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by Suryani Tawali

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TIME SUBMITTED	25-APR-2019 05:48AM (UTC+0700)	WORD COUNT	3497
SUBMISSION ID	1118598124	CHARACTER COUNT	19005

International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-9, Issue-3, 2018, pp161-167
http://www.bipublication.com

Research Article

Changes of Anthocyanin Contents, Radical Scavenging Activities and Total Microbial Counts of Buni Fruit (Antidesmabunius) Juice during Different Storage Times

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ABSTRACT

Background: Cardiovascular disease is still the leading cause of death in the world. Damage to vascular endothels resulted from oxidative reactions caused by excessive exposure to free radicals due to changes in lifestyle and environment are the main cause of the problem. Consumption of foods with high antioxidants content is believed can prevent and ceased the damaging process in the blood vessels. A local Indonesian fruit that has a potentially high antioxidant content is buni fruit (Antidesma bunius).The aim of this study was to investigate the changes ofanthocyanin content , antioxidant capacity, total microbial counts of buni fruit juice during storage time.**Results:** The total anthocyanin content was302.03 - 496.26 mg/ 100 g fresh. The content decreased to 155-166.48 mg/ 100 g after one week storage . Then further decreased to 94.61-136.46; 64.59-100.37; 59.44-81.57 mg/ 100 g after 2, 3, and 4 weeks storage, respectively. The highest antioxidant capacity is in the fresh juice: 66-70%, and it reduced significantly to 2-12% in the 4th week storage time. Total microbial count increased from 0 in fresh juice to 2- 5 x 10^5 CFU / ml in week 3. **Conclusion :** Buni fruit (Antidesma bunius) has a relatively high anthocyanin content and antioxidant capacity, however, during storage, the bioactive contents and activities reduced and contamination of microbes increased. Therefore, further research is required to maintain the quality of buni fruit juice during storage.

Keywords : Bunifruit;Antidesmabunius; anthocyanins; antioxidant activities; antioxidant capacity;total microbial counts.

BACKGROUND

Cardiovascular disease is still the major cause of death in the world, more than 50% of deaths are caused by non-infectious diseases, and 48% are caused by the diseases of the heart and blood vessels. Changes in lifestyle, high exposure to chemicals either from food or from air pollution are believed to be the risk factors for the onset of the disease. This is attributed to the increasing number of free radicals in the body that trigger physiological changes in the level of cells that eventually leads to such diseases [1].Free radicals are naturally produced in the physiological processes in the body. The number of free radicals increases if the body is working beyond its capacity. Besides these free radicals can also come from unhealthy foods, chemicals, exposure to ultraviolet rays, cigarette smoke and air pollution. In normal circumstances free

radicals are neutralized by antioxidants produced by the body's own (endogenous antioxidant). However, if the number of excessive free radicals in the body, the body can not neutralize all free radicals. And this is what causes damage to cells and tissues of the body, accumulation of fat in the arteries, hardening of the arteries that causes heart disease and hypertension [2]. Researchers have found that many plants contain substances that can neutralize free radicals, which can prevent and treat various diseases and cancer. Substances in plants that are believed to neutralize free radical oxidation reaction by most of the class of polyphenolic compounds. The largest part of natural polyphenol compounds are flavonoids. Flavonoids are found in fruits and vegetables that are colorful. Many studies have proven the benefit of flavonoids in preventing a variety of chronic diseases, especially cardiovascular disease and cancer[3,4]. Anthocyanins as one group of flavonoids are abundant in colorful fruits and vegetables. Berries groups are known as sources of anthocyanins such as blueberry, raspberry, blackberry, gooseberry, etc. Many studies have revealed the benefits of fruit and vegetables anthocyanin for health [3,4,5, 6,7,8,9,10].One of the local Indonesian plant that has similar physical appearance with berry group particularly cranberry is Buni fruit (Antidesmabunius). In ripe stage it has dark black-red color[11]. It has been used as traditional remedy for some diseases and consumed raw or processed in relatively small amount particularly in rural places. However it becomes less popular since people tend to consume more “modern, western” fruit such as grapes and apples. One reason why people is becoming less likely to eat this fruit because its taste which is sour , and also the benefit of this fruit has not been explored and promoted. In Thailand, the fruit is known as Mao Luang. Samappito and Butkhup has found that buni fruit in Northeast Thailand contained high amount of flavonoids, they are catechin, procyanidin B1, procyanidin B2 and several organic acids[12,13]. In South Sulawesi, Indonesia, the fruits are harvested from the end of February to April, and they can be found in traditional markets during harvest seasons, however because of the low demand for consumption the amount of buni fruit marketed decreased in the recent decade.

