-0.545**
Redness (a*)	0.706**	0.550**	0.350*
Yellowness (b*)	-0.657**	ns	ns
TAC (mg CGE 100 g^{-1})	1.000	0.705**	0.466**
TPC (mg GAE g^{-1})	0.705**	1.000	0.922**
TFC (mg CE g^{-1})	0.466**	0.922**	1.000
Anti-oxidant activity	0.602**	0.940**	0.938**

Significance correlation *P < 0.05 and **P < 0.01. ns = not significant
TAC = Total anthocyanin content   CGE = cyanidin-3-glucoside equivalent
TPC = Total phenolic content   GAE = gallic acid equivalent
TFC = Total flavonoid content   CE   = catechin equivalent

Flavonoids are a large group of phytochemicals that are widely available in the plant kingdom and are produced from phenylalanine and tyrosine via the shikimic acid pathway [36]. It is important for a wide array of biological functions. The major representative of the flavonoids, quercetin, is reported to prevent the oxidation of low-density lipoprotein by scavenging free radicals [37].

Antioxidant Activity of *D. alata* Flour

The antioxidant activities of *D. alata* flours were evaluated by DPPH radical scavenging assay. The crude extract of *D. alata* flours was diluted in the concentration range which sample solution was colorless. So, there was no bias in the measurement result.

The IC$_{50}$ values of the *D. alata* flour crude extract can be seen in Fig. 1. IC$_{50}$ value is concentration (mg mL^{-1}) of *D. alata* crude extract that is required on decreasing the initial DPPH concentration by 50%. The lowest value of IC$_{50}$ was the most effective concentration. IC$_{50}$ of *D. alata* was ranged from 2.55 to 8.70 mg mL^{-1}. The strongest DPPH radical scavenging activity was *D. alata* flours of Kulonprogo with Na-bisulfite treatment (2.55 mg mL^{-1}). It was followed by Kulonprogo with ascorbic acid and water treatment. Whereas, the weakest DPPH radical scavenging activity was Malang with water treatment (8.70 mg mL^{-1}). These results suggested that Na-bisulfite treatment provided stronger DPPH radical scavenging activity both on Kulonprogo and Malang.

In line with purple-flesh sweet potato flour, although the DPPH assay was not specific to any particular anti-oxidant components, the possible mechanism of hydrogen donation suggested that the radical scavenging activity of *D. alata* flour crude extracts may be due to the hydroxyl groups in the antioxidant [38].

Correlation between Color, Functional Properties and Anti-Oxidant Activities of *D. alata* Flour

Table 4 showed the correlations between parameters. Redness index (a*) has a positive correlation

with all functional properties that measured in this study (TAC, TPC, and TFC). Brightness index (L*) has a negative correlation with TPC and TFC, while yellowness index (b*) has a negative correlation with TAC.

TAC has a positive correlation with two other functional properties (TPC and TFC), as well as TPC and TFC. All functional properties that measured in this study positively correlated with another functional property. Antioxidant capacities of purple *D. alata* showed strongly match with those anthocyanin, phenolic and flavonoid content, with the coefficient correlation (r) was 0.60, 0.94, and 0.94, respectively. These coefficient correlation value indicated that the total anthocyanin, phenolic, and flavonoid contents of these purple *D. alata* flour play an important role in antioxidant activities respectively.

CONCLUSION

Na-bisulfite treatment provided not only better color but also better total anthocyanin, phenolic, flavonoid content of *D. alata* flour on both Kulonprogo and Malang cultivars. IC$_{50}$ of antioxidative activity on *D. alata* flour significantly correlated with total anthocyanin, phenolic, and flavonoid content.

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REFERENCES

[1] Lebot, V., Malapa, R., Molisale, T., and Marchand, J.L., 2006, Physico-chemical characterisation of yam (*Dioscorea alata* L.) tubers from Vanuatu, *Genet. Resour. Crop Evol.*, 53 (6), 1199–1208

[2] Chung, Y.C., Chiang, B.H., Wei, J.H., Wang, C.K., Chen, P.C., and Hsu, C.K., 2008, Effect of

blanching, drying and extraction processes on the antioxidant activity of yam (*Dioscorea alata*), *Int. J. Food Sci. Technol.*, 43 (5), 859–864.

