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The Antioxidant Activity of Fresh Yam and Its Changes During Processing into Flour and Storage

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Abstract: This research aimed to study the antioxidant activity of several cultivar of fresh yam tubers and its flour and to determine the effect of processing an storage on antioxidant activity of yam flour. The research used Complete Randor Design (CRD). This study was conducted in three stages and repeated 3 times. A first stage, antioxidant activities of three types of yam were investigated. The typ of yam tuber which has the highest antioxidant activity was used for second stag At second stage, the experiment was carried out to determine the effect c processing methods on the antioxidant activity of yam flour. The treatment whic produced yam flour with the highest antioxidant activity was further used in th last stage. At last stage, the experiment was carried out to determine the effect c storage on antioxidant activity. At this stage, 3 different storage times was use (0 weeks, 4 weeks and 8 weeks). The results showed that types of yam an processing treatment gave significant effect on antioxidant activity, IC_{50}, L*, a* an b*, and total anthocyanin of yam tuber and flour. However, processing metho gave insignificant effect on value of L* of yam flour. Type of yam with the highes antioxidant activity was purple yam with antioxidant activity 96,35%, IC_{50} 44,9 µg/ml, total anthocyanin content 351,82 mg/100g, L* 46, a* 54, and b* - 21. Th processing method that produce flour with the highest antioxidant activity wa steaming which produce yam flour with antioxidant activity of 83.59%, IC_{50} 67.4 µg/ml, total anthocyanin content 136.94 mg/100g, L* 66, a* 26, and b* -15. Ther was a decrease in antioxidant activity, total anthocyanin content, L*, a* and b* c yam flour during storage of 4 and 8 weeks

Keywords: Antioxidant activity; processing; storage; yam flour yam tuber

Introduction

Yam tubers are a type of tubers that grow a lot in Indonesia, one of which is in Jambi Province. In Jambi Province, the Kerinci Regency area is one of the areas where this plant is commonly found, especially those of purple tubers. Meanwhile, in Bangko district, plants with purple, white and yellow bulbs were also found. The districts of Tanjung Jabung Timur and Muaro Jambi are also the places where plants grow that have purple and yellow tubers (Ulyarti et al., 2015).

Yam tubers contain a number of bioactive compounds that have physiological effects as antioxidants, such as phenol and flavonoid (Wang et al., 2022) and anthocyanin (Lavlinesia et al., 2019). In addition, anthocyanin positively impact human health, including anti-inflammatory action (Qiu et al., 2021), regulating blood lipid and blood sugar (Yan et al., 2016), and protecting cardiovascular system (Luo et al., 2019) .

The antioxidant activity of anthocyanin is influenced by several factors such as tuber type, processing and storage time (Chen et al., 2023; Liao et al., 2019; Palupi et al., 2012; Yun et al., 2023). Previous study on sweet potato showed that the type of sweet potato affects the tuber's antioxidant activity (Endah, 2012). The antioxidant activity is indicated by the color intensity of the tubers. The darker the color, the higher the

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anthocyanin and β-carotene content in the tuber and so the antioxidant activity.

Anthocyanin is an organic pigment found in many color fruit and vegetables. It is water-soluble and gives color ranges from red to blue (Gençdağ et al., 2022; Ngoc Nhon et al., 2022). The actual color depends on the substituent in R1 and R2 (see Figure 1). pH and the presence of other compound and metal ions are other factors affecting the color of anthocyanin (Gençdağ et al., 2022). Yam tubers have a limited shelf life, so they need to be processed in the form of flour so that they can be stored for a period of time. Proper processing is able to maintain the amount of bioactive content in the processed products. According to previous report, the anthocyanin in the flour can be maintained by blanching and steaming process (Lavlinesia et al., 2019; Ulyarti et al., 2019). A study produced yam flour with anthocyanin content of 78.5 mg cyanidin equivalent/100g (Ulyarti et al., 2019). Other study reported the use of different method of processing, yam flour could contained even much higher anthocyanin 175.53 mg/100g (Lavlinesia et al., 2019). Study reported by Immaningsih (Imanningsih et al., 2013), showed that the combination of soaking in 1% citric acid and 10-minute steaming produces a good retention value of bioactive components.