The aim of this study was to investigate the anthocyanin content of buni fruit juice and its antioxidant capacity and the total microbial colony .

MATERIALS AND METHODS

Materials and Sample Preparation

Buni fruits (Antidesmabunius) were purchased from local markets (Makassar, Indonesia). Only the ripe fruits which were completely reddish and red-black in color were chosen. The collected berries were washed, drained, then weighted. Five hundred grams fruit was extracted using water with ratio 1 : 1, then filtered. The extraction was repeated four times until the anthocyanin content could not be detected anymore. Twenty a 150 ml juice samples weretreated differently, 5 samples without added fructose, 5 with fructose 5%, 5 with fructose 10%, and the rest 5 samples with added fructose 15% . The juice samples thenbottled in the dark bottles, pasteurized, and sealed then stored at 10^{o}C until further analysis.

Chemical Analysis

Analysis was conducted for anthocyanin, vitamin C, and TSS content, total microbial count, pH, antioxidant activities, and organoleptic test. All analysis was conducted every week (week 0, week 1, week 2, and week 3) except for organoleptic test which was conducted only in week 0. For each analysis consisted of two replications.

Anthocyanins

The total anthocyanins wereestimated by a pH differential method [14,15,16]. From each sample,0.05 ml was dissolved with buffer solution of potassium chloride 0.025 M (4.95 ml) pH 1.0 and another 0.05 ml with solution of sodium acetate buffer 0.4 M (4.95 ml).Using UV-Visible spectrophotometer, the absorbance for each sample was then read versus the buffer solution pH 1.0 and buffer solution pH 4.5 as the blank at λ = 510 nm (for the cyanidin 3-glucoside) and λ= 700 nm (for correction

factor). The absorbance values were calculated using the equation:

$$A = \{(A516 - A700)\ pH\ 1\ -\ (A516 - A700)\ pH\ 4.5\}$$

Anthocyanin concentration is calculated as sianidin-3-glycosides using a molar extinction coefficient of 29,600 L mol^{-1} cm^{-1} (ϵ)and a molecular weight of 449.2 g mol^{-1}(MW) using the formula:

$$TAC = \frac{A}{C\ L} \times MW \times DF \times \frac{V}{Wt} \times 100\%$$

Where TAC is total anthocyanin content (mg. 100 g^{-1}); A is absorbance value; ϵ is molar extinction coefficient; L is width of cuvette; DF is dilution factor; V is final volume after dilution; and Wt is weight of original extract.

Antioxidant capacity

Antioxidant capacity was measured by the ability of the sample to scavenge the free radicals [15,17]. The free radical used was 1,1-diphenyl-2-picrylhydrazyl (DPPH). The DPPH analysis was carried out using method from Brand-Williams et. al. [18] with modification. To make DPPH solution, 0,0157 gram DPPH dissolved in 100 ml of absolute ethanol in a measured flask. The radical scavenging activity of the buni fruit juice was measured by adding 1 ml DPPH solution into 100 ml juice sample and added with ethanol until the solution reached 5 ml. The mixture then vortexed and allowed for about 30 minutes in a room temperature. The absorbancy then measured at 518 nm. Radical scavenging activity was determined with the formula :

$$\%\ \text{free radical scavenging activity} = \frac{\text{blank absorbancy} - \text{sample absorbancy}}{\text{blank absorbancy}} \times 100\%$$

Total Microbial Counts

Total microbial counts was detected using Plate Count Agar (PCA) method [19, 20,21] with modification.From each treatment, 1 gram sample was taken. Each sample then put into a test tube contained 9 ml distilled water then shaken until suspension was formed at the bottom of the tube.One ml of the suspension was taken from the tube and inserted into another tube and diluted to 10^4 dilution. One ml of the solution from the second tube was pipetted and put into a petri dish aseptically. After that, Fifteen ml liquid agar that had been cooled to 50°C was poured into the dish, then shaken carefully in order that the microbial cells could spread evenly.If the agar become solid, the petri dish then incubated in inverted position for approximately 48 hours at a temperature of 37°C. The total microbes was counted using the formula :

$$\left(\frac{\log}{CFU}\right) = \text{Number of colonies} \times 1/\text{dilution}$$

Statistical Analysis

Values shown are means ± standard deviation from duplicate samples.

