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Effect Of Boiling Time In Saline Solution To Reduce Cyanide Acid Levels Of Selected Indonesian Dried Bamboo Shoots

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Abstract: Bamboo shoots are a potential Agricultural product to be used as a food source. Cyanide acid in bamboo shoots may cause constraints in food processing. One of which can be done through a boiling process in saline solution. This study aims to examine the reduction in cyanide levels in several types of Indonesian bamboo shoots due to the effect of boiling in a salt solution. This study used four varieties of bamboo shoots, namely Betung (*Dendrocalamus asper*), Kuning (*Bambusa vulgaris*), Buluh (*Gigantochloa apus*), and Gombong (*Gigantochloa pseudoarundinacea*)). Results show that boiling time and type of bamboo shoots had a significant effect on cyanide reduction in the dry slices of bamboo shoots. The longer the boiling time, the cyanide in the bamboo shoots will decrease. Betung variety experiencing a decrease in cyanide more than other varieties (0.62%-4.71%), from 426.55mg/1000g to 20.03mg/1000g (cyanide reduction = 95.30%). While the Gombong varied from 454.52 mg/1000g to 24.16mg/1000g (cyanide reduction = 94.68%), Kuning varied from 327.44mg/1000g to 21.66mg/1000g (cyanide reduction = 93.39%), and Buluh varied from 451.4mg/1000g to 42.48mg/1000g (cyanide reduction = 90.59%). This shows that each variety of bamboo shoots has a different rate of cyanide reduction and gives a different reaction to the treatment (boiling in 1% salt solution).

Keywords: Bamboo Shoots, Cyanide Levels, Saline Solution, Boiling

1. Introduction

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Bamboo shoots are one type of vegetable that has been known and consumed by people in several countries, such as China, Thailand, and Taiwan. Bamboo shoots consumption worldwide reaches 2 million tons per year, and around 1.3 million tons have been produced in China [1]. Although there is no recent data on the consumption of bamboo shoots, in 2012, the need for bamboo shoots for consumption reached 37.5 tons [2]. Bamboo shoots have become a traditional food in Indonesia, which is generally used in the culinary or cooking fields [3]. Bamboo shoots are a common name for young bamboo stems, also called young shoots from bamboo. According to Putra [3], there are four genera of bamboo shoots that can be consumed, namely *Gigantochloa, Dendrocalamus, Phyllostachys,* and *Bambusa*.

Bamboo shoots have complete nutritional content, consisting of macronutrients such as protein, carbohydrates, fats, fibers, and micronutrient content such as potassium(K) 117-131 mg/Kg, calcium (Ca) 300-360 µg/kg, zinc (Zn) 670-710 µg/kg, , iron (Fe) 1.750-2.360 µg/kg, natrium (Na) 340-379 µg/kg, and magnesium (Mg) 220-270 µg/kg [4][5]. Also, bamboo shoots contain antioxidants 347.48-2489.60 IC50 (µg/mL), phenols 14.62-27.83 (mg GAE/g), and flavonoids 0.92-12.49 (mg QE/g) [6].

The main component in bamboo shoots is water which is 85.6% [7], which causes it to be easily spoiled. The drying process is one method to extend the shelf life of the product, especially commodities that contain high water content like bamboo shoots. Another component in bamboo shoots that is considered resistant is toxic cyanide acid. Bamboo shoots contain cyanide acid of about 327.44 mg/Kg- 454.52 mg/Kg depending on the type of bamboo [5].

The high cyanide acid content in bamboo shoots causes a bitter taste and is dangerous for consumption. According to Hardjo [8], it has been reported that cyanide generally enters the body through food consumption, and the lethal dose was 0.5 - 0.6 mg/kg body weight. Therefore, it is necessary to study methods for the reduction of cyanide levels in bamboo shoots. In a previous study, Ojha and Pandey [1] reported that boiling for 25 minutes in 1% saline solution (NaCl) in Betung bamboo shoots (*Dendrocalamus asper*) can reduce HCN content from 0.016g / 100g - 0.004g / 100g to 0.001g / 100g (decrease 0.003g / 100g - 0.015g / 100g). According to Anam et al, [9], the boiling process in Ampel bamboo shoots (*Bambusa vulgaris*) can reduce the cyanide content from 730 mg/kg to 440 mg/kg (290 mg/kg decrease). The steaming process on Ampel bamboo shoots (*Bambusa vulgaris*) can reduce cyanide content from 35.77 mg / 100g to 17.07 mg / 100 g (decrease of 18.7 mg / 100g) [3]. Kumalasari, et al [5], compared two methods, boiling and steaming to reduce cyanide in several bamboo varieties, and the result was that the variety of methods used had no significant effect on cyanide reduction. Heat treatment can reduce cyanide significantly, but when comparing the two heat treatment methods (steaming and boiling) these two methods do not provide a significant difference in reducing cyanide.

