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Quality Improvement of White Pepper with Processing through Optimizing the Ratio of Starter Culture from *Acetobacter* sp., *Bacillus subtilis*, and *Bacillus cereus*

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ABSTRACT

Fermentation process using known microbial species can be exploited for the processing of white pepper. It is expected to generate quality of white pepper in a short period soaking time. This research aimed to study characteristics of white pepper through a fermentation process by addition of combination isolates of *Acetobacter* sp., *B. subtilis*, and *B. cereus*. After threshing, 2 kg of fresh pepper berries was soaked in water mixed with starter culture. The experiment used a completely randomized design, two replications. The treatment consisted of: A) starter culture ratio of *Acetobacter* sp.: *B. subtillis*: *B. cereus* (A1 = 0:0:0; A2 = 1:1:1; A3 = 2:1:1; A4 = 1:2:1; and A5 = 1:1:2) and soaking time (B1 = 5 and B2 = 7 days). Fermented pepper was decorticated, washed, and dried. The best treatment was fermented for 7 days with the combination isolates of *Acetobacter* sp., *B. subtilis*, and *B. cereus* with ratio 2:1:1. This condition produced white pepper in fulfilling in requirement of SNI standards with piperine and essential oil contents and TPC of 5.95%, 2.95% and 1.1 x 10^2 CFU/g, respectively. This process is expected to generate high quality of white pepper in a short soaking time.

1. Introduction

Pepper (*Piper nigrum* L.) is one of the oldest spices in the world. It has a mild flavour, pungency and light color (van Ruth *et al.* 2019). Due to their characteristics, there is a growing demand for white pepper in the markets worldwide. According to the maturity level of their harvested time and the treatment given to peppercorn, the product of pepper is classified into green, black, and white pepper. Green pepper is processed, since it is harvested in immature and process to the form of pepper in saline solution or dehydrated pepper, which provides a distinctive fruity flavor and a lower pungency than the other varieties (Favre *et al.* 2020).

The main product is very well-known is black and white pepper (Azman *et al.* 2020). 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 (Li *et al.* 2020).

In 2015, Indonesia became the second-largest producer and exporter of pepper in the world after Vietnam. Furthermore, Indonesia was the third highest white pep- per producer supplying 18,000 tons of white pepper after China and Vietnam (Nedspice 2015). Traditionally, white pepper in Indonesia produced by soaking the fully ripened pepper berries in water for about 2-3 weeks to decorticate the pericarp (Darwis *et al.* 2020). Several factors like safety, hygiene and quality (bulk density, moisture content, light berries, and microbial contamination) of white pepper are often neglected during the water retting process. Consequently, it is important to supply better quality and hygienic white pepper.

Another effective yet affordable alternative was to use microbial for fermentation. The microbial can use to degrade cellulose, pectin, and xylan of agricultural product (Ferbiyanto *et al.* 2015; Helianti *et al.* 2016; Ye *et al.* 2019; de Farias *et al.* 2020). Fermentation process using microbial species can be exploited for the processing of white pepper. Microbes are known to secrete several hydrolytic enzymes, which are capable of degrading of pericarp and produce high

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quality of white pepper with an acceptable in the international white pepper trade (Vinod *et al.* 2014; Sreekala *et al.* 2019).

A natural enzymatic activity that contributes to be decortication process, it will be produced by a combination of water and microorganism. Some literature reported that the processing of white pepper by fermentation can produce high quality of white pepper with minimal microbial contamination if using *Aspergillus niger* (Hu *et al.* 2017), *Bacillus cereus* (Feng 2013), *Bacillus subtilis* (Vinod *et al.* 2014; Sasmitaloka and Hernani 2020), *Bacillus licheniformis* (Vinod *et al.* 2014), *Acinetobacter baumanii* (Vinod *et al.* 2014), *Klebsiella pneumoniae* (Vinod *et al.* 2014), *Microbacterium barkeri* (Vinod *et al.* 2014), and *Bacillus pumilus* (Sreekala *et al.* 2019). However, some a study have examined the processing white pepper by fermentation using combination of isolate (Sato *et al.* 2020). White pepper was produced from black pepper by the fermentation method using the combination isolates of *B. mycoides, B. licheniformis,* and *B. brevis* had high piperine and volatile oil content, but it still contains high moisture content and microbial contamination. Therefore, it is necessary to find the best isolate combination to produce high quality of white pepper.

