

The Correlation of Anthocyanin Levels, Vitamin C Levels, and Antioxidant Activity of Purple Cabbage (*Brassica Oleracea* L.) Juice at Different Temperatures and Storage Periods

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**THE CORRELATION OF ANTHOCYANIN LEVELS, VITAMIN C LEVELS, AND ANTIOXIDANT ACTIVITY OF PURPLE CABBAGE (*BRASSICA OLERACEA* L.) JUICE AT DIFFERENT TEMPERATURES AND STORAGE PERIODS**

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ABSTRACT

Purple cabbage (*Brassica oleracea* L. var. capitata f. rubra) is a plant from the Brassicaceae or Cruciferae family that can be grown in both highlands and lowlands. The amount of purple cabbage production is relatively high, but utilization by consumers is still low. The choice of how to use purple cabbage is to make juice, because it is easy to make and only takes a short time, and the freshness can still be enjoyed. The way of consuming juice is seen as more relevant and enjoyable for the general public than having to eat large amounts of purple cabbage. Anthocyanins and vitamin C are water-soluble compounds that have a major role in providing antioxidant activity of purple cabbage juice. Both anthocyanins and vitamin C are compounds that are easily oxidized by O₂ in the air so that the length of contact with air during storage will affect their stability. Likewise, storage temperature is a factor that affects the stability of anthocyanins and vitamin C which will have an impact on their antioxidant activity. Therefore, this study was conducted to determine the correlation of anthocyanin levels, vitamin C levels, and antioxidant activity of purple cabbage juice at different temperatures and storage periods. Determination of anthocyanin levels, vitamin C levels, and antioxidant activity was carried out by UV-Vis spectrophotometry. On the 7th day of storage, the anthocyanin content decreased by 3.7% at cold temperature and 22.5% at room temperature; vitamin C content decreased by 59.1% at cold temperature and 75.7% at room temperature; antioxidant activity decreased with an increase of 9.8% IC₅₀ at cold temperature and 24.9% at room temperature. Purple cabbage juice decreased its antioxidant activity after the crop was stored for 4 days and 7 days, both stored at cold and room temperature. This was caused by a decrease in the content of the main antioxidant compounds in purple cabbage, namely anthocyanins and vitamin C, during the storage process.

Keywords: antioxidant; anthocyanin; purple cabbage juice; vitamin c

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INTRODUCTION

Purple cabbage (*Brassica oleracea* L. var. capitata f. rubra) is a plant from the Brassicaceae or Cruciferae family that can be grown in both highlands and lowlands. Purple cabbage contains vitamins (A, B, C and E), minerals (K, Ca, P, Na and Fe) sulfofanan and flavonoids including anthocyanins that can support antioxidant activity (Putri et al., 2018). The amount of purple cabbage production is relatively high, but utilization by consumers is still low because it is only used for making pickled vegetables and as a salad mixture. In addition, it is generally also consumed as a vegetable ingredient that is cooked or eaten raw. The choice of a simpler way of consuming purple cabbage is to make juice, because it is easy to make and only takes

a short time, and the freshness can still be enjoyed. The way of consuming juice is seen as more relevant and enjoyable for the general public than having to eat large amounts of purple cabbage.

Anthocyanins are compounds of the pigment group from the flavonoid group that cause red to purple colors, which are located in water-soluble cell fluids. According to Priska et al (2018) anthocyanins as bioactive compounds are able to function as natural free radical scavenging compounds or as natural antioxidants (Priska et al., 2018). Vitamin C has been shown to have very strong antioxidant activity. Vitamin C is also found in many fruit and vegetable-producing plants, including cabbage. Anthocyanins and vitamin C are water-soluble compounds that have a major role in providing antioxidant activity of purple cabbage juice.

