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Beverages

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Antioxidant Activity, Fiber, Acidity (pH), and Population of Lactic Acid Bacteria in Green Juice as Functional Beverages

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Abstract

Bok choy (*Brassica rapa* L) is a vegetable group of *Brassicaceae* which still a family with Chinese vegetables. It contains phytochemical substances such as carotenoids, β -carotene, and glucosinolates which are a source of antioxidant. Bok choy can be processed into juice. The purpose of this research is to determine the content of the antioxidant activity, fiber, acidity (pH) and population of lactic acid bacteria in each formulation. The other ingredients added in making this green juice are yogurt and honey made into 5 formulations. The analysis of this research was the analysis of antioxidant activity content using DPPH method (2,2, -diphenyl-1- picrylhydrazyl), crude fiber contents analysis using gravimetric method, pH analysis using pH meter and analysis of lactic acid bacteria using spread plate method. The result obtained is the highest content of the antioxidant activity was found in formulation 5 consist of 30 g yogurt, 15 g honey and 50 g bok choy with $35,198 \pm 0,057\%$. The content of crude fiber ranged from $(0,447 \pm 0,046)$ g to $(0,507 \pm 0,058)$ g. The highest of acidity (pH) is in formulation 1 which consists of 10 g of yogurt, 15 g of honey and 50 g of bok choy at $5,50 \pm 0,040$. The highest population content of lactic acid bacteria in formulation 5 consists of 30 g yogurt, 15 g honey and 50 g bok choy at $2,8 \times 10^6$ cfu/mL. Therefore, green juice can be used as functional drinks.

Keywords

bok choy, green juice, antioxidant activity, crude fiber, lactic acid bacteria

1 Introduction

In Indonesia, consumption of vegetables and fruit and its processing is still low. Consumption of fruits and vegetables that do not meet the recommendation gives effects on the supply of vitamins, minerals, and fiber needed by the body [1]. The consumption rate of vegetables and fruit in Indonesia is below the recommended 95% per capita per day, the minimum requirement is 120 kcal per capita per day [2]. The research from the Ministry of Health [3] states that 93.5% of Indonesia's population aged > 10 years consume vegetables and fruit under the recommendation. WHO recommends 400-600 grams consumption of vegetables and fruit per person per day consisting of 250 grams of vegetables and 150 grams of fruit for a healthy life [4].

Factors affecting the lack of vegetable and fruit intake include low economic factors, lack of availability of vegetables and fruits [5], environmental problems, delays in the introduction of vegetables and fruit, ways of processing and preparing food [6]. Some effects due to lack of vegetables and fruit such as decreased immunity and immune system, digestive tract disorders, disorders of the eye and high blood pressure [7,8].

Vegetables can be processed by sautéing, boiling and making it in the form of soup, and juice. Juice can be made from one or a mixture of various types of fruit or vegetables. Some studies use vegetables and fruit for juice making such as broccoli juice as an anti-cancer and antioxidant therapy [9], red guava juice [10], watermelon juice as a source of phenolate and antioxidant activity [11], and cucumber juice for lowering blood pressure [12]. Bok choy vegetable is one of the vegetables that can be made in the form of juice as a functional drink. Functional properties in functional foods are due to the presence of bioactive components found in ingredients such as food fiber and antioxidants [13]. Functional beverages are complemented by tertiary functions such as probiotics, increasing vitamin and mineral intake [14].

In Indonesia, bok choy vegetables are processed into various types of dishes, besides being easily cultivated they also have relatively cheap prices. The leaf from bok choy vegetable is a source of

carotenoids, β -carotene, glucosinolate, iron, and calcium [15,16,17]. Making green juice from bok choy vegetables alone results in a bland taste with a distinctive vegetable flavor. Hence, the formulation is made with the addition of yogurt and honey. Antioxidant activity is influenced by total phenolic and flavonoid compounds [18,19,20]. Yogurt is a milk product fermented by lactic acid bacteria (LAB), namely *Streptococcus thermophilus* and *Lactobacillus bulgarius* [21]. Probiotics in inhibiting pathogenic organisms by competing to obtain a number of food ingredients for fermentation. These foods are needed so that probiotics can develop properly. Food that supports the development of probiotics in the digestive tract is called prebiotics. Prebiotics consist of food ingredients which generally contain a lot of fiber. Probiotics that produce lactic acid from the lactobacillus species, produce cellulase enzymes that aid the digestion process [22].