Acetobacter sp. is able to produce cellulase (Ye *et al.* 2019) and pectinase (Aziz *et al.* 2019) enzymes that can decorticate the pericarp in short period. According to Vinod *et al.* (2014), *Bacillus subtilis* can produce pectinase, cellulose, xylanase, amylase, and protease. While *Bacillus cereus* is capable of synthesizing extracellular amylases, proteases, cellulases, and pectinase when tested with corresponding substrates like starch, casein, cellulose and pectin in agar medium (Feng 2013). Aplication of *Acetobacter* sp., *Bacillus subtilis*, and *Bacillus cereus* is expected to generate quality of white pepper in a short period soaking time and minimal microbial contamination. This research aimed to study characteristics of white pepper through a fermentation process by addition of combination isolates of *Acetobacter* sp., *Bacillus subtilis*, and *Bacillus cereus.*

2. Materials and Methods

2.1. Materials

The raw material used was fresh pepper berries (Natar 1 variety) obtained from Sukamulya Experimental Garden, Sukabumi, West Java. The microorganisms used were *Acetobacter* sp., *Bacillus subtilis*, and *Bacillus cereus*, which was a collection of Indonesian Center for Agricultural Postharvest Research and Development, Bogor, Indonesia. The chemical used were nutrient agar "Merck 1.05450.0500" and nutrient broth "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. Methods

2.2.1. Raw Material Preparation

Fresh pepper was harvested at the optimum maturity stage (age of 8-9 months) in the garden based on a study of Megat *et al.* (2019). It was placed in a sack and delivered to the laboratory by car. After arriving at the laboratory, it was threshed to separate the pepper from the stalk.

2.2.2. Isolate Propagation

The isolate used in this research were *Acetobacter* sp., *B. subtillis, B. cereus*. It was cultivated on slant nutrient agar and incubated for 48 hours at room temperature. Isolate propagation were carried out by inoculated each isolates on 9 ml of nutrient broth and incubated for 24 hours, in order to obtain 10 ml of isolate suspension. Then, 10 ml of isolate suspension was inoculated with 90 ml of NB and incubated for 24 hours.

2.2.3. White Pepper Fermentation

As much as 1.2 kg of fresh pepper berries without sorted was soaked in mixture of water and inoculum culture and fermented based on the treatment. The treatment consisted of two factors, namely starter culture ratio of *Acetobacter* sp.: *B. subtillis*: *B. cereus* (A1 = 0:0:0; A2 = 1:1:1; A3 = 2:1:1; A4 = 1:2:1; and A5 = 1:1:2) and soaking time (B1 = 5 and B2 = 7 days). After soaked, fermented pepper was decorticated, washed, and dried by oven-dried at 50°C.

2.2.4. Analysis

White pepper analysed their characteristcs, i.e moisture content, bulk density, blackness berries, foreign matter, unpeeled pepper, colour, piperine content, essential oil content, and total plate count. This research was conducted by Completely Random Design (CRD), two replications. All data were subjected to the analysis of variance (ANOVA) using

SAS version. Differences between mean values were estimated using Duncan's multiple range tests at a confidence level of 95%.

3. Results

3.1. Physical Characteristics

The combination of soaking time and ratio of three isolates affected unpeeled pepper content. Unpeeled pepper content of white pepper without addition three isolates around 22.09 to 38.10%, while with addition three isolates around 0.34 to 32.65%. Long soaking time would produce low unpeeled pepper content. The statistical analysis stated soaking time, comparison of three isolates, and combination of this treatment were significant for their unpeeled content ($p<0.05$).

Indonesian National Standards (SNI) requires some quality characteristics of white pepper such as moisture content, bulk density, light berries, blackness berries, and foreign matter. The physical characteristics of white pepper showed in Table 1. Overall, the moisture content of white pepper fermented by combination isolates in 5-day and 7-day soaking time tended to be constant. Although white pepper soaked for 7 day had moisture content lower than white pepper soaked for 5 day. Statistical analysis showed that the ratio of three isolates, soaking time, and the combination of both were not significant for their moisture content ($p>0.05$). White pepper produced from this research had a moisture content according to the required standards of grade I (moisture content max 13%) (SNI 2013).

Soaking duration affects the bulk density of white pepper. The longer of the soaking duration tends to produce a higher bulk density (Table 1). Bulk density of white pepper soaked for 7 day higher than white pepper soaked for 5 day in each combination. Addition of starter culture combination though to increase their bulk density. Statistical analysis stated that the ratio of three isolates, soaking time, and the combination of both were significant for their bulk density ($p<0.05$). White pepper produced with addition of three isolates had bulk density according to the required standards of grade I (SNI 2013), while without addition of isolate had not required standards.

The light berries are white peppercorns that are lighter than normal weight. A ratio of three isolates had no significance for their light berries content ($p>0.05$), while soaking time and combination soaking time and starter culture ratio were significant for their light berries ($p<0.05$). White pepper produced by soaked for 5 days has light berries content more than 2 %. It is not in accordance with the requirement by Indonesian National Standards (Max 2) (SNI 2013). White pepper fermented for 7 day produces light berries content according to the required standards, which addition of isolate can decrease the light berries content and including the quality grade I (SNI 2013).

Indonesian National Standards require blackness berries content max 1% for grade I and 2% for grade II (SNI 2013). This treatment produce blackness white pepper around 0.18 to 0.59%, in accordance with quality grade I. Statistical analysis stated that the ratio of three isolate, soaking time, and the combination of both were significant for their bulk density ($p<0.05$).