Both anthocyanins and vitamin C are compounds that are easily oxidized by O₂ in the air so that the length of contact with air during storage will affect their stability. Likewise, storage temperature is a factor that affects the stability of anthocyanins and vitamin C which will have an impact on their antioxidant activity. Therefore, it is necessary to conduct research to determine the correlation of anthocyanin levels, vitamin C levels, and antioxidant activity of purple cabbage juice at different temperatures and storage periods.

METHOD

The population used in this study was purple cabbage from the plantation of Dusun Sawit, Girirejo Village, Ngablak District, Magelang Regency. The sample used in this study was purple cabbage which was harvested in the population area at the age of 3 months, with the following criteria: the entire surface of the cabbage crown was dark red purple and there were no black or brown parts, the hard crop layer was crispy and not soft, and there are no bruised, cut, peeled or wilted parts of the crop. Purple cabbage that has been clean and dried is divided into 2 groups. Purple cabbage group 1 was stored at cold temperature (5 °C) in the refrigerator, while purple cabbage group 2 was stored at room temperature. Purple cabbage in each storage temperature group was determined for anthocyanin levels, vitamin C levels were determined, and antioxidant activity was determined on the 1st, 4th, and 7th days of storage.

RESULTS

Anthocyanin Analysis

The results of the qualitative test showed that purple cabbage juice extract was proven to contain anthocyanins. This is indicated by the appearance of a red color after the addition of HCl (figure 1) and a bluish-green color after the addition of NaOH (figure 2).

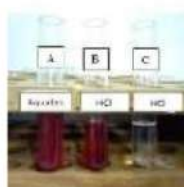


Figure 1. Results of identification of anthocyanins with HCl

A: sample with distilled water,
B: sample with HCl solution,
C: HCl . solution



Figure 1. Results of identification of anthocyanins with HCl

A: sample with distilled water,
B: sample with HCl solution,
C: HCl . solution

The results of determining the maximum wavelength of anthocyanins in purple cabbage juice extract are 525 nm, with the spectrum presented in Figure 3. Anthocyanins are measured as cyanidin-3-glucoside which has a maximum wavelength in the 515 – 545 nm region (Anggraeni, et al, 2018).

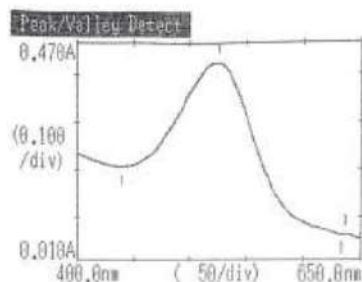


Figure 3. Anthocyanin absorption spectrum

The results of anthocyanin levels at various temperatures and storage times are presented in Table 1, while the relationship between temperature and storage time with anthocyanin levels is presented in Figure 4.

Table 1. Results of determination of anthocyanin levels

Table 1. Results of determination of anthocyanin levels					
Storage	Replikasi	Storage time (days)			Decrease Rate
		1	4	7	
		Vitamin C content (mg/ 100 g juice)			
Cold temperature	1	24,04	18,98	19,43	3,7
	2	17,28	19,15	18,03	
	3	17,91	19,26	19,62	
Average		19,74	19,13	19,02	
Room temperature	1	20,36	17,11	16,48	22,5
	2	18,67	15,32	13,51	
	3	19,08	16,38	15,04	
Average		19,37	16,27	15,01	

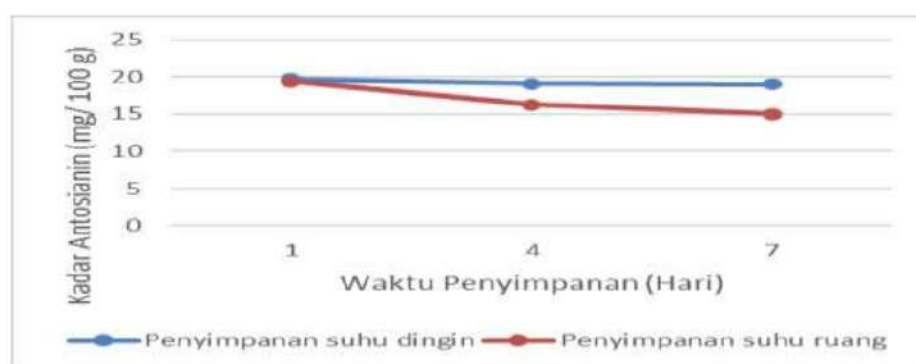


Figure 4. Graph of the relationship between purple cabbage juice anthocyanin levels with temperature and storage time