Honey is a liquid that is produced by bees from plant flower extracts that are collected and stored in the hive for maturation [23] honey has active components that function as antioxidants namely vitamin C, vitamin A, vitamin E, Flavonoids and β -carotene [24,25,23]. So that research was conducted to find out how the antioxidant activity, fiber, acidity (pH) and lactic acid bacteria (LAB) population in green juice formulations as functional drinks.

2 Materials and Methods

2.1 Preparation of Green Juice Samples

The sample used was a green juice, with basic ingredients of bok choy (*Brassica rapa* L), yogurt and honey. Bok choy vegetables were washed thoroughly, cut and then inserted into a blender along with yogurt, honey and water. All ingredients were blended until it dissolved and homogeneous which resulted in juice. The juice that has been blended based on a predetermined formulation as described in Table 1 was then analyzed for its antioxidant activity, crude fiber content, acidity (pH) and LAB population.

Table 1 Green Juice Formulation

Materials	Formulation				
	F1	F2	F3	F4	F5
Bok choy (g)	50	50	50	50	50
Yogurt (g)	10	15	20	25	30
Honey (mL)	15	15	15	15	15
Water (mL)	200	200	200	200	200

2.2 Antioxidant Activity of Green Juice Samples

Antioxidant activity test used the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Merck) [26,27]. One mL of standard solution or sample was added 3 mL of DPPH solution (0.004% in methanol). Then, the solution was left out for about 30 minutes in darkroom temperature during the incubation of DPPH radicals reacting with antioxidant compounds contained in the sample changes in color from purple to fade. Furthermore, the absorbance was measured using a UV-Vis spectrophotometer (Shimadzu UV-1601) with a wavelength of 517nm. The fading of the colors produced the higher the antioxidant activity.

2.3 Crude Fiber of Green Juice Samples

Crude fiber test with modification as suggested by Kusumawati and Yogeswara [28] was conducted in this study. A sample of 10 grams was weighed using a Gewinn Scale and accommodated into an Erlenmeyer flask (Iwaki Pyrex), then added 50 mL of H_2SO_4 - 0.255 N and closed with back cooling. Afterward, it was boiled for 1 hour, the suspension was filtered, and the residue left in the Erlenmeyer was washed with distilled water through filter paper until the washing water was not acidic. The following steps were then performed by transferring back the residue on filter paper quantitatively into the Erlenmeyer using a spatula. The remainder was washed with 50 mL of NaOH - 0.255 N (Merck) until the residue entered the Erlenmeyer. Then it was simmered for approximately 1 hour. The sample was filtered using a filter paper (Whatman 42) which has been known to weigh after drying while washing successively with boiling distilled water solution and alcohol each of 15 mL each. Filter paper and its contents were dried at 70°C to

constant weight for 1 hour. Then, it was cooled again in a desiccator and weighed by reducing the weight of filter paper.

2.4 pH of Green Juice Samples

Acidity test by measuring pH [29]. The pH meter (Hanna) is turned on and calibrated with a buffer of pH 4 and pH 7. Samples were taken as much as 30 mL and placed in plastic cups. Then the pH of the sample is measured for 30 seconds. Every time another pH sample is measured, the pH meter is cleaned with distilled water and dried with a tissue paper.

2.5 Total Lactic Acid Bacteria of Green Juice Samples

Green juice samples were put into 0.1% peptone water (Oxoid) and homogeneous using vortex (Labinco). Homogeneous samples were made into a dilution series in 0.1% peptone water. Then they were spread on MRS agar media (Oxoid) and incubated, for 48 hours at 37° C to see the population of lactic acid bacteria [30].

2.6 Statistical Analysis

Data were analyzed using a one-way analysis of variance (ANOVA) test and if the data obtained showed significantly different then the Tukey method was tested. Data were analyzed using SPSS ver.22 (with statistical significance at $p < 0.05$).

