The improvement of white pepper quality through fermentation process by *Acetobacter* sp

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Abstract. White pepper is a potential plantation commodity. The improvement of white pepper quality has been developed by the fermentation method. This research aimed to study the characteristics of white pepper through fermentation by the addition of *Acetobacter* sp. The procedures of the study consisted of several stages, i.e handling of raw material, fermentation, decorticating, washing, drying and quality analysis. The raw material was used in this study from Sukabumi, Indonesia. After threshing, 1.2 kg of pepper was soaked in water mixed with inoculum culture. The experiment used a completely randomized design (CRD) with two replications. The treatment consisted of: A) concentration of *Acetobacter* sp (A1 = 15, A2 = 20, A3 = 25%) and B) soaking duration (B1 = 3, B2 = 5 and B3 = 7 days). The result showed that the best treatment was the fermentation conducted for 5 days with the addition of 25% of *Acetobacter* sp. This condition produced white pepper in fulfilling in requirement of SNI standards with piperine content of 3.82%, essential oil content of 2.4% and TPC of $1.25 \times 10^2$ CFU/g. These cleaner bio-processes could contribute to the production of pepper providing environmentally friendly and sustainable methods.

1. Introduction
Pepper is an important commodity of Indonesia. In 2015, Indonesia produces 81,501 tons of pepper [1] and increased in 2019 to 85,300 tons [2]. Generally, post-harvested pepper proceeds into black and white peppers [3]. The differences between both products are the maturity level of pepper berries and the processing method. White pepper is produced by removing the outer ripe berry skin (retting method), while black pepper processed by drying the unripe berries until the wrinkled skin is formed [4].

Traditionally, white pepper produced by soaking the fully ripened pepper berries in water for about 2-3 weeks [5][6] to decorticcate the pericarp. During the water retting process, several factors such as safety, hygiene and quality (bulk density, moisture content, light berries, and microbial contamination) of white pepper are often neglected. But, it can delay marketable finish product, profit and produces white pepper with an off-odor caused by the formation of 3-methylindol and 4-methylphenol compounds during the soaking process [3]. Therefore, it is important to supply better quality and hygienic white pepper as well as to increase production.

Aziz et al.,[7] suggested that the application of microbial and enzymatic processes expected to generate high quality of white pepper in a short period soaking duration. It is more convenient, rapid, safer and greener than the traditional method. The enzymatic decorticcation process is a promising
application for white pepper production, but the commercial enzyme is expensive which makes enzymes less likely to be used. Another effective yet affordable alternative was to use microbial fermentation. A combination of water and microorganisms produce natural enzymatic activity that helps to perform the decortication process [8]. Hu et al., [6] reported that pepper could be peeled in <60 hours by fermentation with Aspergillus niger. Furthermore, Vinod et al., [9], Feng et al., [10], and Thankamani and Giridhar [5] mentioned that fermentative study applying Bacillus strain can produce high quality of white pepper with minimal microbial contamination. But no studies have examined the white pepper fermentation by the addition of Acetobacter sp. Acetobacter sp is able to produce cellulase [11] and pectinase [7] enzymes that can decorticate the pericarp from the berries in short period. This research aimed to study characteristics of white pepper through fermentation by addition of Acetobacter sp. Application of Acetobacter sp expected to generate high quality of white pepper in a short period soaking duration and minimal microbial contamination.

2. Materials and Methods

2.1. Materials
The raw material used was fresh pepper obtained from Sukamulya Experimental Garden, Sukabumi, West Java, Indonesia. Microorganism used was Acetobacter sp, isolated from soaking water of pepper. The chemical used were nutrient agar “Merck 1.05450.0500” and nutrient broth (NB) “Himedia M002-500G”. The main equipment used were autoclave “Hiramaya hve-50”, laminar “Thermo scientific 1300 series A2”, incubator “Memmert IN 110”, digital balance “OHAUS PA2202C” and others.

2.2. Isolate propagation
The isolate was cultivated on slant nutrient agar and incubated for 48 hours at room temperature. Isolate propagation was carried out by inoculated Acetobacter sp on 9 ml of NB and incubated for 24 hours, in order to obtain 10 ml of isolate suspension. Then, 10 ml of isolate suspension was inoculated in 90 ml of NB and incubated for 24 hours.