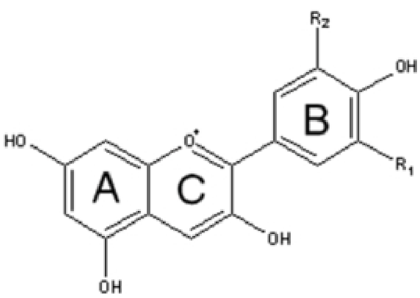


Figure 1. Anthocyanin structure (Yuan et al., 2023)

In addition to processing, storage is another factor that might affect the content of anthocyanin. The decrease of anthocyanin content in food product during storage can be inhibited by combining the food with other compound whose groups capable in forming hydrogen bonds with the hydroxyl groups in the anthocyanin (Yun et al., 2023).

This study aims to determine the effect of the types of tubers (*Dioscorea spp*), namely purple yams, yellow yams and white yams on the antioxidant activity, to determine the effect of processing and storage time on the antioxidant activity of yam flour and to obtain the most appropriate processing and storage time for yam flour.

Method

Material

The main ingredients used in this study were purple, yellow and white yam tubers from Jambi. The chemical used for analysis were distilled water, 1% citric acid, 95% methanol, DMSO solution, DPPH pro-analyst solution, ascorbic acid, nitrogen gas and 1 N HCl.

Research Stages

Antioxidant Activity in Several Types of Tuber

Types of yam tubers (*Dioscorea spp*) namely purple, yellow and white yam tubers are cleaned to remove dirt, washed, and cut into thin pieces for the analysis. The type of yam tuber that perform the highest antioxidant activity was continued to stage II to be processed into flour with three processing methods, namely direct drying in the oven, steaming and soaking in 1% citric acid and by steaming.

Flour Preparation using Several Methods

The direct oven drying method. The yam tubers were cleaned, peeled, washed, then sliced with a thickness of about 2 mm and dried in an oven at 60^{o}C for 12 hours. The dried slices of yam tubers were ground using a blender and sifted through a 60 mesh sieve. The steaming method. Yam tubers were peeled, washed and sliced with a thickness of 2 mm. Then, the slices of yam tubers were steamed at 100^{o}C for 7 minutes. Then, it is cooled and followed by drying using an oven at 60^{o}C (10 hours). The dried slices of yam were ground using a blender and sifted through a 60 mesh sieve. The soaking and steaming method. Yam tubers are washed, peeled and sliced with a thickness of 2 mm. Yam tuber slices soaked in 1% citric acid solution for 30 minutes and washed. Sliced yam tubers are steamed at 100^{o}C for 10 minutes and cooled. Sliced yam tubers that have been steamed are dried in an oven at 60^{o}C (10 hours). The dried slices were ground and sieved with a size of 60 mesh.

Yam Flour Storage

Purple yam tuber flour was packaged in polyethylene plastic, sealed and stored at room temperature for 0, 4, and 8 weeks before its antioxidant activity was analyzed.

Analysis

Total Anthocyanin content (Ulyarti et al., 2019)

As much as 1 gram of powdered fresh starch or tuber sample is dissolved in 10 ml of methanol (95% methanol and 1 N HCl 85:15) in a centrifugation tube. Next, nitrogen gas is flew into the tube and then shaken for 30 minutes. After that, the samples were centrifuged at 3000rpm for 10 minutes. The supernatant obtained was analysed using a Uv-Vis spectrophotometer at both wavelengths 535 nm and 700 nm.

$$\text{Anthocyanin (C)} = \{A/\varepsilon\} \times \text{(total volume of methanol extract)} \times BM \times (1/wt) \times 100 \quad (1)$$

Where:
C = mg cyanidin 3-glucosideε/100g sample
A = (Abs535nm-Abs700nm)
ε = molar absorption coefficient of 25.965/cm/M
BM = molecular weight of cyanidin 3-glucoside (449.2g/mol)
wt = total weight (g)

Antioxidant Activity (Baba et al., 2016)

As much as 1 gram of fresh tuber that has been mashed or flour was first dissolved in a 10 ml solvent (methanol and water), stirred for 2 hours, and centrifuged at 3,500 rpm for 10 minutes. The supernatant obtained was analyzed for its antioxidant activity.