3 Results and Discussion

The results analysis of green juice as a functional beverage can be seen in [Table 2](#).

Table 2 Results analysis of green juice as a functional beverage

Green juice formulation	Antioxidant activity (%)	Fiber crude (g)	pH	LAB population (cfu/mL)
F1	22.395 ± 0.051 ^a	0.490 ± 0.519 ^a	5.50 ± 0.040 ^e	4.60 × 10 ⁵ ± 0.624 ^a
F2	26.224 ± 0.102 ^{ab}	0.503 ± 0.103 ^a	5.26 ± 0.011 ^d	6.93 × 10 ⁵ ± 1.800 ^a
F3	24.009 ± 0.187 ^a	0.493 ± 0.187 ^a	5.03 ± 0.005 ^c	8.26 × 10 ⁵ ± 0.808 ^a
F4	31.002 ± 0.046 ^{bc}	0.447 ± 0.046 ^a	4.92 ± 0.015 ^b	1.10 × 10 ⁵ ± 5.408 ^a
F5	35.198 ± 0.057 ^c	0.507 ± 0.058 ^a	4.74 ± .0115 ^a	2.80 × 10 ⁶ ± 2.645 ^b

Note: Data are expressed as mean ± SD from triplicate experiments. Different letters in the column of each group are significantly different ($P < 0.05$).

3.1 Antioxidant Activity of Green Juice Samples

The results of this study showed the antioxidant activity of green juice ranged from 22.395 ± 0.051% to 35.198 ± 0.057%. The highest antioxidant activity was found in formulation 5 (F5) with the addition of 30 grams of yogurt, namely 35.198 ± 0.057%. Analysis of the content of antioxidant activity in vegetables bok choy without the addition of other ingredients with 50 grams bok choy concentration obtained antioxidant activity content of 56.818%, honey 49.650%, and yogurt 42.307%. The process in making each formulation may damage the structure of the hydroxyl position in the flavonoids in vegetables so that it can reduce antioxidant activity [31]. Juice processing can reduce this antioxidant activity due to the degradation of bioactive compounds. The decrease in vitamin C content and antioxidant activity due to fresh broccoli undergoes several processing processes such as cutting, washing, and excessive destruction can cause antioxidant activity on the material to be easily damaged oxidized and trigger the activity of enzymes such as peroxidase, ascorbic acid oxidase and phenolase [32]. The type of process in making green juice can significantly affect the antioxidant content, especially phenolic content. Polyphenol compounds containing various phenolic hydroxyl groups are responsible for antioxidant capacity and free radicals [33]. The effects of processing by pasteurization and without pasteurization affect the antioxidant activity, processing by pasteurization can reduce antioxidant activity compared to without pasteurization [34]. Yulita et al. states

[35] that the small amount of antioxidants in noni juice syrup is caused by the addition of water, because the higher the dilution of the existing antioxidant compounds, the less.

3.2 Fiber Crud of Green Juice Samples

Crude fiber is a part of the water-insoluble fiber that cannot be hydrolyzed with acid-base. The content of crude fiber in green juice as a functional drink ranges from 0.447 ± 0.046 g to 0.507 ± 0.058 g. The content of pure bok choy fiber without the addition of other ingredients has a fiber content of 0.052 g, honey has a fiber content of 0.067 g, and yogurt has a crude fiber content of 0.034 g. The results of the analysis of crude fiber content in green juice as a functional drink can be seen in [Table 2](#).

The fiber content in the formulation is higher than the fiber in the base material. The increased crude fiber content with increased yogurt used is thought to be due to increased biomass production. Bacterial cell walls are a source of fiber so that the fiber content of the product increases [36]. Bacteria that are classified as lactobacillus can produce cellulase enzymes that can help digest crude fiber, bacteria from lactobacillus produce glucanase enzymes that are able to break down beta-glucans into glucose, glucanase are part of hemicellulose in which hemicellulose enzymes can break down hemicellulose. The processing process can increase crude fiber. The crude fiber content found in the skin of tomatoes is a water-insoluble fiber such as cellulose and hemicellulose so that when blended it does not cause the fiber to dissolve [37]. Crude fiber is a collection of indigestible fibers, fiber components consisting of cellulose, hemicellulose, and lignin [38]. Fiber has prebiotic compounds that are good for digestion usually from fruits and vegetables [39]. Prebiotics are generally carbohydrates that cannot be digested and absorbed, usually in the form of oligosaccharides and food fiber, which are useful for stimulating the growth of beneficial bacteria in the normal flora of small intestines [40].