Several types of bamboo shoots contain different cyanide content, and the response to treatment is presumed to be different. In this study, the effect of boiling time in saline solution on the reduction of cyanide content in bamboo shoots of different varieties was studied. In this study, we observed the changes of cyanide levels per unit time using a method that has been done in previous studies, namely boiling in a salt solution [1][5]. This study aims to examine the reduction in cyanide levels in several types of Indonesian bamboo shoots due to the effect of boiling in a salt solution. Through this research, it will be known the ability of the boiling method in 1% salt solution to reduce cyanide in bamboo shoots, and the optimal time for reducing cyanide in bamboo shoots can be obtained.

2. Materials and Methods

2.1 Materials

Fresh bamboo shoots, namely Betung (*Dendrocalamus asper*), Kuning (*Bambusa vulgaris*), Buluh (*Gigantochloa apus*), and Gombong (*Gigantochloa pseudoarundinacea*) were collected from Dawuan Village, Subang District, West Java. Sodium chloride (NaCl), Chloroform ($CHCl_3$), Hydrochloric acid (HCl), Na-Carbonate (Na_2CO_3), Picric acid ($C_6H_3N_3O_7$), Kaliun cyanide (KCN), n-hexane (C_6H_{14}), 95%

ethanol (C_2H_5OH), and aqua dest (H_2O) for cyanide analysis. The process tools used in this study are knives, basins, filters, pans, stoves, thermometers, stirrers, cutting boards, trays, scales, blenders, and cabinet dryers. While the analytical tools used in this study are Erlenmeyer, beaker, analytical scales, test tubes, funnels, fat flask, soxhlet, condenser, cup, measuring flask, furnace, oven, stove, desiccator, ultraviolet-visible (UV-VIS) spectrophotometer (Shimidzu UV-1900), and dumas (Butchi D-480).

2.2 Preparation of samples

Samples were prepared by peeling, washing, slicing, pre-treatment (soaking in 1% saline solution and boiling), draining, and drying using a cabinet dryer at 45 ºC for 26 hours (Figure 1). Dried bamboo shoots were packed and stored at room temperature until analysis.

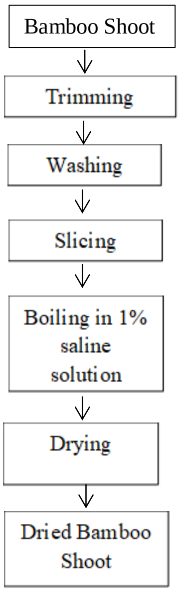

Figure 1. Preparation of samples

2.3 Experimental design

This study used a completely randomized design, two factors, 3 replications. The first factor is the variety of bamboo shoots, and the second factor is the boiling time in a salt solution. the first factor was bamboo shoots varieties, namely *Betung* (A_1), *Kuning* (A_2), *Buluh* (A_3), and *Gombong* (A_4), and the second factor was boiling time consisting of five levels: T_1 = 0 minutes, T_2 = 5 minutes, T_3 = 10 minutes, T_4 = 15 minutes, and T_5 = 20 minutes (Table 1).

Table 1. Experimental design

Boiling time (minute)	Varieties of Bamboo shoots			
	Betung (A_1)	*Kuning* (A_2)	*Buluh* (A_3)	*Gombong* (A_4)
0 (t_1)	A1T1	A2T1	A3T1	A4T1
5 (t_2)	A1T2	A2T2	A3T2	A4T2
10 (t_3)	A1T3	A2T3	A3T3	A4T3
15 (t_4)	A1T4	A2T4	A3T4	A4T4
20 (t_5)	A1T5	A2T5	A3T5	A4T5

2.4 Cyanide acid

The levels of cyanide were determined as described by Bradbury *et al.* [10]. Dried bamboo shoots were accurately weighed (5 g), placed in a test tube, filled with chloroform (1 mL), and covered with picrate paper.

