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Effects of Retting Media Circulation and Temperature on the Fermentation Process in Soft-Texture and Low Cyanogenic Content Cassava Flour Production

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A fermentation process to produce soft-texture and low cyanogenic content cassava flour had been studied, in particular the effects of temperature, circulation of the retting media, and scale to the reduction of cyanogenic content and the structure of cassava. Although the effects of retting media circulation and an increase in the fermentation temperature on cyanogenic content reduction were not clearly seen, they led to more damaged cassava structure and thus improving the overall cassava flour production. On the other hand small scale fermentation led to both cyanogenic content reduction and morphological changes, giving better fermentation performance.

Keywords: cassava flour, fermentation, circulation, retting, cyanogenic

INTRODUCTION

Along with the increase in human population, there is a strong need to supply food in a sustainable way. One way to address this issue is the diversification of staple food. Cassava is one potential alternative staple food. According to Indonesian Statistics Bureau (www.bps.go.id) its productivity was reported to be 17.5 tons/ha. This number is significantly higher than the productivity of sweet potatoes, 12 tons/ha, or rice, 4.6 tons/ha.

The disadvantage of using cassava is its cyanogenic content, mainly linamarin and

lotaustralin. These cyanogenic compounds are very potential in bringing negative effects for human health when consumed. Tewe (1992) showed that there was a tendency that consuming these compounds in high concentration would cause death to animals. Philbrik et al. (1977) and Maduagwu and Umoh (1988) also showed that direct injection of linamarin could cause physiological and biochemical change to mice and chick embryo. Almost every cassava across the globe had both linamarin and lotaustralin, but the amount varies based upon the circumstances on which the cassava was planted on (Bokanga 1994). The cyanogenic content in Indonesia's cassava was reported in the range of 10-60 ppm (Djazuli and Bradbury 1999).

The retting process or submerged fermentation of cassava chips was reported to reduce the cyanogenic content in cassava. This process mainly involved cellulosic bacteria, lactic acid bacteria and yeast (Achi and Akomas 2006). Further Tewe (1992) reported that this process could reduce cyanogenic content in cassava up to 98.6%. The retting process somehow induced the hydrolysis of linamarin by linamarase to HCN, which would then be released to the atmosphere (Giraud et al. 1992).

Unfortunately, the fermentation process is usually time-consuming. Okafor et al. (1984) showed that the process took about 3-4 days. In Indonesia, the fermentation process in modified cassava flour (MOCAF) production usually proceeds for 24 hours. Attempts to reduce the fermentation time have been studied, for example by introducing locally-developed techniques (Ogbo 2006) and by increasing temperature (Ampe et al. 1994). Nevertheless there is still a strong need to significantly reduce the fermentation time.

The cassava fermentation is usually conducted in a open bath where all chips need to be submerged. The hygienic aspect is usually overlooked. Moreover, a large area is required for the fermentation. Literature study revealed that no attempt has been made to improve the design of fermentor.

In this research we developed a circulated bioreactor for cassava fermentation to improve the fermentation process. The retting media was circulated in order to improve the texture softening as well as the cyanogenic compounds removal process. Since there is still little evidence depicting the process of modification occurred inside the cassava and the standardized results, the MOCAF term is not used in this paper. The term chosen is "soft-texture and low cyanogenic-content cassava flour." This article foccused on studying performance of the bioreactor, in particular the effect of temperature and the retting media circulation to the fermentation process which is parameterised as cyanogenic compound removal and chips morphology. Other aspects such as starter composition was out of the scope of this research.

MATERIALS AND METHODS

Materials

Cassava used in the experiments was obtained from local market in Bandung. The cassava was manually chipped to

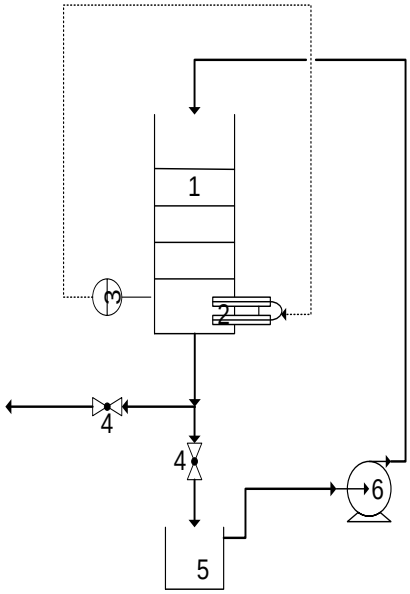

Fig. 1: Schematic diagram of the circulated bioreactor (1. cassava chips holder, 2. Heater, 3. Thermocouple, 4. Valves, 5. Buffer tank, 6. Pump)

average thickness of 1 mm and washed before processed further.