Foreign matter of white pepper had reached the Indonesian National Standards require. The addition of isolates and fermentation time tended to decrease foreign matter content. The statistical analysis stated

Table 1. Physical characteristics of white pepper fermented by combination isolates of *Acetobacter* sp.: *B. subtillis*: *B. cereus*

Comparison of *Acetobacter* sp: *B. subtillis: B. cereus*	Moisture (%)	Bulk density (g/l)	Light berries (%)	Blackness berries (%)	Foreign matter (%)	Unpeeled pepper (%)
Soaking for 5 days						
0:0:0	11.60$^{a(A)}$	506.26$^{b(B)}$	6.53$^{a(A)}$	0.36$^{b(A)}$	1.12$^{a(A)}$	38.10$^{a(A)}$
1:1:1	10.60$^{a(A)}$	630.85$^{a(A)}$	4.32$^{ab(A)}$	0.19$^{b(A)}$	0.46$^{a(A)}$	19.66$^{a(A)}$
2:1:1	11.00$^{a(A)}$	608.32$^{a(A)}$	2.94$^{b(A)}$	0.18$^{b(A)}$	0.75$^{a(A)}$	32.65$^{a(A)}$
1:2:1	10.40$^{a(A)}$	613.68$^{a(A)}$	3.89$^{ab(A)}$	0.30$^{b(A)}$	1.59$^{a(A)}$	22.80$^{a(A)}$
1:1:2	10.75$^{a(A)}$	621.42$^{a(B)}$	4.94$^{ab(A)}$	0.59$^{ab(A)}$	0.61$^{a(A)}$	26.17$^{a(A)}$
Soaking for 7 days						
0:0:0	10.70$^{a(A)}$	10.70$^{a(A)}$	1.45$^{a(A)}$	0.46$^{a(A)}$	1.23$^{a(A)}$	22.09$^{a(B)}$
1:1:1	9.65$^{a(A)}$	9.65$^{a(A)}$	0.13$^{b(A)}$	0.41$^{a(A)}$	0.60$^{ab(A)}$	0.34$^{b(A)}$
2:1:1	9.90$^{a(A)}$	9.90$^{a(A)}$	0.54$^{ab(A)}$	0.23$^{a(A)}$	0.22$^{b(A)}$	0.82$^{b(A)}$
1:2:1	10.15$^{a(A)}$	10.15$^{a(A)}$	0.71$^{ab(A)}$	0.36$^{a(A)}$	0.22$^{b(A)}$	2.91$^{b(A)}$
1:1:2	9.80$^{a(A)}$	9.80$^{a(A)}$	0.57$^{ab(B)}$	0.20$^{a(A)}$	0.25$^{b(A)}$	1.10$^{b(A)}$

the numbers followed by the same capital letter in the same column and the same lowercase letter on the same row are not significantly different based on Duncan continued test at the 5% level

soaking time and ratio of three isolates were not significant for their foreign matter content ($p>0.05$), while combination of this treatment was significant ($p<0.05$).

3.2. Colour

The colour of white pepper tended to be a constant. The highest level of L value was resulted from 7 day soaking treatment and ratio of *Acetobacter* sp.: *B. subtillis*: *B. cereus* inoculums of 1:1:1 (42.57) (Table 2). Soaking time, ratio of three isolates, and combination of this treatment were significant for their lightness value ($p<0.05$).

Addition of three isolates increase °Hue value. Whereas long soaking time would produce high °Hue value. Statistical analysis showed that this treatment and combination of both were not significant for their °Hue (Table 3).

3.3. Piperine and Essential Content

This treatment affected their piperine content (Table 4) and essential oil content (Table 5). Soaking time and addition of *Acetobacter* sp., *B. subtillis, B. cereus* tended to increase their piperine and essential oil content. White pepper without addition of three isolate (ratio of 0:0:0) had piperine content of 3.11 to 3.34% and essential oil content of 2.58 to 2.61%. While white pepper with addition of three isolate (ratio of 1:1:1, 2:1:1, 1:2:1, and 1:1:2) had piperine content of 3.82 to 5.95% and essential oil content of 2.40 to 3.20%.

Statistical analysis showed that soaking time was significant for their essential oil content ($p<0.05$) but not significant for piperine content ($p>0.05$). Starter culture ratio of three isolate and combination of both were significant for piperine and essential oil content ($p<0.05$).