[3] Behera, K.K., Maharana, T., Sahoo, S.L., and Prusti, A., 2009, Biochemical quantification of protein, fat, starch, crude fiber, ash and dry matter content in different collection of greater yam (*Dioscorea alata* L.) found in Orissa, *Nat. Sci.*, 7 (7), 24–32.

[4] Winarti, S., Harmayani, E., and Nurismanto, R., 2011, Karakteristik dan profil inulin beberapa jenis uwi (*Dioscorea* spp.), *Agritech*, 31 (4), 378–383.

[5] Martin, F.W., and Ruberte, R., 1976, Polyphenol of *Dioscorea alata* (yam) tubers associated with oxidative browning, *J. Agric. Food Chem.*, 24 (1), 67–70.

[6] Fang, Z., Wu, D., Yü, D., Ye, X., Liu, D. and Chen, J., 2011, Phenolic compounds in Chinese purple yam and changes during vacuum frying, *Food Chem.*, 128 (4), 943–948.

[7] Ozo, O.N., Caygill, J.C., and Coursey, D.G., 1984, Phenolics of five yam (*Dioscorea*) species, *Phytochemistry*, 23 (2), 329–331.

[8] Champagne, A., Hilbert, G., Legendre, L., and Lebot, V., 2011, Diversity of anthocyanins and other phenolic compounds among tropical root crops from Vanuatu, South Pacific, *J. Food Compos. Anal.*, 24 (3), 315–325.

[9] Lionetto, M.G., Giordano, M.E., Calisi, A., Erroi, E., De Nuccio, F., and Schettino, T., 2011, Effect of the daily ingestion of a purified anthocyanin extract from grape skin on rat serum antioxidant capacity, *Physiol. Res.*, 60 (4), 637–645.

[10] Cornago, D.F., Rumbaoa, R.G.O., and Geronimo, I., 2011, Philippine yam (*Dioscorea* spp.) tubers phenolic content and antioxidant capacity, *Philipp. J. Sci.*, 140 (2), 145–152.

[11] Hu, K., Yao, X.S, Dong, A.J., Kobayashi, H., Iwasaki, S. and Jing, Y.K., 1999, A new pregnane from *Dioscorea collettii* var. *hypoglauca*, *J. Nat. Prod.*, 62 (2), 299–301.

[12] Kelmanson, J.E., Jager, A.K., and van Standen, J., 2000, Zulu medicinal plants with antibacterial activity, *J. Ethnopharmacol.*, 69 (3), 241–246.

[13] Hikino, H., Konno, C., Takahashi, M., Murakami, M., Kato, Y., Karikura, M., and Hayashi, T., 1986, Isolation and hypoglycemic activity of dioscorans A, B, C, D, E, and F; glycans of *Dioscorea japonica* rhizophors, *Planta Med.*, 52 (3), 168–171.

[14] Araghiniknam, M., Chung, S., Nelson-White, T., Eskelson, C., and Watson, R.R., 1996, Antioxidant activity of dioscorea and dehydroepiandrosterone (DHEA) in older humans, *Life Sci.*, 59 (11), 147–157.

[15] Akissoe, N., Hounhouigan, J., Mestres, C., and Nago, M., 2003, How blanching and drying affect the color and functional characteristics of yam (*Dioscorea cayenensis-rotundata*) flour, *Food Chem.*, 82 (2), 257–264.

[16] Almenteros, V.P., and Del Rosatio, R.R., 1985, Phenolic content and polyphenoloxidase activity related to browning in yam (*Dioscorea alata* Linn.), *Philipp. Agric.*, 68, 449–452.

[17] Zhang, X., and Shao, X., 2015, Characterisation of polyphenol oxidase and peroxidase and the role in browning of loquat fruit, *Czech J. Food Sci.*, 33 (2), 109–117.

[18] Severini, C., Baiano, A., De Pilli, T., Romaniello, R., and Derossi, A., 2003, Prevention of enzymatic browning in sliced potatoes by blanching in boiling saline solutions, *LWT Food Sci. Technol.*, 36 (7), 657–667.

[19] Altunkaya, A., and Gökmen, V., 2008, Effect of various inhibitors on enzymatic browning, anti-oxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*), *Food Chem.*, 107 (3), 1173–1179.

[20] Ahmed, M., Akter, M.S., and Eun, J.B., 2010, Peeling, drying temperature, and sulphite-treatment affect physicochemical properties and nutritional quality of sweet potato flour, *Food Chem.*, 121 (1), 112–118.