To determine the antioxidant activity, as much as 3.8 ml of 0.05 μM DPPH solution was added to 0.2 ml of a sample solution. The solution mixture was homogenized and left for 30 minutes in a dark place. Absorption was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. For control, distilled water was used to replace sample solution and treated the same as the samples. Ascorbic acid as the positive control was also treated similar to the sample. Antioxidant activity was presented as percent inhibition. The percent inhibition is calculated using formulated.

$$\%\ \text{Inhibition} = \frac{\text{Control absorbance} - \text{sample}}{\text{Control absorbance}} \times 100\% \quad (2)$$

IC_{50}

IC_{50} is a parameter that shows the concentration of the test sample that is able to capture 50% of the radicals. The IC_{50} value is obtained from the percent inhibition values which is calculated using Linear Regression statistical analysis from the equation Y = a + bX, where Y = dependent variable (% inhibition), X = independent variable (sample solution concentration), a = intercept, and b = regression coefficient.

Color

Measurement of the color of yam tuber and yam tuber flour was carried out objectively using a black box described previously (León et al., 2006). The box is rectangular in shape with black sides measuring 75 x 74 x 30 cm. The box is made of a board consisting of 4 pieces of 10 watt fluorescent lamps with a length of 30cm which are placed on each side of the box with a slope of 45°. The sample is placed in the center of the bottom of the box and taken with a 16 mega pixel camera without a flash with the closed cardboard position at a camera distance of ± 40 cm.

Color measurement

The image is opened with the Adobe Photoshop program, cropped to the same size. Color measurement is carried out using the histogram window to see the color distribution or RGB values (Red, Green, Blue) and L, a, b values.

Data Analysis

The data obtained were analyzed using analysis of variance at 1% and 5% levels. If ANOVA shows a significant effect, the data was further analyzed using the Duncan New Multiple Range Test (DNMRT) at 1% level.

Result and Discussion

The Effect of Tuber Type

The average value of the antioxidant activity of the yam tuber (*Dioscorea spp*) is presented in Table 1. The analysis of variance of antioxidant activity showed that the type of yam tuber (*Dioscorea spp*) had a significant effect on antioxidant activity ($p \leq 0.01$) with purple yam tubers performed the highest antioxidant activity (96.35%) followed by yellow yam tubers (93.58%) and white yam tubers (89.94%). Although the total anthocyanin of purple yam tuber is 27 times higher than the total anthocyanin of yellow yam tuber and white yam tuber, the antioxidant activity of these types of yam tuber does not show much difference. This indicates that there might be other compounds that function as antioxidants in yam tubers other than anthocyanins (Yamuangmorn et al., 2018) such as phenolic acid and flavonoid (Lebot et al., 2022) and dioscin (Wang et al., 2022).

The higher total anthocyanin content also results in a higher antioxidant activity value. Anthocyanin content is a major contributor to antioxidant capacity, similar to study reported previously (Imanningsih et al., 2013). Phenol and flavonoid compounds have stronger correlation with antioxidant activity compare to vitamin C and E (Orsavová et al., 2022). The average IC_{50} value of the yam tuber (*Dioscorea spp*) can be seen in Table 1. The analysis of variance of the IC_{50}.