The starter microorganisms were obtained from local MOCAF producer, Koperasi Gemah Ripah Loh Jinawi (Trenggalek, East Java, Indonesia). The starter solution for cassava fermentation was prepared by mixing 1.5 gram of starter microorganism with media (100 grams of cassava, 1 liter of water, and 3 grams of sugar). The solution was followingly incubated in a 30^{o}C incubator for 24 hours before being used for the fermentation.

Circulated Bioreactor

The bioreactor was designed as such that the retting media could be circulated. The bioreactor was made from fiber glass and was equipped with temperature controlled system and a heater at the bottom and a pump to circulate the media used in fermentation process.

Cassava Fermentation

The fermentation was conducted in the bioreactor described in the previous section. In the preliminary study, we used 300 grams of cassava, 1 L of innoculation solution, and 30 L of water in each batch of fermentation. In this stage, the effect of temperature on the fermentation was studied. The effect of circulation was studied in a larger scale, in which 3750 gram of cassava was used in each batch of fermentation. For this fermentation 1 L of inocculation solution and 30 L of water was used.

In each fermentation, samples were taken every hour for cyanogenic compound analysis. Samples for texture analysis were taken at the end of each experiment.

Cyanogenic Content Analysis

Cyanogenic compound in cassava was analyzed indirectly following the method

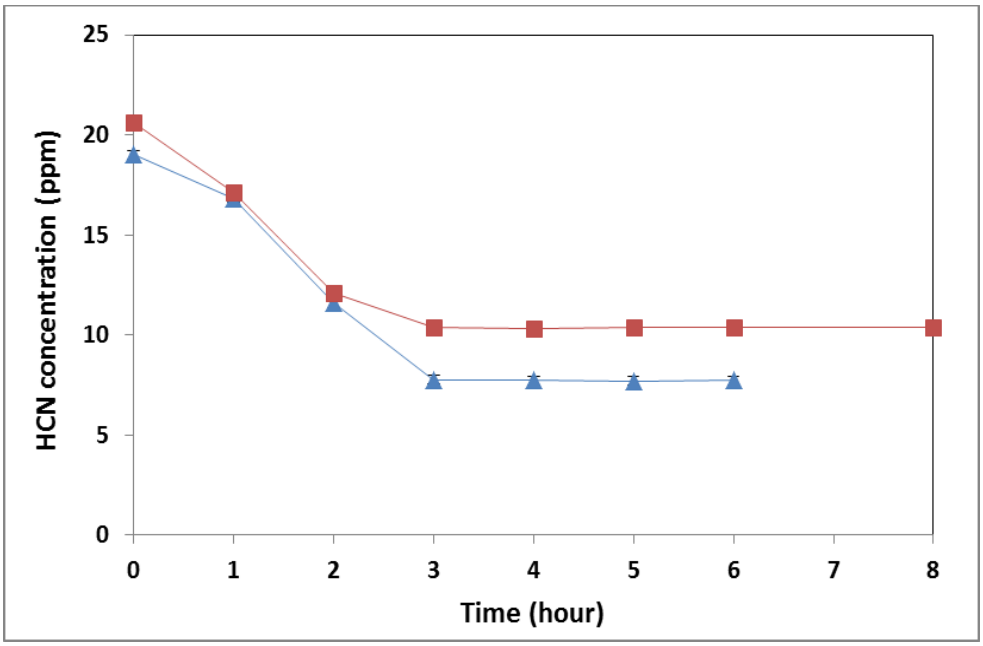

Fig. 2: Effects of circulation on the reduction of cyanogenic content during cassava fermentation (triangles: without circulation, squares: with circulation)