A solution containing 1ml of cyanide standard solutions, 1 ml of distilled water, and 1 ml of 3N hydrochloric (HCL) solution was covered with installed picrate paper and left for 3 hours at 25 ^oC. The picrate filter paper was removed and eluted in 10 mL distilled water. The absorbance of each eluate was measured by spectrophotometer (Shimadzu 1800 UV-Vis, Japan) at λ= 490 nm against a blank. The calculation of cyanide levels was obtained using the following equation (1):

$$\text{Levels of cyanide} = Y \times 1000 \qquad (1)$$

Where:
Y = µg cyanide on a standard curve

2.5-Proximate analysis

Physicochemical properties, as moisture content was determined by direct heating (oven); ash by muffle furnace; protein by DuMaster protein analyzer (Du Master D-480, Buchi, Switzerland); lipid by Soxhlet method; and carbohydrate by Luff Schrool [11].

Moisture Analysis: Gravimetric Method

The dish to be used is heated in the oven and then weighed to a constant weight (A). Weigh the sample 1-2 grams into the cup (B). Then, preheat the cup containing the sample in an oven at 105°C for 3 hours. Cool in a desiccator for 10-15 minutes. Weigh with an analytical balance. Warm up and weigh until a constant weight (C) is obtained. The water content of the sample can be calculated by the formula:

$$\text{Moisture content (\%)} = \frac{(A + B) - C}{B} \times 100$$

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where :
A: Constant dish weight
B: Weight of sample before drying
C: Weight of sample and dish after drying (constant)

Ash Content Analysis: Gravimetric Method

The porcelain dish to be used is heated in an oven and then weighed until its weight is constant (W_0). Weigh the sample ± 2 grams into a porcelain cup (Ws). Then ignite over the flame of the burner. Put in the furnace for 3 hours at a temperature of 550oC until complete ashing. Cool, then put in a desiccator for 15 minutes. Weigh with an analytical balance. Do the weighing until a constant weight (W_1) is obtained. The ash content of the sample can be calculated by the formula:

$$\text{Ash Content (\%)} = \frac{W1-W0}{Ws} \times 100$$

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Where :
Ws: Weight of sample before ashing
W_0: Weight of empty dish
W_1: Weight of sample and dish after ashing (constant)

Protein Content Analysis of the Dumas Method
Prepare nitrogen-free tin foil. Then weigh the crushed sample as much as ± 200 mg into tin foil. Form the foil until the sample that has been weighed is tightly closed. Insert the sample into the tool that has been previously set. The amount of nitrogen obtained is then converted to produce protein content.

Fat Content Analysis: Weibull Hydrolysis Method

Weigh 1-2 g of sample into a beaker (Ws). Add 30 mL of 25% HCl and 20 mL of distilled water. Cover with a watch glass and simmer for 15 minutes until it cools down. Strain in hot conditions and rinse with hot distilled water until it does not react with acid. Dry the filter paper and its contents at a temperature of 100-105oC in the oven. Put the dry filter paper into the sample bag (thimble) and tie it with the mattress thread. Put the thimble into the soxhlet, then fill it with n-hexane and let it flow into a round bottom flask with known constant weight (W0). Do the circulation for 2-3 hours. Take out the n-hexane. Put the pumpkin in the oven at 105oC for 1 hour. Put in a desiccator for 15 minutes. Weigh with an analytical balance. Do the weighing until a constant weight (W1) is obtained. The fat content of the sample can be calculated by the formula:

$$\text{Lipid Content (\%)} = \frac{W1-W0}{Ws} \times 100$$

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Where:
Ws: Sample weight
W_0: Weight of empty flask
W_1: Weight of flask and sample (constant)

Carbohydrate content: by difference method

Determination of carbohydrate content is done by using the calculation of Carbohydrates by Difference. The calculation is not based on chemical analysis but is based on the following calculations:

Carbohydrates (%) = 100 – (water content + ash content + protein content + fat content)

2.4 Statistical analysis

The data are presented in mean ± standard deviation (sd). The data were tested Analysis of Variance (ANOVA) using Ms. Excel. The data was presented in the form of a table and figure (histograms and graphs) and then analyzed for differences using the Duncan test.