Table 1. Total anthocyanin, antioxidant activity, IC_{50} and the color of fresh tuber

Types of tubers	Total anthocyanins (mg/100g)	Antioxidant Activity (% inhibition)	IC_{50} (µg/mL)	Score			Color
				L*	a*	b*	
Purple	351.82 a	96.35 a	44.95 a	46 a	54 a	-21 a	
Yellow	11.29 b	93.58 b	57.39 b	85 b	-4 b	57 b	
White	9.36 b	89.94 c	63.43 c	85 b	0 b	2 c	

Note: Numbers followed by the same lowercase letter in the same column are not significantly different at the 1% level based on the DnMRT test

That the yam tuber (*Dioscorea spp*) had a significant effect on the IC_{50} value ($p \leq 0.01$). According to (Phongpaichit et al., 2007), a compound is declared to contain a high antioxidant capacity if the IC_{50} is between 10-50 µg/mL, moderate if the IC_{50} is between 50-100 µg/mL, and low if the IC_{50} value is lower than 100 µg/mL. Based on the IC_{50} value, purple yam tubers have antioxidants which are classified as high antioxidant capacity, while yellow and white yam tubers are classified as moderate antioxidants.

The average total anthocyanin content of several types of tubers (*Dioscorea spp*) can be seen in Table 1. The result showed that the types of tubers affected total anthocyanin content and color ($p \leq 0.01$). The total anthocyanin of purple yam tubers was significantly different from those of yellow yam tubers and white yam tubers. This type of purple yam tuber has the highest total anthocyanin of 351.82 mg/100g. This value is much higher than the total anthocyanin value as reported previously (Imanningsih et al., 2013), which is 234.46 ± 34.27 mg/100g.

The average value of the color of yam tubers can be seen in Table 1. The analysis variance of color showed that the types of yam tubers had a very significant effect on color parameters ($p \leq 0.01$). The L* and a* values of purple yam tuber were significantly different from those of yellow and white yam tubers. Yellow and white yam tubers had high L* values. The high a* value is owned by the purple yam tuber which reflects to the anthocyanins in the form of cyanidin-3-glucoside (Nadia & Hartati, 2013). The high b* value is owned by the yellow yam tuber which reflects to the carotene found in the yellow yam tuber. The carotene components identified in yellow yam tubers are lutein, zeaxantine, and beta-carotene (Nadia & Hartati, 2013).

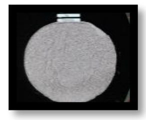
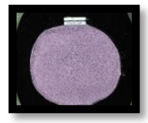
Effect of Processing Method

The average value of anthocyanin content and antioxidant activity of purple yam flour produced using three processing methods are presented in Table 2. The results showed that the processing method of purple yam tuber had a significant effect ($p \leq 0.01$) on the antioxidant activity of the flour. The antioxidant activity of the flour with steaming treatment was the highest among all other treatment. This antioxidant activity of purple yam flour however, is much lower than its tubers. This is due to the processing of yam tubers into flour which undergo several stages which inactivate the antioxidant compounds such as steaming and drying.

There are several processing factors that affect the retention of phenolic components and their antioxidant activity. Increasing the processing temperature will degrade the total phenolics and decrease the amount so that the ability to capture free radicals will decrease (Hsu et al., 2003). However, heating for a short time may reduce peroxidase enzyme activity, while drying can reduce the activity of polyphenol oxidase. These enzymes play a role in the oxidation of phenolic components and produce a brown color. There is a relationship between peroxidase enzyme activity and the browning index of materials containing phenolics compounds that the higher peroxidase enzyme activity, the higher the browning index (Akissoe et al., 2004).

The average IC_{50} value of purple yam tuber flour with three processing methods can be seen in Table 2. The results of the analysis of variance showed that the processing method of purple yam tuber had a very significant effect ($p \leq 0.01$) on the IC_{50} value of purple yam flour. Based on the IC_{50} value, the antioxidant activity of purple yam tuber flour has moderate activity, which is in the range of 50-100 µg/ml.