[21] Arogundade, L.A., and Mu, T.H., 2012, Influence of oxidative browning inhibitors and isolation techniques on sweet potato protein recovery and composition, *Food Chem.*, 134 (3), 1374–1384.

[22] McEvily, A.J., Iyengar, R., and Otwell, W.S., 1992, Inhibition of enzymatic browning in foods and beverages, *Crit. Rev. Food Sci. Nutr.*, 32 (3), 253–273.

[23] Association of Official Analytical Chemist (AOAC), 1990, Official Methods of Analysis of the Association of Official Analytical Chemists, 14th ed., AOAC, Inc., Washington DC.

[24] Giusti, M.M., and Wrolstad, R.E., 2001, "Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy" in Current Protocols in Food Analytical Chemistry, Giusti, M.M., and Wrolstad, R.E., Eds., John Wiley and Sons, Inc., New York, 1–13.

[25] Lee, J., Durst, R.W., and Wrolstad, R.E., 2005, Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study, *J. AOAC Int.*, 88 (5), 1269–1278.

[26] Singleton, V.L., Orthofer, R., and Lamuela-Raventos, R.M., 1999, Analysis of total phenols and other oxidation substrates and anti-oxidants by means of Folin-Ciocalteu reagent, *Methods Enzymol.*, 299 (1), 152–178.

[27] Jothy, S.L., Zuraini, Z., and Sasidharan, S., 2011, Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitory activities of *Cassia fistula* seeds extract, *J. Med. Plants Res.*, 5 (10), 1941–1947.

[28] Zhishen, J., Mengcheng, T., and Jianming, W., 1999, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chem.*, 64 (4), 555–559.

[29] Hsu, C.L., Chen, W., Weng, Y.M., and Tseng, C.Y., 2003, Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods, *Food Chem.*, 83 (1), 85–92.

[30] Chen, Y.T., and K.W. Lin, 2007, Effect of heating temperature on the total phenolic compound, antioxidative ability and the stability of dioscorin of various yam cultivars, *Food Chem.*, 101 (3), 955–963.

[31] Huang, C.C., Lin, M.C., and Wang, C.C.R., 2006, Changes in morphological, thermal, and pasting properties of yam (*Dioscorea alata*) starch during growth, *Carbohydr. Polym.*, 64 (4), 524–531.

[32] Bhandari, M.R., Kasai, T., and Kawabata, J., 2003, Nutritional evaluation of wild yam (*Dioscorea* spp.) tubers of Nepal, *Food Chem.*, 82 (4), 619–623.

[33] Utomo, J.S., Man, Y.B.C., Rahman, R.A., and Saad, M.S., 2008, The effect of shape, blanching methods and flour on characteristics of restructured sweet potato stick, *Int. J. Food Sci. Technol.*, 43 (10), 1896–1900.

[34] Li, Y., and Zhao, M., 2005, Simple methods for rapid determination of sulfite in food products, *Food Control*, 17 (12), 975–980.

[35] Shih, M.C., Kuo, C.C., and Chiang, W., 2009, Effects of drying and extrusion on colour, chemical composition, antioxidant activities and mitogenic response of spleen lymphocytes of sweet potatoes, *Food Chem.*, 117 (1), 114–121.

[36] Khanam, U.K.S., Oba, S., Yanase, E., and Murakami, Y., 2012, Phenolic acids, flavonoids and total antioxidant capacity of selected leafy vegetables, *J. Funct. Foods*, 4 (4), 979–987.

[37] Cartea, M.E., Francisco, M., Soengas, P., and Velasco, P., 2011, Phenolic compounds in *Brassica* vegetables, *Molecules*, 16(1), 251–280.

[38] Soison, B., Jangchud, K., Jangchud, A., Harnsilawat, T., Piyachomkwan, K., Charunuch, C., and Prinyawiwatkul, W., 2014, Physico-functional and antioxidant properties of purple-flesh sweet potato flour as affected by extrusion and drum-drying treatments, *Int. J. Food Sci. Technol.*, 49 (9), 2067–2075.

[39] Nasir, M., Butt, M.S., Anjum, F.M., Sharif, K., and Minhas, R, 2003, Effect of moisture on the shelf life of wheat flour, *Int. J. Agric. Biol.*, 5 (4), 458–459.