2.3. White pepper fermentation
Pepper is harvested at the age of 8-9 months [12] then threshed to separate the stalk and pepper. After threshing, 1.2 kg of pepper was soaked in water mixed with inoculum culture. The treatment consisted of: A) concentration of Acetobacter sp (A1 = 15, A2 = 20, A3 = 25%) and B) soaking duration (B1 = 3, B2 = 5 and B3 = 7 days). Pepper soaked without the addition of inoculum culture used as a control. After soaking, pepper was decorticating, washing, drying (the combination of sun-dried for 2 hours and oven-dried for 10-12 hours at 50-60°C).

2.4. Analysis
White pepper was analysed their quality such as moisture content [13], bulk density [13], light berries [13], blackness berries [13], foreign matter [13], unpeeled pepper, colour [14], piperine content [13], essential oil content [13], and total plate count [15][16]. This research was conducted by Completely Random Design (CRD). All data were subjected to the analysis of variance (ANOVA) using SPSS 21 version. Differences between mean values were estimated using Duncan’s multiple range tests at a confidence level of 95%. All experiments were performed in duplicates.

3. Results and Discussion

3.1. Physical characteristics
White pepper produced from this research had moisture content according to the required standards (Table 1). The moisture content will affect their shelf life. High moisture content in white pepper can accelerate the growth of microbes during storage [17]. Statistical analysis showed that the combination of soaking duration and Acetobacter sp concentrations were not significantly different for moisture
content. This could occur because the white pepper in all treatments was dried by the same method, which sun drying followed by oven drying. It was less than research resulted from Vinod et al. [9] which produce white pepper with a moisture content around 11-12% from fermentation using Bacillus subtilis and Thankamani and Giridhar [5] which had moisture content of 15% from fermentation using combination isolate of B. mycoides, B. licheniformis, dan B. brevis.

### Table 1. Physical characteristics of white pepper fermented by Acetobacter sp.

| Concentration | Moisture content (%) | Bulk density (g/L) | Light berries (%) | Blackness berries (%) | Foreign matter (%) | Unpeeled pepper (%) |
|---------------|----------------------|-------------------|------------------|----------------------|-------------------|-------------------|
| Soaking for 3 days |                      |                   |                  |                      |                   |                   |
| 0             | 11.05±0.49a          | 551.51±5.31a      | 8.52±0.40d       | 0.17±0.08a           | 1.13±0.05a        | 48.87±2.66b       |
| 15            | 10.90±0.57c          | 552.98±4.71c      | 6.46±0.73c       | 0.08±0.00c           | 1.30±0.09c        | 35.58±1.13c       |
| 20            | 10.45±0.49c          | 570.61±2.01bc     | 4.96±0.86bc      | 0.16±0.01a           | 1.23±0.07a        | 19.05±1.54a       |
| 25            | 10.50±0.55c          | 613.45±4.73bc     | 1.19±1.63a       | 0.11±0.08a           | 1.28±0.10a        | 11.13±1.91c       |
| Soaking for 5 days |                      |                   |                  |                      |                   |                   |
| 0             | 10.60±0.71a          | 515.76±1.46a      | 2.44±0.91ab      | 0.33±0.02a           | 1.32±0.07a        | 9.15±0.84a        |
| 15            | 11.15±0.21c          | 592.39±2.43abc    | 3.54±1.92abc     | 0.02±0.00a           | 1.31±0.03a        | 8.57±0.82a        |
| 20            | 10.60±0.14a          | 634.12±4.52a      | 1.14±0.18a       | 0.06±0.00a           | 1.26±0.06a        | 5.76±0.19a        |
| 25            | 10.55±0.35a          | 623.88±0.56bc     | 0.83±0.03a       | 0.07±0.00a           | 1.32±0.06a        | 5.76±0.43a        |
| Soaking for 7 days |                      |                   |                  |                      |                   |                   |
| 0             | 10.75±2.33a          | 570.71±1.73abc    | 3.45±3.26abc     | 0.45±0.05a           | 1.27±0.05a        | 7.59±0.10a        |
| 15            | 11.25±0.78a          | 607.26±2.91abc    | 1.09±0.26a       | 0.17±0.03a           | 1.36±0.09a        | 6.18±0.11a        |
| 20            | 10.60±0.57a          | 601.60±2.58abc    | 1.32±0.24a       | 0.10±0.01a           | 1.21±0.05a        | 4.04±0.19a        |
| 25            | 10.75±0.63a          | 611.54±3.66abc    | 1.01±0.94a       | 0.16±0.02a           | 0.89±0.03a        | 5.85±0.81a        |
| Grade I       | Max 13 Min 600 Maks 1 Max 1 Max 1 - |                   |                  |                      |                   |                   |
| Grade II      | Max 13 Min 600 Maks 2 Max 2 Max 2 - |                   |                  |                      |                   |                   |

Remark: numbers followed by the same letter on the same column are not significantly different based on Duncan's test 5%.