Table 2. Total Anthocyanin, Antioxidant Activity, IC50 Value and Color in Purple Yam Tuber Flour

Processing Method	Total anthocyanins (mg/g)	Antioxidant Activity (% inhibition)	IC50 (µg/ml)	Score			Color
				L*	a*	b*	
Directly dried in the oven	122.36 a	73.99 a	76.58 a	63 a	7 a	0 a	
Steaming	136.94 b	83.59 b	67.48 b	66 a	26 b	-15 b	
Citric acid soaking and steaming	129.67 c	79.88 c	71.22 c	64 a	29 b	-1 a	

Note: Numbers followed by different lowercase letters in the same column are significant at the 1% level based on the DnMRT test

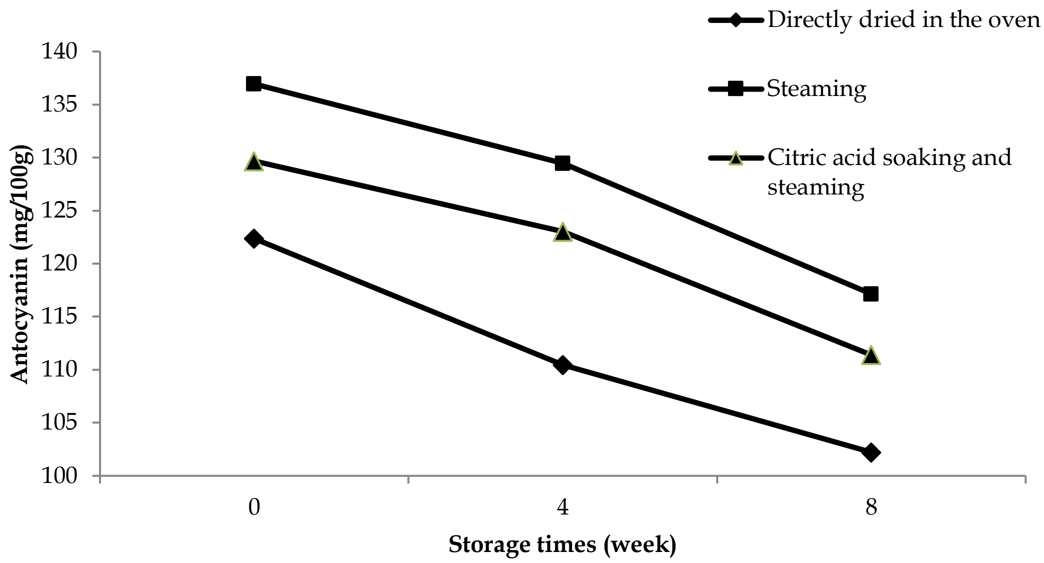


Figure 2. The Total Anthocyanin of Purple Yam Flour During Storage

The processing method of purple yam tuber had a very significant effect on the total anthocyanin of the flour ($p \leq 0.01$). In Table 2, it can be seen that the steaming method produced flour with the highest total anthocyanins content. These results are in accordance with those reported previously (Lavlinesia et al., 2019; Ulyarti et al., 2019). The use of heat in the processing reduces the anthocyanin content. Heat damages the anthocyanin depending on the heating time and the size of the material being processed. Furthermore, the anthocyanin content can actually be preserved by using other type of drying such as vacuum freeze dry (Li et al., 2023).

Color is an important sensory attribute in a food product. This sensory characteristic is one of the attractions of purple yam flour products. The average color value of purple yam flour can be seen in Table 2. The analysis of variance showed that the processing of purple yam tuber had a significant effect on a* and b* values of purple yam flour ($p \leq 0.01$), but had no significant effect on the L* value of purple yam tuber flour. The difference in color of purple yam flour is caused by the treatment or processing method of purple yam flour. Steaming could maintain the purple color. However, when steaming was combined with soaking in 1% citric acid solution, the purple color changed to reddish purple. The purplish color of yam flour always coincides with a decrease in brownish color and vice versa.

Effect of Storage

The storage of purple yam tuber flour at room temperature reduced the total anthocyanin content of the flour. The changes in total anthocyanin content of purple yam tuber flour during storage can be seen in Figure 2. The decrease in total anthocyanin during storage was due to the presence of light and oxygen in contact with the material for a relatively long time.

