The effect of fermentation period of yellow bamboo shoots (B. vulgaris Striata) using L. plantarum starter on physical and chemical properties of its flour as dietary fiber source

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Abstract. Young shoots of bamboo plants (Bambusa vulgaris) are widely used as filler of lumpia, the icon of Semarang City traditional food. It is naturally fermented by producers as a pre-cooking process. This research aimed to analyze the effect of fermentation period using L. plantarum starter on physical and chemical properties of yellow bamboo shoots flour. Experiments were conducted using a completely randomized design (CRD) with two factors (fermentation periods and methods), five treatments (1, 3, 6, 9, and 12 days fermentation period), and four replications. Collected data were analyzed using analysis of variance (p<0.05) followed by Duncan’s multiple range test. The results showed that fermentation periods had a significant effect on total fiber (TDF), insoluble dietary fiber (IDF), soluble dietary fiber (SDF), hydrogen cyanide (HCN) content, and pH (p<0.05). It was suggested that fermentation might improve bamboo flour quality. Twelve days fermentation resulted in shoots flour with TDF of 73.79±0.15%, IDF of 71.45±0.17 %, SDF of 2.34 ± 0.02%, hydrogen cyanide (HCN) of 22.43 ± 2.84 ppm, and moisture content of 2.63 ± 0.26 %.

Keywords: Bamboo shoots; Lumpia; L. plantarum; dietary fiber; HCN

1. Introduction
Bamboo shoot is one of the popular vegetable widely used in Central Java, Indonesia. It has high moisture content (>89%), moderate protein of 2.3-3.9%, 4-5% carbohydrates, 1-1.5% mineral content, while low in fat (< 0.3%) and cholesterols. It also contains several nutritious and active compounds such as vitamins, essential amino acids, and antioxidants [1,2]. Bamboo shoot reportedly contains high total dietary fiber, but mainly as insoluble form [3]. In Central Java, its Ampel Gading variety (Bambusa vulgaris var. Striata) has long been consumed, either as fresh or preserved vegetables. Previous research reported that supplementation unfermented bamboo shoots flour on cookies less than 6% was acceptable [4]. Preserved bamboo shoot is prepared using approximately 8 % brine solution [2, 5, 6]. Bamboo shoots fermentation can be done by two methods of spontaneous and starter addition. The previous is commonly used by local people as traditional methods.
Fermented-sliced bamboo shoots, or bamboo pickle, is widely used as filler of Lumpia, a traditional specialty of Semarang, Indonesia. Rachmadi (2011) reported that fermented bamboo shoot has potential as a dietary food source [3]. Bamboo shoots is fermented in facultative anaerobic condition, either homo-fermentative or hetero-fermentative, using lactic acid bacteria (LAB) strains, such as L. plantarum, L. brevis, and L. pentosaceus [5,7,8]. It was reported that flour prepared from spontaneous fermented bamboo shoots has higher total dietary fiber than fermented-sliced bamboo flour produced by LAB starter, but has lower starch content [3]. However, the research related to characteristics of fermented-sliced shoots flours as a source of dietary fiber is still limited. The objective of this research was to compare the characteristics of bamboo shoots flours produced by two different fermentation methods.

2. Materials and methods

2.1. Materials

Fresh bamboo shoots var. “Ampel Gading” was obtained from local farmers in Mranggen District, Central Java, in December 2018. Lactobacillus plantarum FNCC 0027 culture was obtained from Laboratory of Microbiology, Universitas Gadjah Mada. Analytical weight, stainless steel knife, cabinet dryer, Minolta Chromameter, Tyler 60 mesh sieve, 5 liter plastic jars, and glassware were used.

2.2. Methods

2.2.1. Sample preparation. Approximately 7.5 kilograms of var. “Ampel Gading” bamboo shoot blade and sheath were removed using a stainless steel knife, resulted in approximately 5 kilograms (70%) edible part, which then washed with running water before drained and sliced. Fresh–sliced bamboo shoots were analyzed for proximate content. Sliced bamboo shoots were boiled by submersion in boiling water for 10 minutes before immediately cooled at ambient temperature. The samples were divided into two groups, each of 2.5 kilograms. The first was prepared for 12 days fermentation by L. plantarum, while the other was prepared for spontaneous fermentation (without starter addition).

2.2.2. Proximate analysis. Proximate analyses of moisture content, protein, fat, ash, hydrogen cyanide, and carbohydrate were performed on fresh sliced bamboo shoots cover according to AOAC procedures [9].