Bulk density is one of the parameters that determine the quality characteristics of white pepper in the market. Light berries content affects the bulk density. White pepper with high bulk density content shows that it is heavier and contains less light pepper [18]. White pepper produced on 3 day soaking duration had not reached Indonesian National Standards (SNI) required because the bulk density was less than 600 g / L. While, white pepper produced on 5 and 7 days soaking duration had bulk density according to SNI criteria (more than 600 g/L). This could be caused by high light berries content on white pepper produced on 3 days soaking duration on various Acetobacter sp concentrations (Table 1). Whereas in the longer soaking duration process, the light berries content is lower than short soaking duration because the light berries become soft during the soaking duration process and destroyed in the decorticating process. Statistical analysis showed that the combination of soaking duration and Acetobacter sp concentrations were significantly different for bulk density and light berries content.

Indonesian National Standards require blackness berries content max 1% for grade I and 2% for grade II. Based on Table 1, white pepper had blackness berries content according to Indonesian National Standards (less than 1%). Blackness berries are influenced by the pepper maturity level [8, 12]. Ripe pepper physiologically will produce white pepper with low blackness berries content. Furthermore, Amala Dhas and Korikanthimath [19] reported that blackness berries are caused by enzymatic browning by fermentation and oxidation of phenolic compounds. Statistical analysis showed that this treatment was not significantly different for blackness content. It was confirmed that blackness berries in this research caused by ripe pepper physiologically level, which is the raw material used in this research unsorted between ripe and unripe (also picked by farmers) fresh pepper. Usually, a stalk of fresh pepper can bear both unripe and ripe fruits at the same time.
Foreign matter of white pepper had reached the Indonesian National Standards require (Table 1). The statistical analysis stated that this treatment was not significantly different for foreign matter content. It was suspected that foreign matter in this product comes from the pepper stalks carried during the fermentation, washing and drying processes.

Aziz et al., [7] reported that fresh pepper berries consist of three pericarp layers, namely exocarp (the outer layer which includes skin or peel), mesocarp (middle layer fleshy), and endocarp (innermost layer). White pepper is produced when the outer pericarp has been decorticated. Specific bacteria can help the decortication by producing enzymes [9]. Cellulase is responsible for the degradation of the exocarp layer, whereas pectinase is responsible for the degradation of the mesocarp layer. Cellulase and pectinase which were produced by Acetobacter sp could help the decortication process.

The combination of soaking duration and Acetobacter sp concentration affected unpeeled pepper content. Generally, the longer the soaking duration and the higher Acetobacter sp concentration, it would cause less unpeeled pepper content (Table 1). The dissolution of pectin and cellulose due to enzymatic reaction will cause initial loosening of the cells structure and softening of the pepper skin. Further dissolution of the middle lamella layer of pectinase enzymes causes separation of the cells that resemble the skin loosening of mature ripe fruits [20]. The longer soaking duration causes longer decortication and had produced more and more white pepper. Furthermore, the more concentrations of Acetobacter sp were added, the more enzymes are produced and for decorticating the pepper skin. The statistical analysis stated that this treatment was significantly different for unpeeled pepper content. In the present study, the cellulose and pectinase enzymes produced by Acetobacter sp might have been responsible for the reduction in the time of the retting period.

3.2. Colour

Table 2 shows the color values of white pepper. The L value represents the degree of brightness with the numbers 0-50 indicating dark colours and the number 51-100 indicating light colour [21]. Colour analysis indicated that pepper treated with a fermentation process showed a dark colour (Table 2). The blackening may be attributed to the oxidation of phenols or the Maillard reaction during the fermentation process [15].

