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Effect of immersion-fermentation on decreasing of cyanide acid and physicochemical content of local *hevea brasiliensis* seeds from borneo Indonesia

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Immersion-fermentation is a fermentation method using a liquid substrate with solid materials that immersed to the liquid. The process is very important to get food products to increase the nutritional value. Through the fermentative process, the seeds will change into valuable materials that had higher physicochemical content. Cyanide acid is the most important components contained in the *Hevea brasiliensis* seeds. *Hevea brasiliensis* plants found abundantly in West Borneo Indonesia. This fermentative process is also could decrease the cyanide acid content of *Hevea brasiliensis* seeds. The cyanide acid present in the cell walls which are bound in glicosidic chain and should be degraded by the used of microbes through this fermentation. The aim of this study was to investigate the type of microbe and fermentation time through immersion-fermentation to produce *Hevea brasiliensis* seeds with higher physicochemical content and low cyanide acids. A nested design with two factor was used. The main factor is the type of microbe (*Lactobacillus* sp. and *Saccharomyces cereviseae*) and the second factor is the fermentation time (24, 36, and 48 h) nested on the main factor.The best treatment in this study was fermentation that carried out for 48 h with *Lactobacillus* sp. as a microbial culture. This treatment produce *Hevea brasiliensis* seeds containing cyanide acid, total protein, and total fat were 64.7 mg; 18.1%, and28, respectively.

Keywords: immersion-fermentation, cyanide acid, *Lactobacillus* sp., *Saccharomyces cereviseae*, *Hevea brasiliensis*

INTRODUCTION

Immersion-fermentation (IF) is a type of fermentation method using a liquid substrate with solid materials that immersed to the liquid. This fermentation method has several advantages like the SSF fermentation method by Mushollaeni et al., (2017). This fermentation had increased the anthocyanin levels and improves the nutritional value of fermented lebui bean. In the previous studies concluded that fermentation showed many positive effects such as more simpler process, less energy stated by Prado et al. (2005), gain the stability and physicochemical quality of the final products (Hölkerand Lenz, 2005; Chawla et al., 2017), and also could increase the total of anthocyanin and phenolic (Lee et al., 2008).However, the influence of microbial type on the decrease of cyanide acid content in *Hevea brasiliensis* seeds especially from West Borneo Indonesia has not been studied.

Hevea brasiliensis plant is the common plant species in West Borneo Indonesia. Presently, *Hevea brasiliensis* plant only used as a plant to produce raw material for rubber and the production in 2016 is 592.324 tons/year (Agricultural Directorate, 2015). Part of *Hevea*

brasiliensis plant that is the seeds. The seeds found abundantly, left to fall on the ground, and as plant waste. Local people processed the *Hevea brasiliensis* seeds as grounded bean and roasted it as snack.The main obstacles encountered in processing *Hevea brasiliensis* seeds is the nut covered with a very hard shell and high cyanide acid content. To get the edible part of *Hevea brasiliensis* seeds, it takes an optimal effort to break the shell and it will takes a long time. In addition, the high cyanide acid content, can lead to poisoning.Cyanide acid (HCN) levels greater than 100 ppm, can result in poisoning and are highly toxic, so it need the best treatment to break the cell walls and less the poisonous components.

Cyanide acid is a product of secondary metabolites in plant and found as a compound in plant tissue. According toSiegień and Bogatek (2006), Kamei et al., (2014), cyanide is produced in plants as a secondary metabolites of the ethylene and cyanogenic biosynthesis. Cyanide in plants used as a toxic compound against the insects and weeds. This phenomena is in the line with the bioactive compounds, such as anthocyanin, phenolic compounds and others. However, in the same condition, cyanide is present in the cell walls which are bound with other compounds (Huang et al., 2013). This linkage should be hydrolized by the used of microbes through fermentation. Presently, the experiment aimed to find out the best type of microbes especially *Lactobacillus* sp. or *Saccharomyces cerevisiae* that could break out and hydrolyzed the linkage of cyanide from the cell walls of *Hevea brasiliensis* seeds found at Borneo Indonesia has not been done. This experiment aimed to find out and investigate the best type of microbe combine with fermentation time through immersion-fermentation (IF) to produce *Hevea brasiliensis* seeds with higher physicochemical content and low cyanide acids.