Table 2. Lightness (L) of white pepper fermented by combination isolates of *Acetobacter* sp.: *B. subtillis*: *B. cereus*

Soaking time (day)	Starter culture ratio of *Acetobacter* sp.: *B. subtillis*: *B. cereus*					Averages
	0:0:0	1:1:1	2:1:1	1:2:1	1:1:2	
5	$36.51^{a(A)}$	$36.50^{a(B)}$	$34.80^{a(A)}$	$34.37^{a(A)}$	$34.81^{a(A)}$	35.40^{B}
7	$34.80^{b(A)}$	$42.57^{a(A)}$	$38.80^{ab(A)}$	$43.96^{a(A)}$	$37.01^{ab(A)}$	39.43^{A}
Averages	35.65^{b}	39.53^{a}	36.81^{ab}	39.16^{ab}	35.91^{ab}	

The numbers followed by the same capital letter in the same column and the same lowercase letter on the same row are not significantly different based on Duncan continued test at the 5% level

Table 3. °Hue of white pepper fermented by combination isolates of *Acetobacter* sp.: *B. subtillis*: *B. cereus*

Soaking time (day)	Starter culture ratio of *Acetobacter* sp.: *B. subtillis*: *B. cereus*					Averages
	0:0:0	1:1:1	2:1:1	1:2:1	1:1:2	
5	$79.26^{ab(A)}$	$79.73^{ab(A)}$	$81.81^{a(A)}$	$78.40^{b(A)}$	$80.33^{ab(A)}$	81.25^{A}
7	$80.23^{a(A)}$	$82.05^{a(A)}$	$81.19^{a(A)}$	$82.07^{a(A)}$	$80.71^{a(A)}$	79.90^{A}
Averages	79.75^{a}	80.89^{a}	81.50^{a}	80.24^{a}	80.52^{a}	

The numbers followed by the same capital letter in the same column and the same lowercase letter on the same row are not significantly different based on Duncan continued test at the 5% level

Table 4. Piperine content of white pepper fermented by combination isolates of *Acetobacter* sp.: *B. subtillis*: *B. cereus*

Soaking time (day)	Starter culture ratio of *Acetobacter* sp.: *B. subtillis*: *B. cereus*					Averages
	0:0:0	1:1:1	2:1:1	1:2:1	1:1:2	
5	$3.34^{c(A)}$	$4.75^{a(A)}$	$4.86^{a(B)}$	$4.35^{b(A)}$	$4.69^{a(A)}$	4.40^{A}
7	$3.11^{d(A)}$	$3.82^{dc(A)}$	$5.95^{a(A)}$	$5.13^{ab(A)}$	$4.48^{bc(B)}$	4.50^{A}
Averages	3.23^{d}	4.28^{c}	5.40^{a}	4.74^{b}	4.58^{bc}	

The numbers followed by the same capital letter in the same column and the same lowercase letter on the same row are not significantly different based on Duncan continued test at the 5% level

Table 5. Essential oil content of white pepper fermented by combination isolates of *Acetobacter* sp.: *B. subtillis*: *B. cereus*

Soaking time (day)	Starter culture ratio of *Acetobacter* sp.: *B. subtillis*: *B. cereus*					Averages
	0:0:0	1:1:1	2:1:1	1:2:1	1:1:2	
5	$2.58^{d(A)}$	$3.20^{a(A)}$	$2.59^{d(A)}$	$3.05^{b(A)}$	$2.79^{c(A)}$	2.84^{A}
7	$2.61^{b(A)}$	$2.42^{c(B)}$	$2.95^{a(A)}$	$2.60^{b(A)}$	$2.40^{c(B)}$	2.59^{B}
Averages	2.59^{b}	2.81^{a}	2.77^{a}	2.82^{a}	2.59^{b}	

The numbers followed by the same capital letter in the same column and the same lowercase letter on the same row are not significantly different based on Duncan continued test at the 5% level

3.4. Total Plate Count (TPC)

Total plate count was increase during soaking time. The longer of soaking time, the higher of total plate count. The fermentation process by addition of three isolates could reduce the total plate count of white pepper. White pepper produced by fermentation process day without addition isolate (0:0:0) for 7 day had total plate count of 6.58 log CFU/g, while for 5 day fermentation had total plate count of 2.53 log CFU/g. The lowest total plate count was 2.00 log CFU/g which produce on the treatment of 5 day soaking time with starter culture ratio of 2:1:1 (*Acetobacter* sp.: *B. subtillis*: *B. cereus*). Statistical analysis showed that only soaking time was significant for total plate count ($p<0.05$).

3.5. Flavor

The best treatment was fermented for 7 days with the combination isolates of *Acetobacter* sp., *B. subtilis*, and *B. cereus* with a ratio 2:1:1. From the best treatment was identified for the aroma such as, caryophyllene, 3-carene, D-limonen, sabinene, delta-eIemene, α and β-phellandrene, 4-chlorobuten-3-yne, (E)-.beta.-famesene, β-Phellandrene, Isospathulenol, o-Cymene, Trans-β-ocimene, copaene, L-α-terpineol and caryophyllene oxide (Figure 3). Therefore, it was also identified for an unpleasant odor less than 0.3 %, such as p-cresol, hexanoic acid, butanoic acid, piperonal, 1H-Indole, 2-methyl, linalool.