2.2.3. Bamboo shoots fermentation. Approximately 10 liters of 5% brine solution (NaCl) was prepared and put into 10 plastic jars followed by addition of 0.5 mL L. plantarum starter on each jar only in first group samples. About 0.5 kg sliced bamboo shoots was put into jars so that all the bamboo submerged perfectly. Jars were closed with screen-cloth, continued by 12 days fermentation at room temperature (30 ±2 °C; 85-90 % relative humidity). Sampling was conducted at 1, 3, 6, 9 and 12 days according to the treatment where the shoots were taken from fermenter jars. The sample's acidity at the end of the fermentation period was also determined.

2.2.4. Drying and grinding. The fermented-sliced bamboo shoots were taken out from fermenter jars, drained by centrifugation, dried by a mechanical dryer (60±5 °C, 16-18 hours) until 13% moisture
content, ground into the powder by mill, and sieved by 60 mesh screen. Crude, insoluble, and soluble dietary fiber content, as well as proximate content, was determined.

2.2.5. Determination of crude, insoluble and soluble dietary fiber. Defatted samples were analyzed for their crude, insoluble, and soluble dietary fiber content using enzymatic and gravimetric method according to the methods by Association of Official Analytical Chemists (AOAC) [9].

2.2.6. Determination of hydrogen cyanide content. Hydrogen cyanide content was determined by alkaline titration method described by AOAC (2005) official methods [9].

2.2.7. Statistical data analysis. Data collected from chemical and physical analyses were subjected to statistical analysis using SAS 9.2 (SAS Institute). Kolmogorov-Smirnov test was first carried out to assess data normality followed by Analysis of variance (ANOVA). Statistical significant effect of variables was analyzed using Fisher’s least significant differences (LSD) to separate means. A comparison of the two group means was performed using T-test. Statistically significantly different was determined at p<0.05.

3. Results and discussion

3.1. Proximate content

Fresh bamboo shoots contained 90.96 % moisture content (g/100 g wb) as shown in table 1, comparable to the results of previous research of 90.70 % [1], 92.6% [10] and 93.35% [11]. Carbohydrate content was 4.27%, similar to those of previous studies i.e. 2.6-5.1% [1,10]. Cyanide level of fresh bamboo shoots was 0.58 %, higher than those previously reported of 0.056% [12] and of 0.007- 0.14% [13]. Its protein content of 2.34% was also similar to previous research i.e. 2.59% [10], 2.06-2.58 % [1] and 1.98-3.29% [7]. Fresh bamboo shoots were rich in dietary fiber (3.90±0.27) and protein (3.72±0.32 %) [4]. The difference of proximate analyses and cyanide level results was probably due to genetic differences or environmental factors, such as plantation location, season, part of the shoots, and harvest period [7].

| Table 1. Proximate composition of fresh bamboo shoots. |
|-------------------------------------------------------|
| Component    | Percent (%)                  |
|--------------|------------------------------|
| Protein      | 2.34 ± 0.09                  |
| Lipid        | 0.86 ± 0.09                  |
| Carbohydrate | 4.27 ± 0.05                  |
| Water        | 90.96 ± 0.48                 |
| Ash          | 1.06 ± 0.03                  |
| HCN          | 0.058 ± 0.001                |
| n=4          |                              |

3.2. The changes in acidity

Acidity is an important indicator that the fermentation process has occurred. Lactic acid bacteria are known to be the main microorganism in bamboo shoots fermentation. Setiadi, (2012) reported that more than 8 strains of lactic acid bacteria (LAB), such as L. plantarum, L. brevis and L. pentosaceus, were isolated from bamboo shoots pickle [5]. Similarly, a total of 327 strains of LAB were isolated from “Mesu”, traditional fermented bamboo shoots from India [7,14].

There were significant differences in acidity among samples within the same column (p<0.05), indicated increasing acidity with a longer fermentation period, both in spontaneous and fermentation by a starter. The lowest acidity was obtained on the first day of 7.02±0.1 and 6.75±0.10, respectively, while the highest was obtained at 12th day of 2.62 ±0.05 and 4.03±0.01, respectively. Wasis et al (2019) reported that LAB isolated from bamboo shoots pickle showed strong acidification properties, lowering the pH to less than 4 [8,14]. Lactic acid bacteria in bamboo pickle could synthesize extracellular enzymes that able to break glycoside bonds in dextran molecule, pectic acid, and soluble starch, producing simple
sugars and organic acids, including lactic acid [7]. Dietary fiber can be partially or completely fermented to short chain fatty acid (SCFA), acetate, and butyrate acid [4]. Hence decreasing pH with higher lactic acid and acetate [7,15].