RESULT AND DISCUSSION

Anthocyanins

It shown in table 1. and figure 1 that the highest total anthocyanin content of buni fruit juice was found in the fresh fruit juice whether with or without added fructose that was302.03 - 496.26 mg/ 100 g fresh fruit. It averagely similar with the anthocyanins content of cultivated blueberry and wild blueberry which were 365 mg and 487 mg per 100 g respectively [23]. The concentration of anthocyanin in buni fruit juice consistently reduced during storage time. After seven days the anthocyanin concentrations decreased to 135.55-166.48 mg/ 100 g . Then further decreased to 94.61-136.46; 64.59-100.37; 59.44-81.57 mg/ 100 g after 2, 3, and 4 weeks storage respectively.

The reduction of anthocyanin concentrations during storage time are influence by many factors. It is known that anthocyanins are unstable compounds. Light, storage, pH, oxygen, heat-humidity, enzymes, and the presence of ascorbic acid, sugar, sulfur dioxide, sulfite salts, metal ions and copigments influence the concentration of anthocyanin in food [24,25,26].

Table.1. Changes of anthocyanin content (mg/ 100 gr) of buni fruit (Antidesmabunius) during 4 weeks storage time

Fructose concentration (%)	Storage time (Week)				
	0	1	2	3	4
0	496.26±21.38	166.48±3,64	136.46±0.91	83.09±35.78	81.57±37.30
5	379.36±99.16	151.92±19.1	126.45±35.48	100.37±6.37	67.32±5.46
10	371.17±58.22	135.55±40.94	94.61±4.55	79.75±23.35	58,53±21.53
15	302.03±14.56	120.54±15.01	84.18±3.21	64.59±33.66	59,44±26.69

** mean value± sd (n = 2)

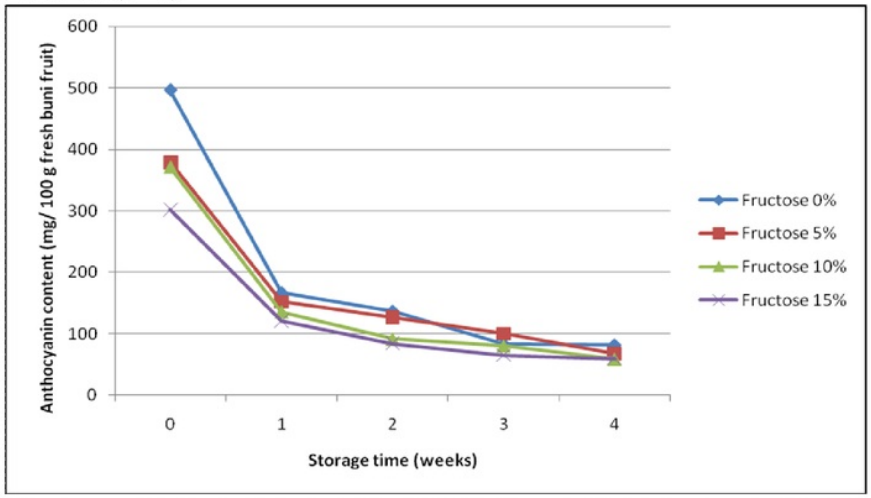

Figure 1. The decrease of anthocyanin content of buni fruit (Antidesmabunius) during 4 weeks storage time

Antioxidant capacity

Antioxidants are chemical compounds that can donate one or more electrons to free radicals, so the free radicals that can be neutralised. Antioxidant activity testing methods used in this study are DPPH (1,1-Diphenyl-2 picrylhidrazyl). DPPH method is a simple method to determine the antioxidant activity of a food using DPPH radical [18]. DPPH test is a colorimetric method that is effective and quick to estimate anti-radical activity. DPPH radical is an organic compound containing nitrogen unstable with strong absorbance at = 517 nm and dark purple [17].

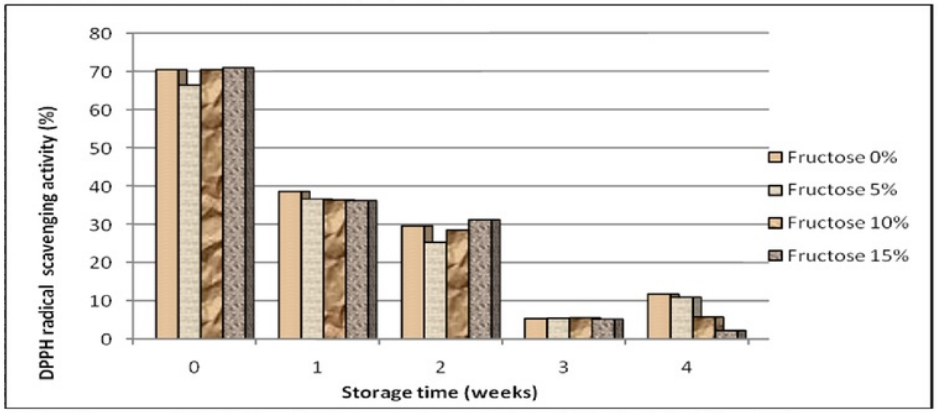

Figure 2. Changes of DPPH radical scavenging activity of buni fruit juice during storage time