4. Discussion

4.1. Physical Characteristics

The pericarp of a fresh pepper berries consists of three sections which are the outer layer which includes skin or peel (exocarp), middle layer fleshy (mesocarp) and innermost layer (endocarp) (Rosnah and Chan 2014; Aziz *et al.* 2019). In white pepper production, the outer pericarp will be decorticated to obtain the pepper seeds to produce white pepper. According to Vinod *et al.* (2014) and Wang *et al.* (2019), specific microbes can help the decortication process by producing enzymes. Enzymes that work on the decortication process are pectinase and cellulose. Cellulase is responsible for the degradation of the exocarp layer, while pectinase is responsible for the degradation of the mesocarp layer (Aziz *et al.* 2019).

The pectin present in the middle layer fleshy (mesocarp) area of pepper skin begin to degrade enzymatically and break apart from the core (Geron *et al.* 2019). The dissolution of pectin due to enzymatic reaction will cause initial loosening of the structure of the cells and softening of the pepper skin. Further dissolve of the middle lamella layer by pectinase enzymes causes separation of the cells that resembles the skin loosening of mature ripe fruits (Aziz *et al.* 2019).

At the same time, the cellulosic cell wall starts to degrade during retting by cellulose enzymes. The pepper berries skin cells are exposed to disrupt and weaken the bonds structure by enzymatic activity. Prolonged soaking time will eventually increase the water uptake by osmosis through a semi-permeable membrane into the cell causing the cell to be stretched and swelled to its limit of elasticity (Hu *et al.* 2017). The longer the soaking time, the deeper of enzymatic reaction acts to degrade the pericarp cells. When the extracellular matrix (i.e. cellulose, pectins, hemicellulose) is weakened and removed by the enzyme, the protoplast of the plant cell will burst due to soak in culture solution of low osmotic potential (Aziz *et al.* 2019), which will lead to major damage to the structure of the plant cell wall and cause the berries skin to totally loosen from its core. It can cause the pepper berries skin to become softer and enable the peeling of pepper skin by abrasion. Pectinase and cellulose enzymes produced by *Acetobacter* sp., *Bacillus subtilis*, and *Bacillus cereus* could use in the decorticating process.

Generally, the length of the soaking duration would produce less unpeeled pepper content. It causes longer decortication and had produced more and more white pepper. In the present study, the cellulose and pectinase enzymes produced by combination isolates might have been responsible for the reduction in the time of the retting period.

Moisture content is an important factor affecting its tenderness, taste, freshness, and flavor (Song *et al.* 2019). It will affect their shelf life. High moisture content in white pepper can accelerate the growth of microbes during storage (Hidayat *et al.* 2009) and shortens its shelf life (Kusmiadi *et al.* 2017). The high and low moisture content of the white pepper is affected during the drying process. In this study, white pepper in all treatments was dried by the same method. This causes the moisture content of white pepper in all treatments was not significantly different It is less than research resulted from Vinod *et al.* (2014) which produce white pepper with a moisture content around 11-12% from fermentation using *Bacillus subtilis*, Thankamani and Giridhar (2004) which had moisture content of 15% from fermentation using combination isolate of *B. mycoides*, *B. licheniformis*, and *B. brevis*, and Ashari *et al.* (2014) with a moisture content of 15% by fermentation using commercial enzyme.

Bulk density is one of the parameters that determine the quality characteristics of white pepper in the market. According to Hidayat *et al.* (2009), light berries can affect the bulk density. White pepper with low bulk density contains high light berries. It is make white pepper become lighter than white pepper with high bulk density.

Soaking time and ratio of isolate addition tends to affects the bulk density and light berries content. The length of the soaking time is expected to produce white pepper with high bulk density and low light berries content (Table 1). White pepper produces on 5 day soaking time had bulk density lower than 7 day soaking time. This could be caused by high light berries content on white pepper produced on 5 day soaking time on various starter culture ratios. The longer soaking time process, the light berries content become soften during the retting process and destroyed in the decorticating process. This result also shows that the addition of combination isolate in various comparisons can accelerate the decorticating process. It can speed up the softening process of the light berries and destroy during decorticating and washing process. Eventually, the light berries content in white pepper becomes low and bulk density become high. The bulk density of white pepper in this study was higher than Vinod *et al.* (2014), which produce white pepper by fermentation process using *Bacillus subtilis* with bulk density around 557 to 570 g/l. This shows that the use of isolate combinations with various ratio can improve the quality of the white pepper produced.

