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The Effect of Purple Yam (*Dioscorea Alata*, L) Blanching Time on Anthocyanins Content and Antioxidant Activity

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Abstract:
This study aimed to determine the effect of purple yam blanching treatments on anthocyanin content, phenolics content and antioxidant of activity. Blanching was done by steam blanching for 4, 8, and 12 minutes and without blanching as a control. The results showed that blanching time affected anthocyanins level, phenolics content and antioxidant activity as determined by DPPH and radical scavenging activity (RSA) methods. Blanching for 8 and 12 minutes increased anthocyanins level (2.4 to 2.5 times), phenolics content (1.3 to 1.6 times), and antioxidant activity of purple yam compared to those without blanching. Antioxidant activities of purple yam blanching were 8-minute blanching ≥ 12-minute blanching = BHT > without blanching > 4-minute blanching . The level of total anthocyanins and phenolics content were significantly correlated with antioxidant activity as measured by radical scavenging activity.

Keywords*: blanching, anthocyanin, phenolic, % RSA*

1.Introduction

Anthocyanins are natural pigment which found in plants. Anthocyanins are beneficial to health due to their ability as antioxidant, anti-inflammatory and anticancer. As antioxidant, they have high reactivity by taken role as hydrogen or electron donors, and polyphenols ability to stabilize and displace unpaired electron, as well as ability to chelate metal ions (Rice-evans *et al*., 1997).

Purple yam (*Dioscorea alata L*) is the Indonesian natural food source which has a potential source of anthocyanins, antioxidants and natural food coloring. Anthocyanins content in purple yam is 31 mg/100 g of dry matter (Fang *et al*., 2011). Some other materials that contain anthocyanins are black potato by 21 mg/100 g dry matter (Kita *et al*., 2013), black/red rice with anthocyanins contain 26,5 mg/100 g dry matter (Shao *et al.*, 2014), sweet potato which harvested from Cilembu West Java Indonesia with anthocyanins content 22,5 – 37,8 mg/100 g dry matter (Mahmudatussa'adah *et al.*, 2014).

Purple yam has not been widely used as a source of anthocyanin and antioxidants. The yam's consumption which have been conducted so far is only form as boiled, steamed, fried, or grilled yam. The fresh purple yam with high water content (about 80%) will be perished if it's not immediately processed. Process to make purple yam become a powder is one of the way to make the material easier to use, more flexible and storable. The first process to make powdered purple yam was given purple yam heat treatment called *blanching*. In the *blanching* process, there will be occurred enzyme inactivation, softening material structures and changes in mechanical properties of the materials (Amin and Lee, 2005).

Some research suggested that *blanching* treatment will have some effect on the changes of nutritional components. Lemos *et al*. (2013), reported that a *Purple Majesty potato* which was boiled for 25 minutes and steamed for 35 minutes will have some increases in its active components (i.e., total phenolics, total anthocyanins and antioxidative activity). According to Burgos *et al*. (2013), a purple potato (*Solanum andigenum*) which was boiled will have some increases in the total phenolic contents, total anthocyanins, chlorogenic acid and antioxidative activity. A research conducted by Burgos *et al*. (2012), indicated that the boiled Andean potato showed the higher levels of *lutein* and *zeaxanthin* compared with fresh potato. The boiling process will have some effect on cell structural changes which facilitates liberation of cell components content.

The purpose of this research is to find out the effect of purple yam blanching time on anthocyanin components, phenolic compounds and antioxidative activity (% RSA/% Radical scavenging activity).

2.Materials and Methods

2.1. Materials

Raw material used in this research was purple yam which bought from Godean Traditional Market, Sleman, Yogyakarta. Each purple yam weight was about 2 kg. The chemical substances used are free radical *2,2-diphenyl-1-picrylhydrazyl* (DPPH), Folin–ciocalteu reagent, GA (*gallic acid*) from Sigma Chemical Co., St Louis, ethanol, methanol, HCl, $NaCO_3$, $NaNO_2$, $AlCl_3.6H_2O$, NaOH (E. Merck).

The research instrument used are UV vis 1240 spectrophotometer, Ohaus analytical balance, HI 2210 pH meter.