3. Results and Discussion

3.1 Cyanide acid content of fresh-cut bamboo shoots

The cyanide acid content of fresh-cut bamboo shoots is shown in Table 2. Based on Table 2, it is seen that bamboo shoots cyanide content is different for each variety (with a range of 327 - 455 mg/Kg). In this study, cyanide in *D. asper* and *G.apus* bamboo shoots was higher than the results of other studies conducted by [1, 3]. In previous studies, it was reported that cyanide in *D.asper* bamboo shoots was 150 mg/Kg [1] and cyanide in *G.apus* bamboo shoots was 202.5 mg/Kg [3]. The cyanide content in Table 1 is higher than the maximum limit of cyanide acid in foodstuffs set by FAO at 50 mg/Kg. Cyanide levels in *B.vulgaris* bamboo shoots were lower than Putra's research [3], which was 357.7 mg/Kg. These differences can be influenced by innate factors such as genetic and environmental factors such as soil conditions of growth, climate, and human treatment.

Table 2. Cyanide acid in fresh-cut of bamboo shoots

Varieties	Cyanide level (mg/Kg)
Gombong (*Gigantochloa pseudoarundinacea*)	454,52±53,42
Betung (*Dendrocalamus asper*)	360,96±8,96
Kuning *(Bambusa vulgaris)*	327,44±63,50
Buluh (*Gigantochloa apus*)	451,40±14,45

Data were presented mean±standard deviation (SD) (n=3).

3.2 Determination of drying time limits

The moisture content of drying time limits treatment are shown in Table 3. Table 3 shows that the drying time for 26 hours has the lowest water content of 6.54%, and has fulfilled the Indonesian Nasional Standard (SNI 01-7084-2005) regarding simplicia with a standard maximum limit of the water content of 10% [12]. Decreasing water content in the material during the drying process is caused by the process of moving air heat in the dryer and the diffusion of water from the material. This causes a phase change of water from liquid to vapor [13]. Martunis [14] reported that a longer drying time results in lower water content of the material, therefore the 26-hour drying time will be used for the main research.

Table 3. The moisture content of dried bamboo shoots

Drying Time	Moisture content (%)
25 hours	8,65±11,67
26 hours	6,54±13,51

Data were presented mean±standard deviation (SD) (n=3).

3.3 Cyanide reduction in dried bamboo shoot slices

A decrease in cyanide content in the slices of several dry bamboo shoot varieties at various boiling times is presented in Figure 1.

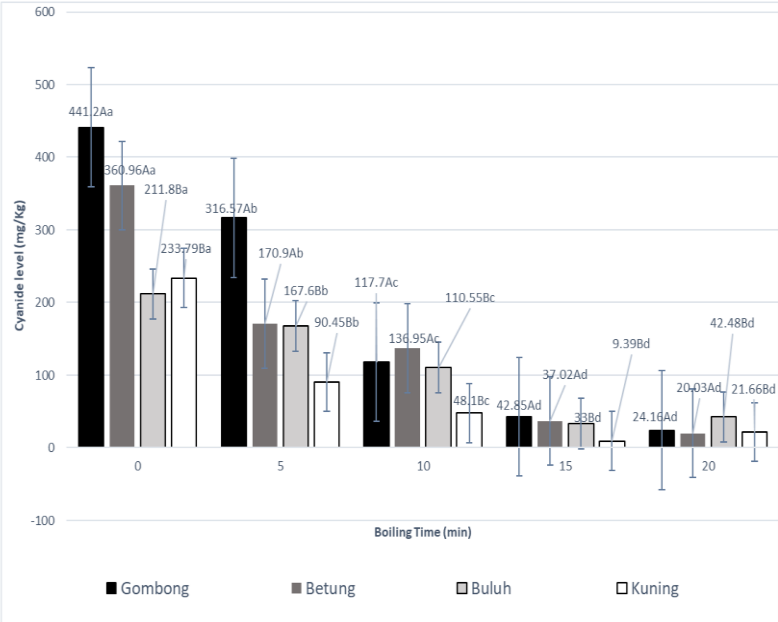

Figure 2. The decreasing cyanide levels in several Indonesian dried bamboo shoots at various boiling times (n=3)

(*)Mean value with a different uppercase letter is significantly different for varieties factor, mean value with different lowercase latter is significantly different for boiling time factor (Duncan P<0.05).