| Table 2. Colour characteristics of white pepper fermented by Acetobacter sp. |
|-----------------|-----|-----|-----|
| Concentration (%) | L   | C   | %hue|
| Soaking for 3 days |     |     |     |
| 0                | 28.11±1.57a | 25.75±1.75b | 78.19±0.62b |
| 15               | 32.76±1.11a | 25.68±1.73b | 78.94±1.75b |
| 20               | 28.56±0.11a | 25.09±0.28b | 78.36±0.81b |
| 25               | 30.09±1.99a | 24.23±1.60b | 79.57±0.36c |
| Soaking for 5 days |     |     |     |
| 0                | 36.48±0.99a | 21.22±1.70ab | 81.15±1.59cde |
| 15               | 34.89±0.58a | 22.61±0.16b | 80.30±0.16cde |
| 20               | 30.93±2.32a | 23.35±0.45b | 79.38±0.27c |
| 25               | 38.46±2.01a | 20.13±0.94ab | 82.72±1.43d |
| Soaking for 7 days |     |     |     |
| 0                | 35.79±1.98a | 20.60±1.83a | 81.44±1.72cde |
| 15               | 25.74±1.49a | 30.87±1.87c | 77.13±0.58a |
| 20               | 30.24±1.31a | 25.96±1.30b | 77.15±1.07a |
| 25               | 29.40±2.00a | 32.55±1.85c | 79.31±0.62c |

Remark: numbers followed by the same letter on the same column are not significantly different based on Duncan's test 5%.

Decay activity in the fermentation process occurs by microorganisms that produce oxygen in the metabolic process. Browning reaction that produces a reddish-brown colour, it occurs because microbes
can convert diphenol, amino-phenol and diaminobenzen compounds to produce melanin (brown pigment) [22]. The statistical analysis stated that this treatment was not significantly different for L value.

C (Chroma purity) is a parameter that shows the intensity of colour sharpness. The higher the C value, the sharper the colour will be. Soaking duration is thought to cause a decrease in colour sharpness in white pepper (Table 2). The longer the fermentation time, the more brown pigment is formed. Hue is a value that describes a range of colours. White pepper fermented by *Acetobacter* sp had Hue value around 77-82.72 °Hue. Based on the chromaticity color range, the Hue value in the range of 54-90° is in the reddish-yellow chromaticity area. The closer to the 90°, the closer to the yellow color the chromaticity will be. The statistical analysis stated that this treatment was significantly different for C and Hue value. Therefore, it is necessary to combine isolates in the fermentation process.

### 3.3. Piperine and essential oil content

White pepper quality is constituted by the essential oil and the pungent principles [23] [24]. Vishvnath and Jain [25] stated that piperine is the main alkaloid content as well as the identity compound in white pepper. Based on Figure 1, piperine and essential oil content from this research had a tendency to increase in line with the addition of *Acetobacter* sp, which secrete hydrolytic enzymes such as, cellulase, amylase, xylanase, protease, and pectinase that act meticulously on the component of the pericarp. The uses of enzymes are specific [7] [26]. They only degrade the pectin and cellulose to softening the pepper skin without degrades the volatile compounds. The statistical analysis stated that this treatment was not significantly different for piperine and essential oil content.

![Figure 1. Piperin content (a) and essential oil content (b) of white pepper.](image)

### 3.4. Total plate count (TPC)

The results analysis showed that the soaking duration and *Acetobacter sp* concentration affected the TPC value. The addition of up to 20% *Acetobacter sp* concentration could reduce the TPC, and would be increased in addition of 25% isolates. This happened during soaking for 3, 5, and 7 days. The fermentation process with the addition of 25% isolate could degrade the cell wall of pepper berries skin optimally, making it more possible for the decorticating process by microbes. During the decorticating process, many substrates will be available for microbial growth [6], so that the TPC value of white pepper becomes high (Figure 2).

When compared with the TPC value without isolates addition, it appeared that the total microbe value would be higher. This means that more microbes did the decorticating process which had implications for the increasing availability of substrate produced by metabolism for the development of microbes [27], so that the microbial population would be higher. The lowest TPC value was found in the treatment of soaking duration for 5 days with the addition of 25% *Acetobacter sp*.
Figure 2. Total plate count of white pepper fermented by *Acetobacter* sp.

4. Conclusions
Application of *Acetobacter* sp on white pepper production is expected to generate high quality of white pepper in a short period soaking duration. The best treatment was fermented for 5 days with the addition of 25% of *Acetobacter* sp. This condition produced white pepper in fulfilling the requirement of SNI standards with piperine content of 3.82%, essential oil content of 2.4% and TPC of $1.25 \times 10^2$ CFU/g. These bio-processes could contribute to the production of pepper providing environmentally friendly and sustainable methods.

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