Analysis of vitamin C

The results of the qualitative test with Fehling's reagent are presented in Figure 5. The test solution that has been added with Fehling's reagent shows a positive result containing vitamin

C because a brick red precipitate is formed with similar results to the positive control. The results of the qualitative test with iodine reagent are presented in Figure 6. The test solution that has been added with iodine shows a positive result containing vitamin C because it has decreased the intensity of the iodine color which was originally purplish brown, like the color of the negative control solution, to purplish yellow, with similar results to the control positive.



Figure 5. Results of identification of vitamin C with Fehling

A: test solution, B: test solution + Fehling reagent

C: positive control, D: negative control



Figure 6. Results of identification of vitamin C with Iodine

A: test solution, B: test solution + Iodine

C: positive control, D: negative control

The spectrum of the maximum wavelength determination using a standard solution of vitamin C with a concentration of 2 g/ml. is presented in Figure 7.

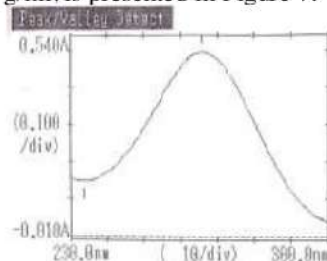


Figure 7. The absorption spectrum of vitamin C

The linear regression equation obtained from the determination of the standard curve in Figure 8 was used to calculate the vitamin C content in the test solution. The results of the determination of vitamin C levels of purple cabbage juice at various temperatures and storage times are presented in table 2, while the relationship between temperature and storage time with vitamin C levels of purple cabbage juice is presented in Figure 8.

Table 2. Results of determination of vitamin C . levels

Table 2. Results of determination of Vitamin C levels					
Storage	Replikasi	Storage time (days)			Penurunan Kadar
		1	4	7	
		Vitamin C content (mg/ 100 g juice)			%
Cold temperature	1	17,47	12,46	6,79	59,1
	2	17,12	12,29	7,08	
	3	17,30	11,82	7,36	
Average		17,30	12,19	7,08	
Room temperature	1	17,12	9,72	4,28	75,7
	2	17,69	10,72	3,93	
	3	16,96	10,12	4,38	
Average		17,26	10,19	4,19	

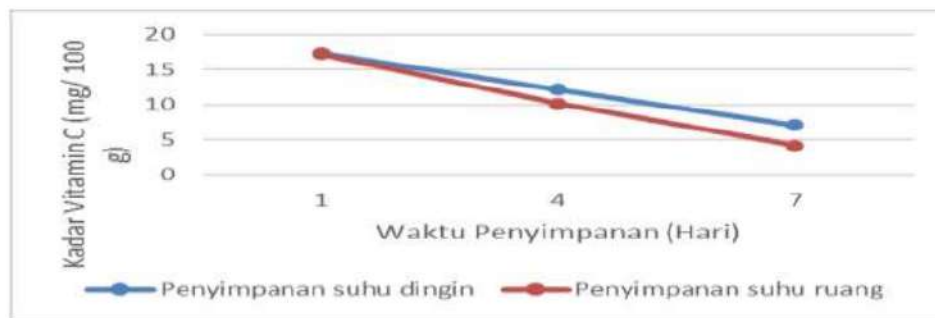


Figure 8. Graph of the relationship between purple cabbage juice anthocyanin levels with temperature and storage time

C. Antioxidant activity analysis

In this study, a stable absorbance value was obtained at 5-8 minutes, which was then used as the incubation time of the test solution after being reacted with ABTS radicals. This is similar to Pulungan's research (2018) which uses operating time at the 6th minute. The maximum wavelength of ABTS radicals obtained in this study is 733.5 nm with an absorption spectrum as shown in Figure 9.