Bamboo shoot varieties and boiling time had a significant effect on reducing cyanide levels in the dry slices of bamboo shoots. Each variety gave a different response to the treatment (boiling in a salt solution), this was shown by the *Betung* variety experiencing a decrease in cyanide more than other varieties, from 426.55mg/Kg to 20.03mg/Kg (cyanide reduction = 95.30%). While the *Gombong* variety from 454.52 mg/Kg to 24.16mg/Kg (cyanide reduction = 94.68%), *Kuning* variety from 327.44mg/Kg to 21.66mg/Kg (cyanide reduction = 93.39%), and *Buluh* variety from 451.4mg/Kg to 42.48mg/Kg (cyanide reduction = 90.59%).

Boiling in a salt solution can reduce cyanide in all types of bamboo shoots, it can be seen from Figure 3. The boiling process in this study was proven to increase the rate of cyanide reduction, as seen in Figure 3. At 0 minutes of boiling the cyanide levels have decreased, according to Arianto et al. [15] that soaking in an 8% salt solution (NaCl) for 5 days can reduce the cyanide of Koro beans from 38.27 mg / Kg to 15.46 mg / Kg or reduce 59.60%. Within 20 minutes of cyanide in slices of dry bamboo shoots, an average of 93.49% was reduced (33.89% more than the results of Arianto et al. [15]). The results of this study are also more effective than other methods that use a combination of soaking in husk ash and boiling [16]. This study took about 30 minutes to reduce about 93.51% cyanide in the *Gadung* tuber slices. The boiling process can reduce the content of cyanide in bamboo shoots, the process has a dual function in decreasing cyanide acid, which is to activate the ß-glucosidase enzyme in the formation of cyanide acid (HCN). The enzyme cannot catalyze cyanogenic glycosides (compounds that have the potential to break down into cyanide acid). In line with [17], the boiling process can evaporate the formed cyanide acid because it has volatile properties at a temperature of 26ºC. The addition of sodium chloride (NaCl) in the solution has affected the nutrients of the material in the boiling process, through the process of osmosis and diffusion. Meanwhile, diffusion is the process of transferring solvent molecules from a solution that has a high concentration to a solution that has a low concentration. Fluctuations in the content of cyanide acid in the boiling process will continue until it has reached the equilibrium between the solute molecules in the solution. This is supported by the statement of Karmana [18] osmosis is the process of transferring solvent molecules from a low concentration solution to a high concentration solution. According to Asmadi [19] that the process of fluid and electrolyte movements must be maintained in a balanced state both diffusion and osmosis. Saskia, et al [18] explains that cyanide acid comes out of the material and is dissolved in a salt solution (NaCl), which then evaporates into the air causes a decrease in cyanide acid. The process of diffusion and osmosis is what causes the release of cyanide compounds from the shoots tissue out and dissolved in water. The boiling process can decompose cyanide compounds and the addition of salt in the water makes the solution more saturated so that more cyanide compounds can be drawn out. This is what causes this method to be more effective than the previously reported method.

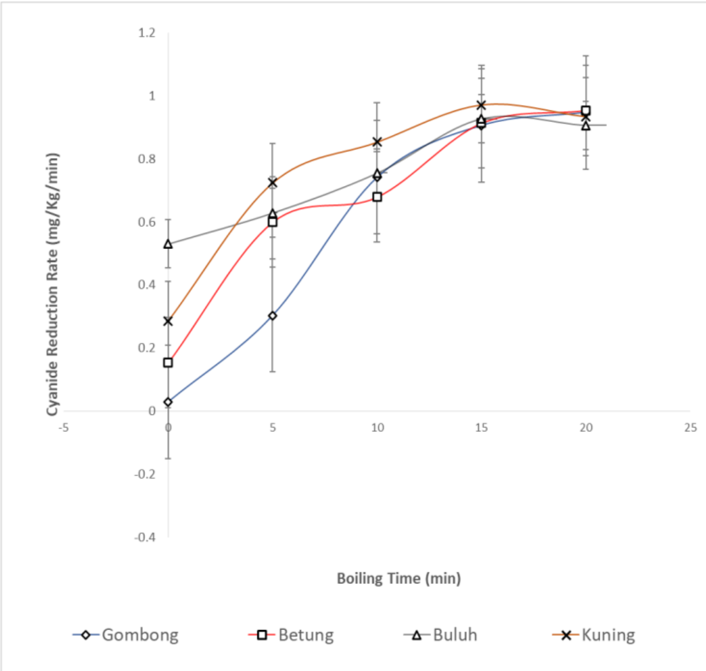

Figure 3. The cyanide reduction rate of selected Indonesian bamboo shoots at a various boiling time

The final product of this study still contained a certain amount of cyanide but the figure was already lower than the maximum limit set by FAO of 50 mg/1000g.