Table 2. Acidity changes of liquor in bamboo shoots fermentation.

| Fermentation period (day) | Liquor acidity (pH) |
|--------------------------|---------------------|
|                          | fermentation by starter | spontaneous fermentation (pH) |
| 1                        | 6.75 ± 0.10[a]     | 7.02 ± 0.10[b]       |
| 3                        | 3.62 ± 0.05[a]     | 5.24 ± 0.04[b]       |
| 6                        | 3.30 ± 0.14[a]     | 4.22 ± 0.02[b]       |
| 9                        | 2.72 ± 0.12[b]     | 4.07 ± 0.00[b]       |
| 12                       | 2.62 ± 0.05[a]     | 4.03 ± 0.01[b]       |

Note: Different superscript within the same column and different superscript in parenthesis [x] within the same row indicates significant difference (p<0.05, n=4)

3.3. Moisture content

Fermented bamboo shoots flour moisture content obtained from two fermentation methods had no significant difference among treatments at p>0.05 as shown in table 3. However, there was a significant difference among the fermentation period (p<0.05), both in spontaneous fermentation and fermentation by a starter. Fresh bamboo shoots had high moisture content (90.96 ± 0.48%). Fermentation reduces the water content of food through a hydrolytic mechanism [1]. Fermented bamboo shoots drying followed by grinding reduced moisture content by 94-97 %. The flour (60 mesh) had 2.63-4.62 % moisture content, lower than tapioca flour standard by SNI 3451-2011 of 14% [16]. On the other hand, the chairman of the National Standardization Agency of Indonesia mentioned Indonesia has no standard for fermented bamboo shoots flour [17].

Table 3. Moisture content of fermented bamboo shoot flour.

| Fermentation period (days) | Moisture content of bamboo shoots (%) |
|----------------------------|--------------------------------------|
|                            | Fermentation by starter (%) | Spontaneous fermentation (%) |
| 1                          | 4.62 ± 0.22[a]       | 4.62 ± 0.22[a]       |
| 3                          | 3.82 ± 0.20[a]       | 3.92 ± 0.04[a]       |
| 6                          | 3.54 ± 0.16[a]       | 3.59 ± 0.07[a]       |
| 9                          | 3.15 ± 0.25[a]       | 3.27 ± 0.04[a]       |
| 12                         | 2.63 ± 0.26[a]       | 2.82 ± 0.05[a]       |

Note: Different superscript within the same column and different superscript in parenthesis [x] within the same row indicates significant difference (p<0.05, n=4).

On the other hand, an unfermented bamboo powder that was produced in similar methods contain 13.63% moisture [4], higher than fermented bamboo powder. Rachmadi reported that mechanical drying can decrease moisture content of bamboo shoots flour less than 2% [3]. Bamboo flour with low moisture content is easier to handle and weighed with longer shelf life. In the future, processing bamboo shoots into flour is quite prospective.

3.4. Dietary fiber

Results indicated that TDF, IDF and SDF of fermented shoots flour obtained from two fermentation methods varied significantly between treatment (p<0.05). Longer fermentation period resulted in increasing TDF, IDF and SDF content, but the increasing pattern was not similar among different methods (table 4). The flour obtained from fermentation by starter had increasing TDF, IDF, and SDF of 21.3%, 25%, and 47.7%, respectively, while those of spontaneous fermentation had 6%, 7.8%, and 41%, respectively. Higher dietary fiber content of bamboo shoot flour obtained from fermentation using starter compare to spontaneous fermentation indicated the previous as a better dietary fiber source than the latter.
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Table 4. TDF, ISD and SDF content of fermented bamboo shoots flour.

| Period (day) | Dietary fiber content of bamboo shoots flour (%) | Fermentation using starter | Spontaneous fermentation |
|--------------|-----------------------------------------------|---------------------------|-------------------------|
|              | TDF (%) | ISD (%) | SDF (%) | TDF (%) | ISD (%) | SDF (%) |
| 1            | 57.31 ± 0.16[^a][x] | 55.66 ± 0.19[^a][x] | 1.63 ± 0.15[^a][x] | 57.31 ± 0.16[^a][x] | 55.66 ± 0.19[^a][x] | 1.63 ± 0.15[^a][x] |
| 3            | 62.93 ± 0.10[^a][x] | 61.27 ± 0.12[^a][x] | 1.65 ± 0.22[^a][x] | 58.31 ± 0.20[^b][x] | 56.53 ± 0.10[^b][x] | 1.77 ± 0.19[^b][x] |
| 6            | 65.32 ± 0.18[^a][x] | 63.61 ± 0.09[^b][x] | 1.70 ± 0.12[^a][x] | 59.27 ± 0.76[^b][x] | 57.39 ± 0.70[^b][x] | 1.87 ± 0.10[^b][x] |
| 9            | 70.65 ± 0.14[^a][x] | 68.78 ± 0.12[^a][x] | 1.86 ± 0.02[^a][x] | 61.28 ± 0.12[^b][x] | 59.21 ± 0.07[^b][x] | 2.06 ± 0.10[^b][x] |
| 12           | 73.79 ± 0.15[^a][x] | 71.45 ± 0.17[^b][x] | 2.34 ± 0.02[^a][x] | 63.17 ± 0.90[^b][x] | 60.80 ± 0.68[^b][x] | 2.36 ± 0.22[^b][x] |