2.2. Methods

The fresh purple yams were peeled, washed and cut into cubes with a thickness about 3 cm, then was steamed (blanching) at various time (4, 8, 12 minutes and without blanching). The blanched purple yams then extracted with 1% Methanol-HCl. Extraction was conducted as follows; 1 g of material was macerated with 10 ml of 1% Methanol-HCl for 12 hours at a temperature of 4^{o}C, in a dark condition. The extract which obtained then filtered using Whatman filter paper no. 1. The extraction was repeated on the residue for 2 times with 1% Metanol–HCl of 10 ml and 5 ml, respectively, for 30 minutes each. The extract which obtained has been collected and the combination was measured with a 25-ml flask and was accomplished to 25 ml. This extract was then tested for its total anthocyanins, antioxidant activity.

2.2.2. Determination of DPPH radical scavenging activity

The antioxidative activity was conducted by finding out the DPPH free radicals scavenging capacity. A total of 0,2 ml sample was added by 3,8 ml of 0,1 mM DPPH solution, then was vortexed for 3 minutes and observed for its absorbance in every 5 minutes using spectrophotometer at a wavelength of 517 nm for 1 hour (Yun *et al*. 2003). The comparison reference material of 100 ppm BHT solution was used and methanol was used as the control. The free radicals scavenging was calculated and expressed in percent (%) RSA = % *Radical Scavenging Activity* is the % of DPPH discoloration.

$$\%\ RSA = 1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ blank}$$

2.2.3. Determination of total anthocyanins

Total anthocyanins was determined by the method proposed by Giusti and Wrostald (1996), with slight modifications. A total of 0,4 ml of each extract was put into 2 test tubes. The first test tube was added by 2,6 ml potassium chloride buffer (0,025 M) pH 1. The second test tube was added by 2,6 ml sodium acetate buffer solution (0,4 M) at 4,5. The absorbances of both samples were calibrated using a spectrophotometer at a wavelength of 520 and 700 nm after having rested for 15 minutes. The absorbance values were calculated with the formula A = (A520 - A700)pH1 – (A520 - A700)pH4,5. The concentration of anthocyanins was calculated as cyanidin-3-glycosides using a molar extinction coefficient of 26.900 L cm-1 and a molecular weight of 484,82. The concentration of anthocyanins (mg/L) = (A X BM X FP X 1000)/ (e x 1), where A is the absorbance, BM is the molecular weight (484,82), FP is the dilution factor (3 ml / 0,4 ml) and e is the molar extinction coefficient (26.900 L cm-1).

2.2.4. Determination of Total Phenolic Content

The total phenolic content was determined using Folin-Ciocalteu method (Roy *et al.*, 2009), using the gallic acid as the standard. A sample of 50 µl was added by 250 µl Folin-ciocalteu solution, then rested for 1 minute and was then added by 750 µl $NaCO_3$ 20 %, then was vortexed and added by aquades till reaching volume 5 ml. After getting incubated for 5 minutes at room temperature, the absorbance was calibrated at λ 760 nm. The gallic acid was used as the standard and the calibrating curve was made by 31,875 gallic acid till reaching 510 mg/L with r = 0,99. The total phenolic results was calculated as mg Gallic Acid Equivalent (EAG) per gram of dry extract.

2.2.5. Statistical analysis

The results were presented as mean and standard deviation, all of the analyses were done in triplicate. All data obtained were tabulated and analyzed using variance analysis (ANOVA). The result differences would be tested using Duncan Multiple Range Test (DMRT)

3.Results and Discussions

3.1. Anthocyanins Content

Anthocyanins content of purple yam due to blanching time can be seen in Figure 1. It shows that blanching time for 8 and 12 minutes was increase anthocyanins contents. Anthocyanins content in purple yam was increase at 8 and 12 minutes of blanching time, respectively are 2,4 to 2,5 times compared with non-blanched yam (fresh yam). It is in line with the research which conducted by Tokusoglu and Yildirim (2012), the blanching treatment on sweet potatoes will increase anthocyanins contents for 1,14 times higher than the fresh sweet potatoes.