3.3 pH of Green Juice Samples

The results of this study indicate that the average pH value of green juice as a functional beverage ranges between 4.75-5.50. The highest pH value is in formulation 1 (F1) with the addition of yogurt as much as 10 grams with a pH of 5.50 ± 0.040 while the lowest pH is found in the formulation (F5) with the addition of yogurt as much as 30 grams with a pH of 4.74 ± 0.0115 . The results of the analysis of the acidity (pH) of green juice as a functional beverage can be seen in [Table 2](#).

The acidity (pH) decreases with the addition of different amounts of yogurt in each formulation, 10 g, 15 g, 20 g, 25 g, and 30 g. An increase in acidity or a decrease in pH will help inhibit pathogenic bacteria [42]. Plain yogurt used has a pH of 4.28 and kapok honey has a pH of 3.56. The pH value of yogurt ranges from 3.8 -4.6. Thus, the pH of plain yogurt is still according to established standards. The decrease in pH is also influenced by the acidity of honey [41]. Honey is an acidic liquid with a pH of 3.9, thus the addition of honey can affect the decrease in pH [42]. The acidity of the pH is determined by the amount of yogurt added to each formulation.

3.4 Total Lactic Acid Bacteria (BAL)

The viability of lactic acid bacteria in juices varies between formulations ranging from 4.6×10^5 cfu/mL to 2.8×10^6 cfu/mL. The highest viability of lactic acid bacteria reached 2.8×10^6 cfu/mL with a pH of 4.74 which was obtained in formulation 5 (F5) with the addition of 30 grams of yogurt while, the lowest amount of lactic acid bacteria was 4.6×10^5 cfu / mL with a pH of 5.50, obtained in formulation (F1) with the addition of yogurt as much as 10 grams. The results of the population analysis of lactic acid bacteria in green juice as functional drinks can be seen in [Table 2](#).

[Table 2](#) shows that the population of lactic acid bacteria found in green juice has increased in formulation 5, this is due to the addition of different yogurt in each formulation. The higher the formulation the more yogurt is added as well as the pH, the higher the yogurt content added the lower the pH of each formulation [43]. Lactic acid causes a decrease in the pH value, the value is determined due to the presence of H^+ ions so that the pH decreases [44].

Plain yogurt used in the manufacture of juice contains lactic acid bacteria which is 8.7×10^6 cfu/mL. Yogurt which is used as a control is higher than the formulation, this is because the content in different samples containing different nutrients is one of the factors that influence cell growth [45]. Yogurt by giving green grass jelly extract produces lactic acid bacteria that are 1.05×10^6 cfu/mL [46]. Yogurt with the addition of honey pineapple has a total amount of lactic acid bacteria that is 1.59×10^4 cfu/mL, lactic acid bacteria can grow maximum in the growth medium depending on the available nutrient content that can be directly used for growth [47]. Both *S. thermophilus* and *L. bulgaricus* can grow together in a symbiotic

mutualism. *Streptococcus thermophilus* grows because it is stimulated by the presence of lysine and histidine from protein degradation by *L. bulgaricus* while *L. bulgaricus* grows rapidly after *S. thermophilus* reaches stationary phase. The amount of lactic acid bacteria that must be contained in a probiotic food product in order to provide health effects for the human digestive tract is 10^7 cells/mL [46].

4 Conclusions

From the research results obtained, it can be concluded that formulation 5 consisting of 30 g yogurt, 15 g honey and 50 g bok choy has the highest antioxidant activity that is equal to $35,198 \pm 0.057$ and population of lactic acid bacteria 2.8×10^6 cfu/mL, fiber content roughly 0.507 ± 0.058 and the acidity value is $4.74 \pm .0115$.

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