3.4. Macronutrients of dried bamboo shoots

Table 3 has presented macronutrient content from four dried bamboo shoot varieties. The water content obtained in each variety is following the standards, concerning SNI-01-7085-2005 regarding Simplicia, but for ash content in each variety is higher than the standard [12]. Based on Table 3, the highest protein content (24.95%) was produced by *Buluh* bamboo shoots, and the highest carbohydrate content was produced by *Betung* bamboo shoots. Kencana, et al [20] stated that bamboo shoots have higher carbohydrate content, especially dietary fiber (2.56%) than other types of tropical vegetables.

These variations results may be caused by factors such as different species of bamboo shoots, geographical location of growth, age of harvesting, and drying method techniques. According to Shonte et al. [21], variations in nutrients that accumulate in plants are greatly influenced by variations in pre-harvest conditions such as growth conditions, climatic conditions, and differences in genotypes.

The drying of bamboo shoots using a cabinet dryer affects the nutritional content of bamboo shoots flour. The nutritional content of dried bamboo shoots increases compared to fresh-cut bamboo shoots. This is due to the reduced moisture content in the dried bamboo shoot so that it seems other nutrients are increasing. According to Rodrigues et al [22], the selected drying method has an impact on nutrient degradation and retention. The use of high drying temperatures will cause protein denaturation in food products [23].

Table 3. Macronutrients of fresh-cut and dried bamboo shoots

Constituent	Values (%)	
	Fresh	Dried
Gombong (*Gigantochloa pseudoarundinacea*)		
Moisture content	91.61±0.11	7.36±1.89
Ash	1.25±0.08	15.38±0.73
Protein	2.33±0.36	23.66±3.54
Fat	0.48±0.13	6.42±0.31
Carbohydrate	4.33±0.02	47.18±2.12
Betung (*Dendrocalamus asper*)		
Moisture content	93.17±0.08	7.55±0.98
Ash	1.01±0.10	15.97±0.86
Protein	1.68±0.34	19.21±1.64
Fat	0.64±0.01	7.2±0.59
Carbohydrate	3.5±0.07	50.07±1.46
Buluh (*Gigantochloa apus*)		
Moisture content	92.17±0.15	5.78±1.18
Ash	0.95±0.03	16.78±1.20
Protein	1.89±0.30	24.95±1.90
Fat	0.89±0.06	6.89±0.27
Carbohydrate	4.1±0.28	45.6±1.84
Kuning *(Bambusa vulgaris)*		
Moisture content	91.75±0.13	7.22±0.96
Ash	1.08±0.13	16.16±0.97
Protein	3.64*±0.10	22.35±0.18
Fat	0.96±0.04	7.05±0.22
Carbohydrate	2.57±0.09	47.22±0.86

Data were presented mean±standard deviation (SD). *) (Source: [24])

4. Conclusion

Boiling in a 1% salt solution significantly reduced cyanide in several Indonesian bamboo shoot varieties with an average reduction of 93.49%. The addition of salt in the water makes the solution more saturated so that more cyanide compounds can be drawn out due to the osmotic diffusion process. Boiling time and type of bamboo shoots had a significant effect on cyanide reduction in the dry slices of bamboo shoots. The highest cyanide reduction was in Betung bamboo with a decrease reaching 95.30% for 20 min of boiling. The combination of high temperature and the addition of 1% salt was shown to increase the reduction of cyanide compounds in bamboo shoots. Dry bamboo shoots still contain nutrients, carbohydrates 45.60% - 50.07%, protein 19,21% - 24.95%, fat 6.42% - 7.20%, ash 15.38% - 16.78%, and moisture 5.78% - 7.55% . Bamboo shoot drying can be used as an alternative to bamboo shoot preservation and as an intermediate product.

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