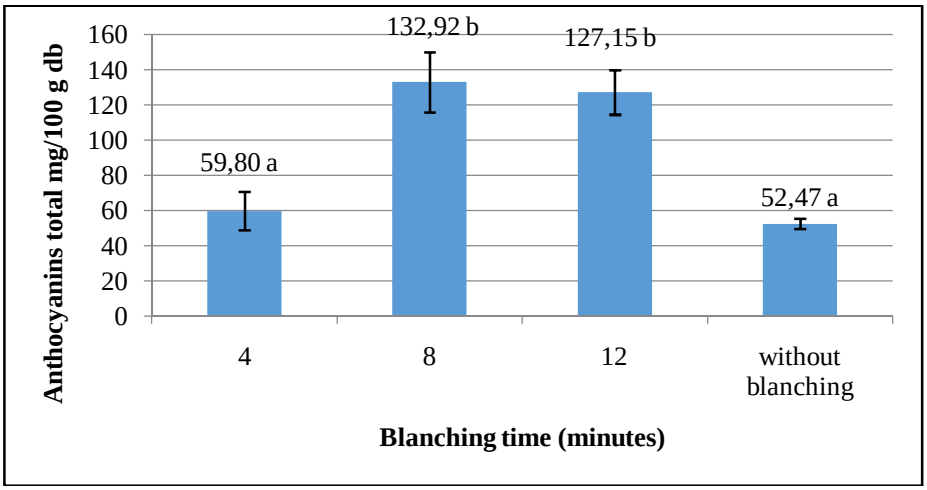

Figure 1: Effect of blanching time on total anthocyanins of purple yams

Huang *et al*. (2006), also state that the blanching treatment can increase anthocyanins content in sweet potatoes. Blanching treatment will perish the cell structure in the materials, so the cell components content will be more easily extracted, including anthocyanins. According to Ozo *et al*. (1984); Fang *et al*. (2011), type of anthocyanins contained in yam is sianidin-3-glucoside and acylated with sinapic and ferulic acid components. Most of anthocyanins contained in the tubers are acylated anthocyanins which are stable (Lachman *et al*, 2009).

3.2. Total Phenolic Content of Blanched Purple Yam

Total phenolic of blanched purple yams due to the blanching time can be seen in Figure 2. It shows that the total phenolic contents of the blanching treatment of 8 and 12 minutes are 1,6 to 1,3 times higher than the non-blanched yam (fresh yam), respectively.

This result is in line with some previous research, including the research conducted by Huang *et al*. (2006), the blanching treatment will increase total phenolic in sweet potatoes. Oboh (2005) has been done research by blanching some green vegetables, and showed that the total phenolic will increase due to blanching treatment.

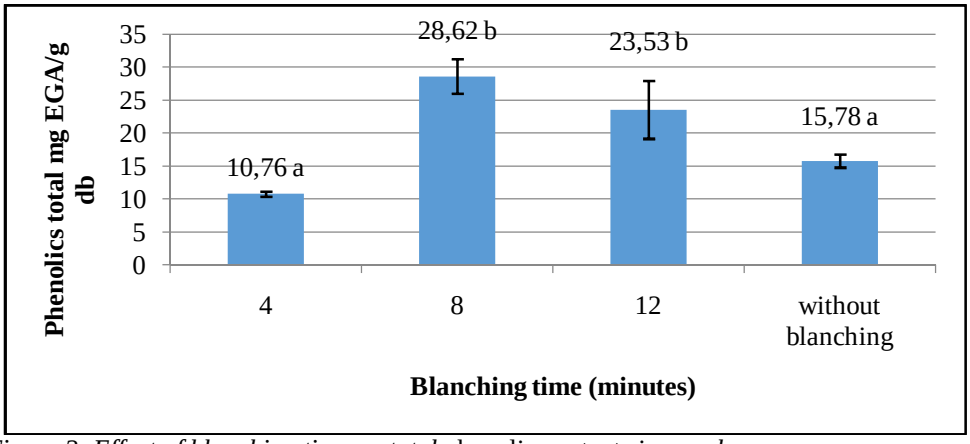

Figure 2: Effect of blanching time on total phenolic contents in *purple yams*

A research conducted by Dwiyati *et al*. (2010), the blanching treatment on white saffron also showed increasing content of phenolic compounds. The increase in total phenolic was considered relate to the damaged tannins into phenol. In addition, phenolic compounds does not oxidized by enzyme due to blanching treatment.

3.3. Antioxidative Activity (DPPH, Radical Scavenging activity) of the Blanched Purple Yam

DPPH radical absorbance was decrease due to scavenging of free radicals by the extract of 1g/100 ml Me-HCl 1% of purple yam in the blanching treatment can be seen in Figure 3. It shows that all types of extracts showed decrease absorbance rate which indicate the free radicals scavenging rate, especially at 10th minute.