Anthocyanin degradation can occur during processing and storage. Exposure to light can increase the degradation of anthocyanin molecules. The main cause of color pigment loss is related to anthocyanin hydrolysis (Enaru et al., 2021). Most of the decomposition was due to photooxidation and p-hydroxybenzoic acid was identified as a minor degradation product. The ability of light to excite anthocyanins through electron transfer causes anthocyanin pigments to a photochemical decomposition.

Storage of purple yam tuber flour at room temperature educed the antioxidant activity of the flour. The antioxidant activity of purple yam flour during storage can be seen in Figure 3. The decrease in antioxidant activity of flour stored at room temperature may be due to the presence of residual oxygen compounds in the packaging, both arising from oxidative and anaerobic pathways. In general, the constant rate of anaerobic degradation of antioxidant compounds will be up to two or three times faster than oxidative degradation. The presence of these residual oxygen compounds can cause phenol compounds to donate their hydroxyl groups (-OH) to maintain product stability. The phenolic compounds eventually lose the –OH group which results in a decrease in antioxidant activity during storage.

Figure 4 shows the increase in the IC_{50} value of purple yam tuber flour during storage. An increase in the IC_{50} value during storage indicates a diminishing antioxidant activity. Overall, the antioxidant activity were proportional to the decrease in anthocyanin content of each type of flour preparation. The dominant antioxidant activity is contributed by the anthocyanin content.

The quality of a food ingredient is determined not only by nutritional elements, but also by color. Table 3 presents the changing of the color of purple yam tuber flour during storage. The color of yam tuber flour tends to be darker than the initial color.