MATERIALS AND METHODS

Raw materials and pre-treatment procedures

Hevea brasiliensis seeds were collected from Notrh Kayong, West Borneo of Indonesia. This pre-treatment is based on the process of preparing lebui bean by Mushollaeni et al., (2017) with slight modification. The outer shell of the *Hevea brasiliensis* seeds is removed by splitting or peeling, then the inner of *Hevea brasiliensis* seeds are sorted out of the damaged and moldy parts. The inner part of *Hevea brasiliensis* seeds are then washed thoroughly, boiled for 60 minutes at 100°C until cooked. Then keep it in the dry place to avoid contamination and ready for the next step of fermentation.

Figure 1.*Hevea brasiliensis* seeds

The pre-fermentation of *Hevea brasiliensis* seeds

The next step after the pre-treatment is immersion-fermentation (IF). *Hevea brasiliensis* seeds is then immersed in aquadest (1:1 w/v) for 48h. During the fermentation, the water should be change at least 6 times for every 8h. This process is pre-fermentation and let the indigenous microbes to participate in this step. After this process, *Hevea brasiliensis* seeds is then washed thoroughly, dried in the room temperature, roughly grinded, and stored in a tightly closed glass container as the grinded *Hevea brasiliensis* (GHb).

Figure2.*Hevea brasiliensis* seedsin pre-fermentation process

The IF process of *Hevea brasiliensis* seeds

The next step after the pre-fermentation is immersion-fermentation (IF).For the each treatment, placed the GHb in the different container and homogenized with *Lactobacillus* sp. (A1) or *Saccharomyces cerevisiae*(A2) stock culture. This each treatment is then separated in 3 container for the different fermentation time (24h,

36h, and 48h). After the IF completed, then the GHb is washed thoroughly, rinsed, dried in the room temperature, and stored in a tightly closed glass container for the physichocemical and cyanide acid analysis.

Figure3.The IF process of *Hevea brasiliensis* seeds

General experimental procedures

A nested design with two factor was used in this experiment. The type of the microbes as the main factor (*Lactobacillus* sp. Referred to A1 and *Saccharomyces cerevisiae* referred to A2). The secondfactor is the fermentation time (24h, 36h, and 48h) nested on the main factor.Observation parameters were cyanide acid, total protein, fat, carbohydrate, water and ash. The proximate contents analysis and cyanide acid were measured by AOAC (2000). The research data were analyzed using nested ANOVA.

RESULTS AND DISCUSSIONS

Cyanide acid levels

The IF fermentation process is aimed to hydrolized the linkage bond that bind secondary metabolites such as cyanide compounds, so this process will change the form of binded cyanide to free forms of cyanide. Stated by Mushollaeni (2018), the secondary metabolites and bioactive compounds found in the plants cell walls should hydrolyzed to make the free form and could be extracted. In addition, fermentation is also to gained the nutritional content of the final products.

Cyanide acid levels of *Hevea brasiliensis* seeds after IF fermentation is lower than in the raw material. As raw material, the *Hevea brasiliensis* seeds has 300 mg of cyanide acids and become 80% lower after the IF fermentation. The cyanide acids levels was in the range of 64.7-92.2 mg. *Hevea brasiliensis* seeds that fermented with A1 has lower cyanide levels (the average content of cyanide acids was 78 mg) than fermented with A2(the average content of cyanide acids was 83 mg).

The cyanide levels for all the treatments tend to decreased in line with the lenght of fermentation time. This condition is the same with the experiment done by(Mushollaeni et al., 2017), the anthocyanin levels in lebui beans (*Cajanus* sp.) fermented by *Rhizopus* sp. or *Lactobacillus* sp.tend to decrease with increasing length of fermentation time. Balik (2006) also stated that during fermentation could degradate the chemical constituents in the cell walls by microbes to produce free form of this contituents and decrease several compounds, including secondary metabolites (Hornedo-Ortega et al., 2017). This condition is occurs by the activities of microbes, the change of temperature and pH (Afoakwa et al., 2012).Based on the results of experiment, fermentation treatment up to 3 days by A1 gave better results than A2. This related to the increasing of temperature and pH.