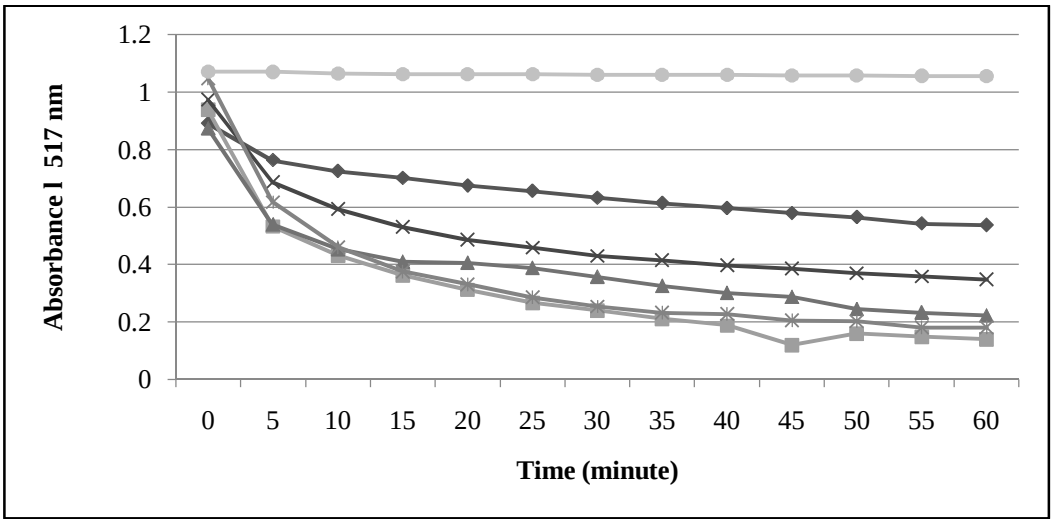

Figure 3: DPPH radical absorbance was decrease due to the free radicals scavenging activity by the extract of Methanol-HCl 1% from 1 g/100 ml purple yam given for the blanching time.

Information: = 4-minute blanching, = 8-minute blanching, = 12-minute blanching, = without blanching, = BHT, = blank

Free radical scavenging activity of the purple yam extracts in the first 10 minutes of blanching treatment of 4 minutes < without blanching < 12-minute blanching < BHT ≤ 8 minutes.

Similarly, it is shown in percentage of inhibition test on the blanched purple yam extract on antioxidative activity test with DPPH in Figure 4. At the 30th minute observation, it showed the percentage of inhibition as follows: 8-minute blanching ≥ 12-minutes = BHT > without blanching > 4 minutes.

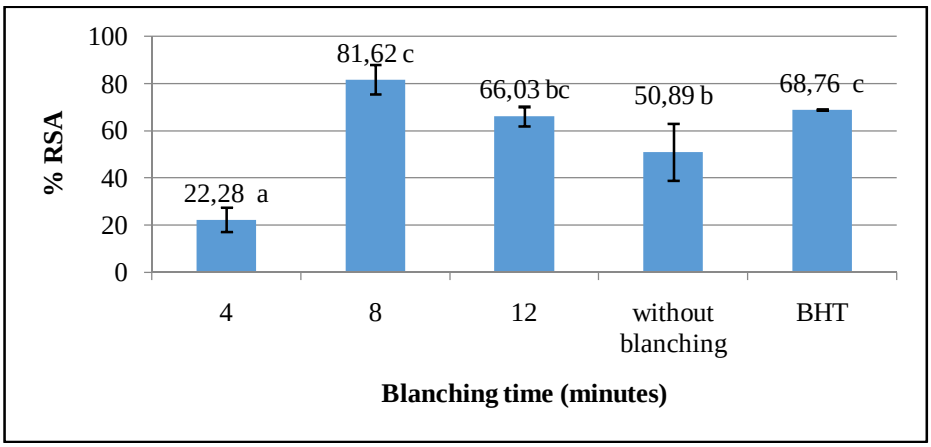

Figure 4 : % RSA of the blanched purple yam extract

Anthocyanins and total phenolic content of the 8 and 12-minute blanched purple yams are higher than other treatments. Both components act as natural antioxidants. It is in line with the research conducted by Oki *et al*. (2002), which stated that the anthocyanins and phenolic compounds in purple sweet potatoes are the components which act in scavenging activity DPPH free radicals.

3.5.The Correlation between total phenolic vs antioxidative activity (%RSA) and total anthocyanins vs antioxidant activity

The correlation between DPPH-method in antioxidant activity with total phenolic and correlation between antioxidant activity with total anthocyanins can be seen in Figure 5 and 6. The concentration of total phenolics and antioxidant activity (DPPH method,% RSA) from blanched purple yam which extracted by Methanol-HCl 1%, showed a strong correlation (r^2 = 0,95), the correlation between antioxidative activity and total anthocyanins is r^2 = 0,69, lower than the correlation between total phenolic and antioxidative activity.