The maturity level of fresh pepper as raw material can affect the formation of blackness berries. Megat *et al.* (2019) and Kusmiadi *et al.* (2017) reported that mature pepper physiologically will produce white pepper with low blackness berries content. It was confirmed that one of the causes of the formation of blackness berries in this study is the maturity level of fresh pepper berries, which was the raw material used in this research unsorted between mature and immature (also picked by farmers) fresh pepper. Generally, a stalk of fresh pepper can bear both immature and mature fruits at the same time. Besides that, blackness berries also caused by enzymatic browning by fermentation and oxidation of phenolic compounds (Rathnawathie and Buckle 1984; Dhas and Korikanthimath 2003; Kusmiadi *et al.* 2017). White pepper produced by fermentation for 5-day soaking time had blackness pepper higher than 7-day soaking time. According to Usmiati and Nurdjannah (2006), too short soaking time can cause the outer pepper skin become not soft enough, so that a browning process occurs during the peeling and produces white pepper with a brownish colour.

Foreign matter is other things that are not white pepper seeds, such as stalks, skin, rocks, sand or soil. It was suspected that foreign matter in this study comes from the pepper stalks carried during the fermentation, washing and drying processes. Nevertheless, the foreign matter of white pepper had reached the Indonesian National Standards require which is max 1 for grade I and max 2 for grade II (SNI 2013).

4.2. Colour

Colour is an early indicator to assess the quality of white pepper. The desired colour of white pepper is yellowish white or cream, which means it has a high lightness value. According to Megat *et al.* (2019), maturity of fresh pepper berries affect to the colour of white pepper produced. Furthermore, colour of products can occurs by many reactions during food processing, such as pigmen degradation, browning reactions, changes in the distribution and structure of the bioactive compounds, and others (Bayram *et al.* 2004). Generally, white pepper production by retting process of fresh pepper berries is the formation of blackness berries colour.

The color of white pepper product by fermentation process was giving little brownish, according to the L value, °Hue, and visual appearance. Baldevbhai and Anand (2012) and Hunter Laboratories (2012) stated that the L numbers of 0-50 indicating dark colour and 51-100 indicating light colour. Based on Figure 1, white pepper treated by fermentation process showed a dark colour. This is confirmed by the results of the L value 34.37 to 42.57 (dark colour). The blackening may be attributed to the oxidation of phenols or the Maillard reaction during the fermentation process (Waje *et al.* 2008). Furthermore, Mustafa *et al.* (2015) state that decorticating activity in the fermentation process occurs by microorganisms would produce oxygen in the metabolic process. It can convert diphenol, amino-phenol and diaminobenzen compounds to produce melanin (brown pigment) and occurs browning reaction that produces a reddish-brown colour.

White pepper fermented by combination isolate had Hue value around 79.26-82.07°Hue. The Hue value in the range of 54-90° is in the reddish-yellow chromaticity area (Hunter Laboratories 2012). The closer to the 90°, the closer to the yellow color the chromaticity will be. Soaking time could increase Hue value. Long soaking time could soften the pepper skin and inhibit the browning reaction. The blackness berries color is the pepper that unpeeled. It has difficult to decorticate the pepper skin because the pepper's skin is not soft. The highest Hue value is found in treatment 7-day soaking time and combination of

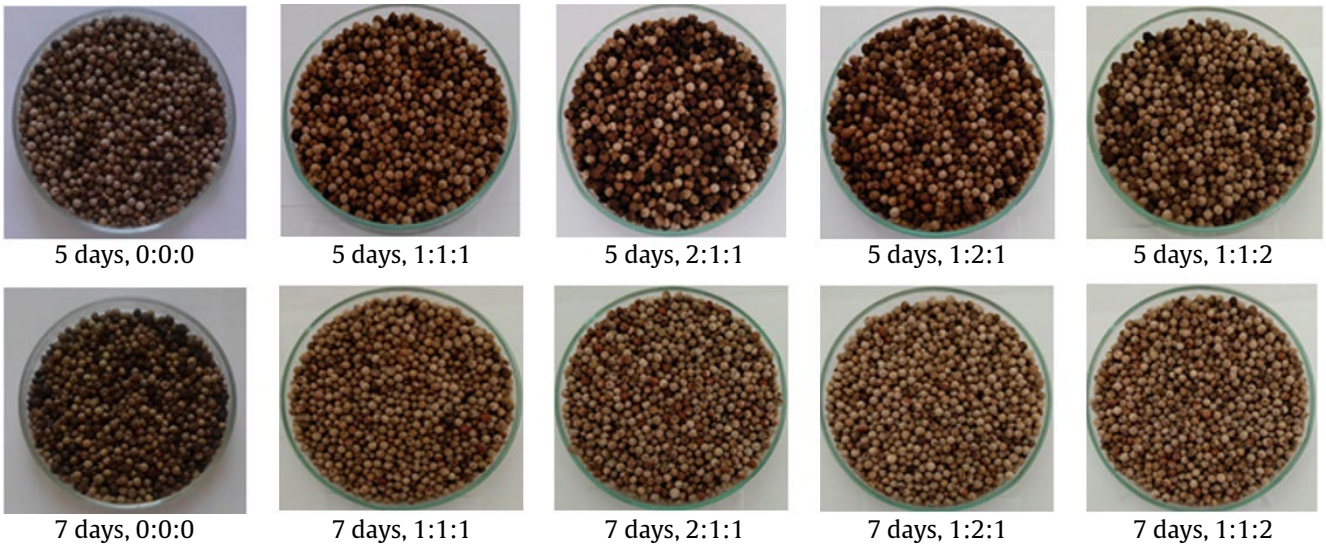

Figure 1. *White pepper* produced from various fermentation times and combinations of isolates (*Acetobacter* sp.: *B. subtilis*: *B. cereus*)