Note: Different superscript within the same column and different superscript in parenthesis [x] within the same row indicates significant difference (p<0.05, n=4)

Food high in TDF has various health benefits, including promoting weight loss and improve digestive system [1,4]. Thus, bamboo shoots fiber is now a common ingredient in breakfast cereals, fruit juices, bakery and meat products, sauces, shredded cheeses, cookies, pastas, snacks, frozen desserts, and many other food products in Pakistan [4].

3.5. Hydrogen cyanide content

The result of HCN analysis of fermented shoots flour showed a significant difference among treatment (p<0.05). Longer fermentation period resulted in decreasing HCN content, with a 90% decrease of the flour obtained from starter fermentation compare to a significantly lower rate of 78.9% of the other. Fermentation using starter had a better result than spontaneous fermentation on decreasing HCN content. Fresh bamboo shoot with 580 ppm HCN level was decreased into 22.48 ppm and 48.48 ppm of starter fermented and spontaneous fermented flour, respectively. Previous research reported that traditional processing of bamboo shoots fermentation (spontaneous fermentation) effectively reduced cyanide [1,3,5], while boiling and drying also contributed to lowering HCN content [1,4,15]. Certainly, HCN content from the result was above the recommended level (10 mg HCN/kg) for foods but was considered safe with regard to cyanide toxicity based on the fact that the bamboo powder was used as a food additive [18].

Table 5. HCN content of fermented bamboo shoot flour.

| Fermentation period (days) | HCN of bamboo shoots flour by starter (ppm) | HCN of bamboo shoots flour by spontaneous fermentation (ppm) |
|---------------------------|---------------------------------------------|----------------------------------------------------------|
| 1                         | 229.47 ± 14.0[^a][x]                        | 229.47 ± 14.0[^a][x]                                      |
| 3                         | 133.11 ± 15.12[^a][x]                       | 189.10 ± 9.4[^a][x]                                      |
| 6                         | 111.87 ± 6.42[^a][x]                        | 97.58 ± 1.49[^b][x]                                      |
| 9                         | 99.52 ± 5.10[^a][x]                         | 73.67 ± 1.09[^b][x]                                      |
| 12                        | 22.43 ± 2.84[^a][x]                         | 48.48 ± 0.78[^b][x]                                      |

Note: Different superscript within the same column and different superscript in parenthesis [x] within the same row indicates a significant difference (p<0.05, n=4)

3.6. Lightness

The lightness of bamboo shoots powder was expressed as lightness score (L*), determined using Chromameter at range score between 0 (dark) to 100 (transparent). Lower lightness score means darker color and vice versa. The results indicated no significant difference among fermentation methods as well as period (p>0.05). It is clear that the color of bamboo shoots flour was light brown at the value of 4.4-4.8 (table 4). Ambarwati et al (2019) used sodium metabisulfite (Na2S2O5) as a whitener so that the flour produced more light (L*=14)[12]. Satya et al (2014) reported that colour of shoots dried using superheated steam is darker that shoots dried using hot air [1].
Table 6. Lightness of fermented bamboo shoots flour.

| Fermentation periods (day) | Lightness of bamboo shoots flour by starter (%) | Lightness of bamboo shoots flour by spontaneous fermentation (%) |
|---------------------------|-----------------------------------------------|---------------------------------------------------------------|
| 1                         | 4.39 ± 0.32<sup>a</sup>                        | 4.39 ± 0.32<sup>a</sup>                                       |
| 3                         | 4.25 ± 0.27<sup>a</sup>                        | 4.65 ± 0.18<sup>c</sup>                                       |
| 6                         | 4.66 ± 0.27<sup>a</sup>                        | 4.25 ± 0.17<sup>b</sup>                                       |
| 9                         | 4.68 ± 0.26<sup>a</sup>                        | 4.65 ± 0.2<sup>b</sup><sup>c</sup>                           |
| 12                        | 4.73 ± 0.45<sup>a</sup>                        | 4.81 ± 0.03<sup>c</sup>                                       |

Note: Different superscript within the same column and different superscript in parenthesis [x] within the same row indicates significant difference by T-test (p<0.05, n=4)

4. Conclusion
Both spontaneous fermentation and fermentation by starter on bamboo shoots could improve several properties of the flour. The flour produced by starter fermentation had much higher food fiber increase than that of spontaneous fermentation. While hydrogen cyanide was also successful reduced to almost zero limit. However, fermentation subsequent of drying and grinding could not improve shoots flour color.

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