The value of antioxidant activity of buni fruit juice with different concentrations and different storage time can be seen in Figure 2. The graph shows that the highest antioxidant activity is in the fresh fruit juice that is 66-70%, while the lowest antioxidant activity is in fruit juice that stored for three weeks that is 2-12% . The antioxidant activity is likely to experience continued decline over time. The ability of the fruit juice to neutralize free radicals depends on the antioxidant concentrations in it. In buni fruit, even though many plant phenolic compounds were present,however, the prominent antioxidant found was anthocyanin, The radical scavenging activity of buni fruit juice reduced concurently with the reduction ofthe anthocyanin concentrations.On the other hand, the concentration of added sugar did not influence the antioxidant capacity As a part of phenolic compounds, anthocyanins has aromatic rings that can donate its electron to stabilized the free radicals in a solution. However, the contribution of other phenolic compounds in buni fruit juice in the antioxidant activities could not be overseen. The phenolics contents of colorful fruits and vegetables are proven to be beneficial for health [24,27]..

Total microbial counts

From figure 3, it can be seen that the effect of fructose concentrations and storage time significantly high to the total microbial counts. In fresh fruit juice, the total microbial counts is 0, therefore, the fresh fruit juice after processing without storage is still considered safe from microbial growth. However, the microbes had started to grow and continuously increased every week. After seven days storage, the total microbial counts was 1-7 x 10^4 CFU/ ml. The density of microbial growth was the highest in the third week after storage: 2-4 x 10^5 CFU/ ml. However, there was an inverted association between concentrations of added sugar with the growth of microbes. The less concentration of the sugars, the more likely the microbes to grow. There are two factors that affect microbial growth are intrinsic factors (pH, aw, nutrient content, biological structure and content of anti-microbial) and extrinsic factors (storage temperature, relative humidity, type and amount of gas in the environment).

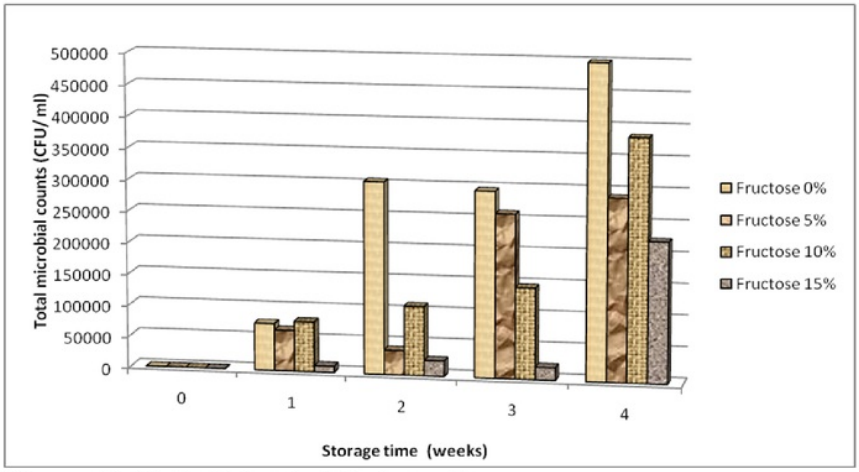

Figure 3. Changes of total microbial counts duringstorage time

This is consistent with argument from Buckle, which stated that a sugar solution with a high concentration (TSS> 70 ° Brix) will be able to provide stability of microorganisms on a product [28]. Manufacture of a product with high sugar concentrations is one important food preservation technique. This is because the decrease of water activity (aw) is associated with the increase in the concentration of sugar itself, so that the water becomes less available for the growth of microorganisms. However, one should consider other food preservation

methods according to the characteristics of the bioactive compounds in a product in order to preserve the maximal nutritional benefits of it.

CONCLUSION

Buni fruit (Antidesma bunius) has a relatively high anthocyanin content and antioxidant capacity with averagely similar with other fruits with high anthocyanin content such as blueberry. However the concentration of anthocyanin and the fruit radical scavenging activity showed a marked reduction during storage time in temperature of 10^{o} C. Therefore, further studies have to be done to find out the appropriate processes and storage methods in order to preserved the anthocyanin content and it antioxidant capacity.

ACKNOWLEDGEMENT

This research was funded by Directorate of Higher Education , The Ministry of Research and High Education Grant.

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