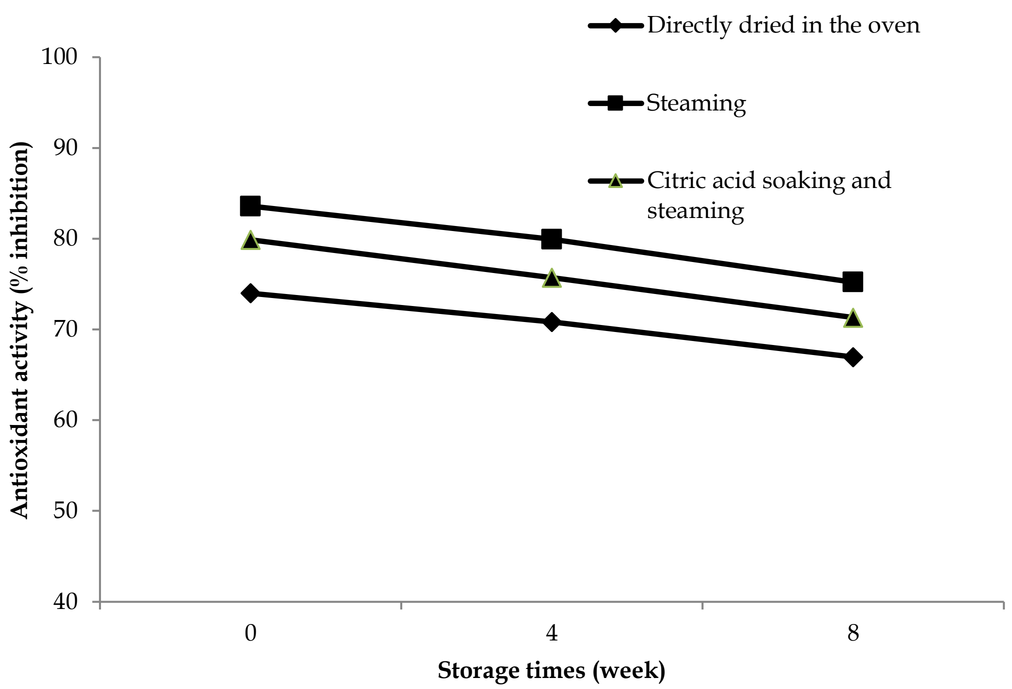


Figure 3. Antioxidant Activity of Purple Yam Flour During Storage

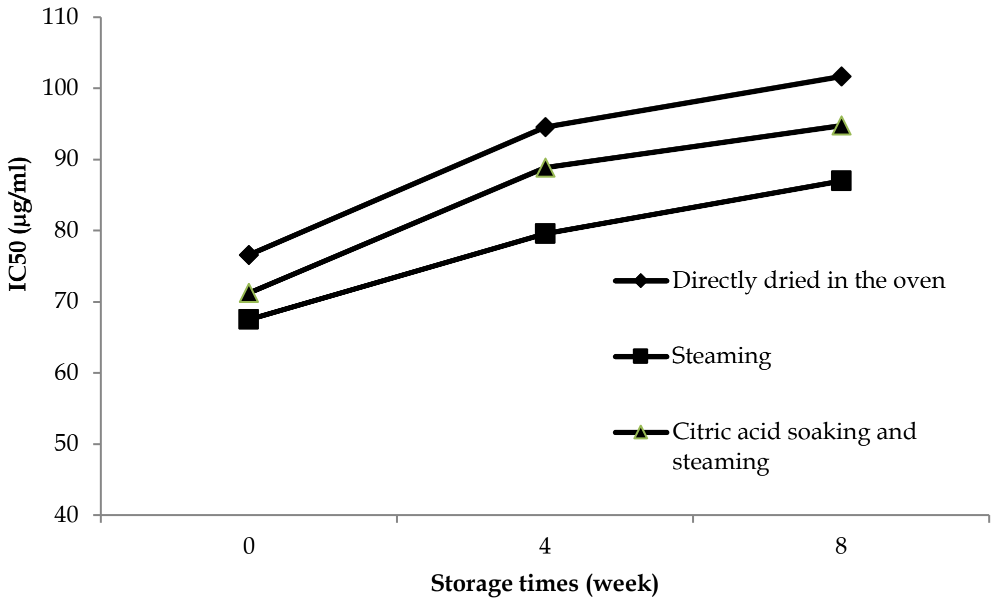


Figure 4. IC_{50} of Purple Yam Flour During Storage

Table 3. L* a* b* Value of Purple Yam Flour During Storage

Processing Method	Storage Time (weeks)	Score L*	a*	b*	Color
Directly dried in the oven	0	63	7	0	
	4	59	6	-1	
	8	51	8	-2	
Steaming	0	66	26	-15	
	4	62	23	-15	
	8	61	20	-12	
1% citric acid soaking and steaming	0	64	25	-1	
	4	62	22	-1	
	8	61	22	0	

Conclusion

The type of yam tuber gives a very significant effect on the antioxidant activity, IC_{50} value, total anthocyanins, L*, a* and b* values. The purple tuber contains the highest antioxidant activity of 96.35%, IC_{50} value of 44.95 µg/ml, total anthocyanin 351.82 mg/100g, L* value 46, a* 54, and b* -21. The processing method affected the antioxidant activity, IC_{50} value, total anthocyanins, a*, and b* values of purple yam flour, but not its L* value. Processing method that produces flour with the highest antioxidant activity was "steaming" which produces flour with antioxidant activity of 83.59%, IC_{50} value of 67.48 µg/ml, total anthocyanin 136.94 mg/100g, L* value 66, a * 26, and b* -15. A decrease in total anthocyanin, L*, a* and b* values and antioxidant activity of yam tuber flour were observed during storage for 4 weeks and 8 weeks at room temperature.

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Author Contributions

This research was technically carried out in the laboratory by Elsa Monica. Elsa Monica was also in charged for writing the first draft of the manuscript. Lavlinesia was responsible for the conceptualization and methodology of the research. Ulyarti was responsible for review and finalization of the manuscript and publication.

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Conflicts of Interest

Authors declare that no conflict of interest in the research.

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