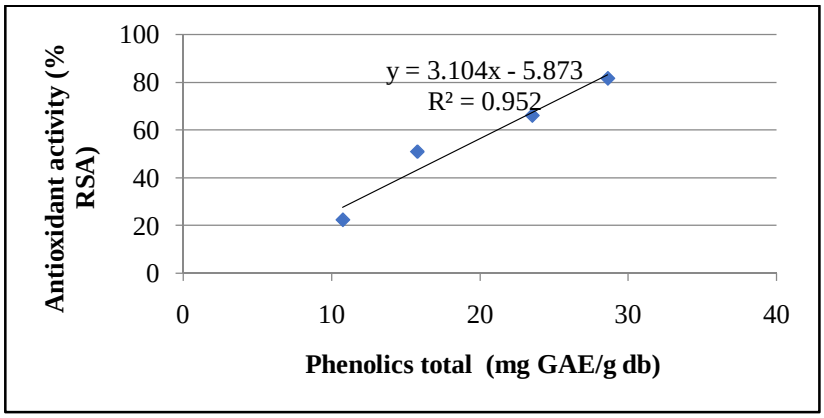

Figure 5: Correlation % RSA vs total phenolics content

This result was in line with the results of some reseach. Pantelidis *et al*. (2006), conducted a research on antioxidative activity with FRAP method, on phenolic compounds and anthocyanins in raspberries, blackberries, red currants, gooseberries and cornelian cherries. The research showed a strong correlation between the value of FRAP with phenolic content of (r = 0,95), and a lower correlation between the antioxidative activity and anthocyanins content of (r = 0,64).

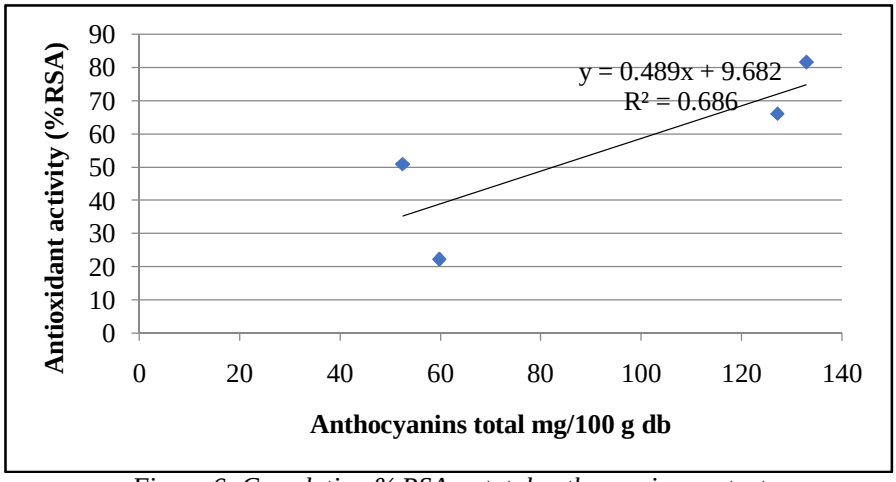

Figure 6: Correlation % RSA vs total anthocyanins content

A research conducted by Deighton *et al.* (2000), on the antioxidant activity in Rubus species, showed that the correlation between antioxidant activity (FRAP method) and total phenolic is (r = 0,97), while the correlation between anthocyanins content and antioxidant activity is (r = 0,59).

4. Conclusion

Blanching treatment on purple yam will increase the total anthocyanins, total phenolic and DPPH-method antioxidant activity (% of radicals scavenging activity, % RSA). The 8 and 12-minute blanching will increase the anthocyanins for 2,5 to 2,4 times higher than non-blanching treatment. The 8 and 12-minute blanching will increase the total phenolic for 1,6 to 1,3 times higher than non-blanching treatment. Antioxidant activity otherwise as DPPH radicals scavenging on the 30-minute observation is as follow; 8-minute blanching ≥ 12-minute blanching = BHT > without blanching > 4-minute blanching. The correlation between total phenolic vs antioxidative activity is (r^2 = 0,95), which is higher than the correlation between total anthocyanins vs antioxidant activity (r^2 = 0,69).

5.Acknowledgements

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