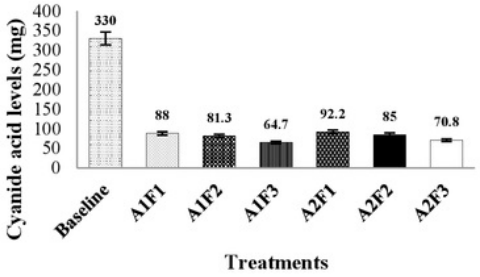

Figure 4.Cyanide acid levels of *Hevea brasiliensis* (GHb). A1:*Lactobacillus* sp.; A2: *Saccharomyces* cerevisiae; F: Fermentation time (F1: 24h, F2: 36h, F3: 48h)

Physicochemical compounds

Protein content (%)

There is an influence between microbial type and duration of fermentation nested on microbial type to protein level of *Hevea brasiliensis* (GHb) is shown in Figure 5. Total protein content is in the average of 5.5%-18.1%. This protein content is lower than the raw material, caused by the action of microbes that breakdown the protein and used it for their metabolism during fermentation. The lower protein content for all type of microbes is shown after 24h of fermentation and started to rise after 24h of fermentation time. (Kasprowicz-Potocka et al., 2016) stated that due to fermentation time, an increase in the mass of microbes will hydrolyze the proteinon the substrate or product. This protein then hydrolyzed and become free amino acids also nitrogen to

support its growth.

After 48h of fermentation, it shown that the protein content is raising up and higher than the raw material (baseline). This condition proves that fermentation could improve protein quality and also stated by Igbabul et al., (2014) that along fermentation process on mahogany bean could increase the protein content of the final product from 21.88% to 22.43-26.8% in 72h. This caused by the activity of microbes that can lead to hydrolyze of protein molecules, resulting in amino acids and nitrogen.

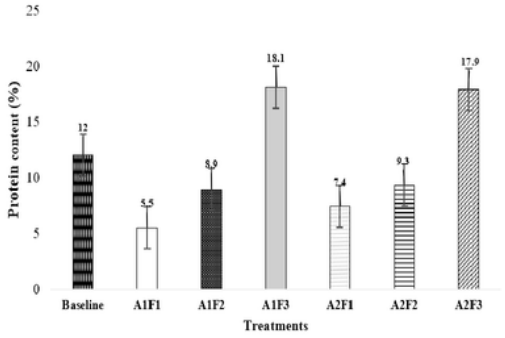

Figure 5.Proteins content of *Hevea brasiliensis* (GHb). A1:*Lactobacillus* sp.; A2: *Saccharomyces* cerevisiae; F: Fermentation time (F1: 24h, F2: 36h, F3: 48h)

Fat content

The average fat content of *Hevea brasiliensis* (GHb) fermented with A1 and A2, respectively are 27.7% and 28.57%.Fermentation using culture A1 produced GHb with less fat content than A2. It showed a different activity of the microbes to breakdown the large molecules of fat and also could indicate that A1 needs more fatty acids for its metabolism than A2, so the final content of fat in the product fermented with A1 is less than A2.

The decrease in fat content compare with its raw material is possible because of microbial growth, therefore the fat molecules that have been hydrolyzed into simpler compounds are used to support the microbial life. Complex fat molecules decreased by the activity of lipolytic enzymes produced by microbes during fermentation time and resulted in decreased levels of fat in product (Obadina et al., 2013).For each treatment by A1 or A2, showed no significant level of fat content (Figure 6).This condition is in line with the experiment by Osman (2011), no noticeable change in the fat content of the final product during fermentation.

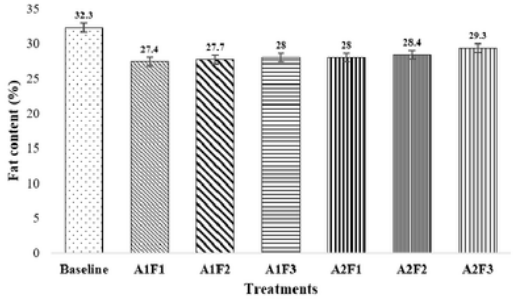

Figure6.Fat content of *Hevea brasiliensis* (GHb). A1:*Lactobacillus* sp.; A2: *Saccharomyces* cerevisiae; F: Fermentation time (F1: 24h, F2: 36h, F3: 48h)

CONCLUSION

The IF method used in this study has been able to decrease 80% of the cyanide acids level of *Hevea brasiliensis* seeds by the activity of *Lactobacillus* sp. This IF is carried out for 48h. IF method also could increase the total protein and decrease the fat content of final product.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

WM, RS, RMY, and TMM has performed the experiments and also wrote the manuscript. WM prepared and runned the materials, general experimental procedures, analysis the data from parameters, and wrote the discussion. RS, RMY, and TMM runned the experimental procedures. All authors read and approved the final version.

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