Acetobacter sp., *Bacillus subtilis*, and *Bacillus cereus* with ratio 1:2:1. Almost the same as research result of Rosnah and Chan (2014), which produce white pepper by enzymatic retting for 15 day soaking time with Hue value of 83.77. White pepper produced by 7-day soaking time with combination isolate addition have a colour of creamy white (Figure 1), while white pepper produced by 5-day soaking duration with combination isolate addition have a dark colour (Figure 1).

4.3. Piperine and Essential Oil Content

The quality of white pepper depends on the content of piperine for the pungent test (Mustafa *et al.* 2015) and essential oil for aroma (Mamatha *et al.* 2008) (Sreekala *et al.* 2019). Piperine, as the most abundant alkaloid in pepper, has beneficial health and therapeutic effects. Fermentation process of white pepper tends to increase the piperine content. *Acetobacter* sp., *B. subtilis*, and *B. cereus* can secrete hydrolytic enzymes specific to degrade the pectin and cellulose of pepper skin (Mohammadkazemi *et al.* 2015; Aziz *et al.* 2019). It would soften the pepper skin without degrades the alkaloid compounds. White pepper produced by suitable soaking time and combination of isolates can produce high levels of piperine. The higher piperine content in white pepper was found in the treatment of 7-day soaking duration and combination of isolates *Acetobacter* sp., *B. subtilis*, and *B. cereus* with ratio of 2:1:1 (5.95%). The piperine content produced in this study was higher than other study. Vinod *et al.* (2014) produces white pepper from fermentation process using Bacillus subtilis WP38 isolate with a piperine content of 3.4%. While Zhihao *et al.* (2011) produces white pepper fermented using *Bacillus cereus* with a piperine content of 4,25%. Furthermore, Sasmitaloka and Hernani (2020) produces white pepper with piperine content of 1.45% by fermentation process of *Bacillus subtilis*. This suggests that the use of a combination of isolates is proven to be effective in producing white pepper with high piperine content.

The essential oil of white pepper is a mixture of many volatile chemical compounds in while pepper. The component consists of α-pinene, β-pinene, limonene, myrcene, linalool, α-phellandrene, sabinene, β-caryophyllene and germacrene-D which contribute to the aromatic and flavour properties of pepper (Singh *et al.* 2013). As well as piperine content, fermentation process also tends to increase the essential oil content. Combination of isolate suspected to produces high essential oil content. The specific characteristics of the enzymes secreted by each isolate are thought to cause the degradation process only occurs on pectin and cellulose without essential oil. The higher essential oil content was found in the treatment of 5-day soaking time and combination of isolates *Acetobacter* sp., *B. subtilis*, and *B. cereus* with ratio of 1:1:1 (3.20%). The length of the soaking time, the lower of the essential oil content will be. The results of this study were not much different from the results of Feng (2013), which produced white pepper by fermentation process using *Bacillus cereus* with essential oil content of 3.26%. While, Sreekala *et al.* (2019) produced white pepper fermented by *Bacillus pumilus* with essential oil content of 2.95%.

4.4. Total Plate Count (TPC)

White pepper has to be free from pathogenic and non-pathogenic contamination i.e., bacteria, mold and

yeast. They can cause deterioration in pepper quality. In addition, contaminated pepper can also serve as disease transmission vehicles and cause death if contaminated with harmful microorganisms, microbial toxins or environmental contaminants (Darwis *et al.* 2020). According to Kaur and Gautam (2019), food is considered to be spoiled when the appearance, texture, flavor and odor are changed because microbes could have entered the food.

The length of soaking time showed increasing the number of TPC. The decorticating process can degrade the cell wall of pepper skin and many substrates will be available for microbial growth (Hu *et al.* 2017), so that the TPC value of white pepper produced in the 7-day soaking time becomes high. This means that more microbes carry out the decay process, which has implications for the increasing availability of metabolic substrates for microbial development, making the microbial population will be highest. However, it will be decreased by the addition of a combination isolate. The most remarkable feature of white pepper produced by the fermentation process was its extremely low microbial contamination (Figure 2). They can inhibit another microbial growth during soaking. Furthermore, decreased microbial count in the final product would enhance the shelf life significantly (Thankamani and Giridhar 2004).