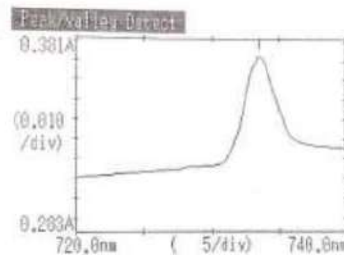


Figure 9. ABTS . radical absorption spectrum

The results of measuring the IC₅₀ value of purple cabbage juice at various temperatures and storage times are presented in Table 3, while the relationship between temperature and storage time with antioxidant activity of purple cabbage juice is presented in Figure 10.

Table 3. Results of determination of antioxidant activity

Table 3. Results of determination of antioxidant activity					
Storage	Replikasi	Storage time (days)			Penurunan aktivitas
		1	4	7	
IC ₅₀ (µg/ml)					%
Cold temperature	1	169,76	175,90	186,32	9,8
	2	171,04	180,03	187,46	
	3	170,04	179,57	187,31	
Average		170,28	178,50	187,03	
Room temperature	1	173,49	202,37	216,34	24,9
	2	174,02	201,91	217,19	
	3	173,08	201,76	216,93	
Average		173,53	202,01	216,82	



Figure 10. Graph of the relationship between the IC50 value of purple cabbage juice with temperature and storage time

DISCUSSION

Determination of anthocyanin levels was carried out with the principle of differences in anthocyanin structure at pH 1.0 and pH 4.5, so that the difference in anthocyanin absorbance between pH 1.0 and pH 4.5 was equivalent to monomeric anthocyanin pigments. The absorbance measurement by UV-Vis spectrophotometry was carried out at a maximum wavelength of 525 nm anthocyanins and 700 nm as a correction. Anthocyanin levels were calculated using the Lambert-Beer equation which states that the absorbance is the product of the absorptivity value, solution thickness, and concentration. Determination of vitamin C levels was carried out by UV-Vis spectrophotometry at a maximum wavelength of vitamin C 266 nm. Vitamin C levels were calculated using the linear regression equation $Y = 0.0426X + 0.3886$, with a correlation coefficient of 0.9997. Determination of antioxidant activity was carried out by UV-Vis spectrophotometry at the ABTS radical wavelength (2,2 azinobis (3-ethylbenzothiazoline)-6-sulphonic acid 733.5 nm when the operating time was reached 6 minutes. The parameters used in measuring antioxidant activity were the value of Inhibition Concentration 50% (IC50), which is a concentration capable of reducing 50% of ABTS free radicals.

The temperature and storage time of purple cabbage sprouts affected the anthocyanin levels, vitamin C levels, and antioxidant activity of purple cabbage juice. Purple cabbage juice made from sprouts stored at cold temperatures can produce higher levels of anthocyanins, vitamin C levels, and antioxidant activity than purple cabbage juice made from sprouts stored at room temperature. Purple cabbage juice decreased anthocyanin levels and vitamin C levels after the crop was stored for 4 days and 7 days. This happens in the crop that is stored at cold temperatures and at room temperature. Likewise, the antioxidant activity of purple cabbage juice decreased after the crop was stored for 4 and 7 days, both stored at cold and room temperature. This was caused by a decrease in the content of the main antioxidant compounds in purple cabbage, namely anthocyanins and vitamin C, during the storage process.

CONCLUSION

Storage at cold temperatures was able to maintain the stability of antioxidant activity, because it correlated with the stability of anthocyanins and vitamin C, as the main antioxidant compounds in purple cabbage.

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