4.5. Flavor

The odor of white pepper gave an unpleasant aroma note, which is often described as cow shed-like or fecal-like. The chromatogram analysis of white pepper from the best treatment showed Figure 3. It has been reported by Liu *et al.* (2013) that compound gave the odor like that is 3-methylindole, p-cresol, a-pinene, linalool, b-damascenone, eugenol, and skatole. Aroma is one of the essential components of spices quality, include in pepper, because it will attract consumers. The volatile aroma compounds contain in pepper species more than 270 compounds (Steinhaus and Schieberle 2005; do Carmo *et al.* 2012). The important compounds which gave the flavor to the pepper is limonene, α-pinene, D-3-carene, β-pinene, 4-carene, terpinolene, α-copaene,

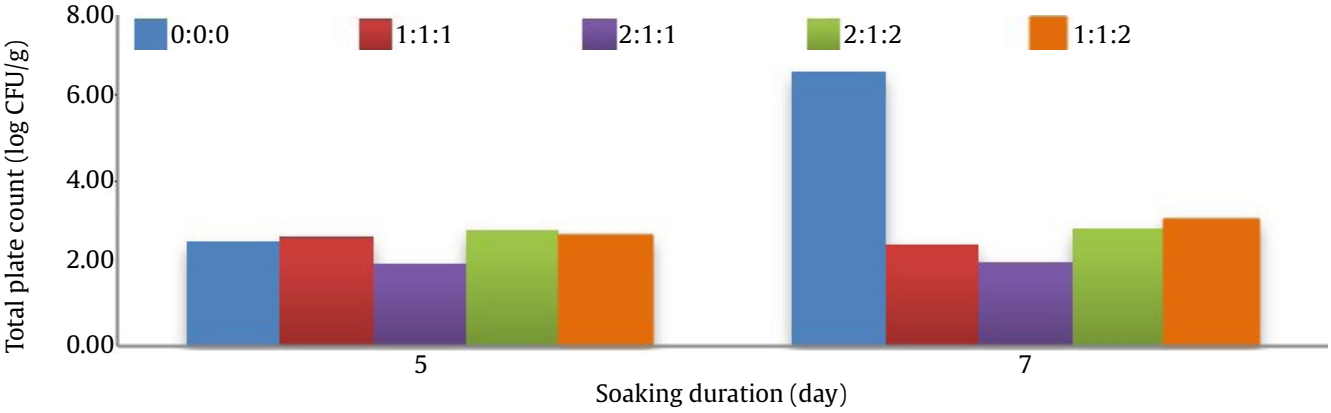

Figure 2. Total plate count of white pepper fermented by combination isolates of *Acetobacter* sp.: *B. subtillis*: *B. cereus*

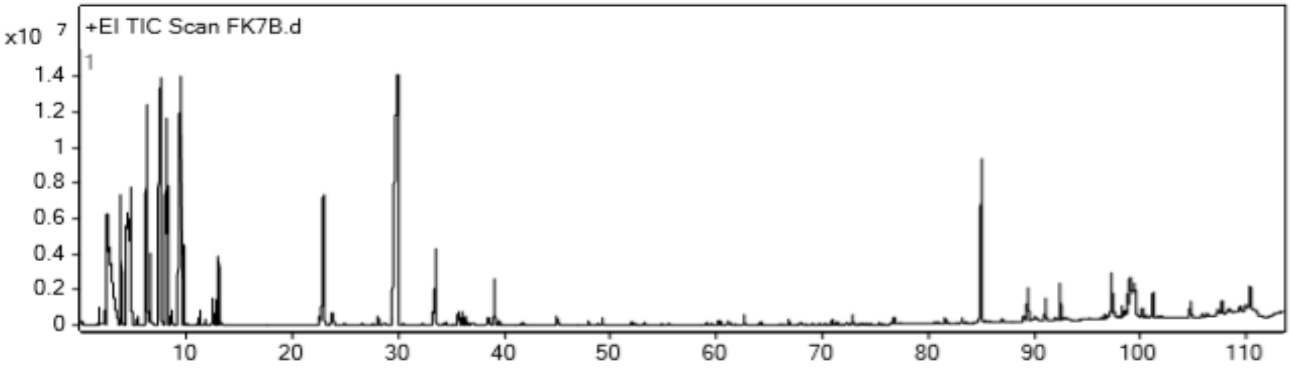

Figure 3. Chromatogram analysis of white pepper from the best treatment

β-caryophyllene, αcaryophyllene, and D-elemene (Hao *et al.* 2018).

In conclusion, combination isolates of *Acetobacter* sp., *B. subtilis*, and *B. cereus* 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 7 days with the combination isolates of *Acetobacter* sp., *B. subtilis*, and *B. cereus* with a ratio 2:1:1. This condition produced white pepper in fulfilling in requirement of SNI standards with piperine, essential oil contents and TPC of 5.95% 2.95%, and 1.1 x 10^2 CFU/g, respectively.

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