described by Rasulu et al. (2012). The amount of cyanogenic compounds was represented by HCN concentration that was calculated by steps described below. The analysis was conducted based upon 10 grams of cassava chip being fermented in the reactor. The chip was firstly crushed with mortar with addition of 10 mL of water. The mix (crushed chips and water) was then placed in a glass with an addition of 90 mL of water and to be held about 2 hours. After being held, the water in the glass was subjected to distillation after 100 mL of water was further added. The distillate was then collected in an erlenmeyer flask that had been filled with 20 mL of NaOH 5% until the volume had reached 150 mL. Having been collected, the condensate was titrated with $AgNO_3$ 0.004 N after an addition of 3 mL of KI 5% as indicator. The titration would be stopped if the color of the condensate became yellowish.

Morphology Analysis

Morphology analysis was conducted by using scanning electron microscopy (SEM). SEM analysis was conducted at Indonesian Institute of Science (LIPI) Bandung. The samples were coated with gold and the magnification used is 1000.

RESULT AND DISCUSSIONS

Effects of Media Circulation

In order to study the effects of media circulation on cassava fermentation, we performed fermentations with and without media circulation. Both were performed at 35°C. Followingly samples were taken from each fermentation and analysed for the cyanogenic content as well as morphology. The time profile of cyanogenic content of cassava chip throughout the fermentation process was presented in Figure 2 whereas the morphology was presented in Figure 3.

As was suggested by Tewe (1992) and Girraud et al (1992), the cyanogenic content of the cassava decreased along the fermentation. Figure 2 shows that in the beginning of the fermentation, 0 – 2 hours, the reduction of cynogenic content occurs at similar rate between the fermentation with and without media circulation. At 3 hour the steady state concentration had been achieved. However, the final HCN concentration of the fermentation with circulation was observed to be higher than the one without (Figure 2). The final HCN concentration in the fermentation with retting media circulation was observed to be 60%, whereas the one without retting media circulation was observed to be 40%. Should the fermentation only be parameterized by cyanogenic content reduction, the presented results suggested that this process could be ended only in 3 hours which is much faster the fermentation time reported in the literature, 3 – 4 days (Okafor et al. 1984), or applied in industries, 24 hours. The reduction of cyanogenic content was, however, much lower than reported in literatures. For example, Tewe (1992) reported up to 98.6% reduction of cyanogenic content after the fermentation.

On the other hand, Figure 3 shows that the morphology of cassava chips fermented with media circulation was more damage compared to cassava chips fermented without media circulation. This indicated that the circulation of media also affected the fermentation performance.

Several possibilities relating media circulation with fermentation performance are listed here. The first possibility is that media circulation contributes to the improvement of aeration process, hence improving cell growth, increasing the number of microorganisms, and enhancing the fermentation process. The second possibility is that media circulation improves the transport process from the liquid phase to the solid phase, thereby enhancing the hydrolysis process. The third possibility is that media circulation improves temperature distribution within the bioreactor. This would further contribute to the increase of microorganism activity.

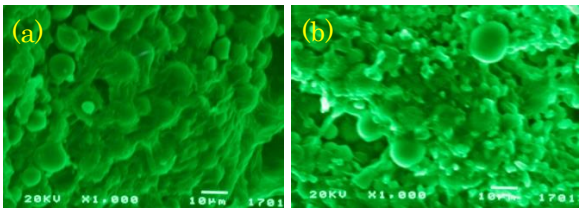

Fig. 3: SEM profile of chipped cassava fermented at reactor performance tests (a. without circulation, b. with circulation)

Effects of Temperature

In order to study the effect of temperature on the fermentation process, the temperature of the fermentation process was set to be either 30, 35, or 40°C. Followingly samples were taken from each fermentation and analysed for the cyanogenic content as well as morphology. The time profile of cyanogenic content of cassava chip throughout the fermentation process was presented in Figure 4 whereas the morphology was presented in Figure 5.

At a glance, it seems that the increase in temperature does not effect the cyanogenic compounds reduction. The rate of cyanogenic compound reduction in

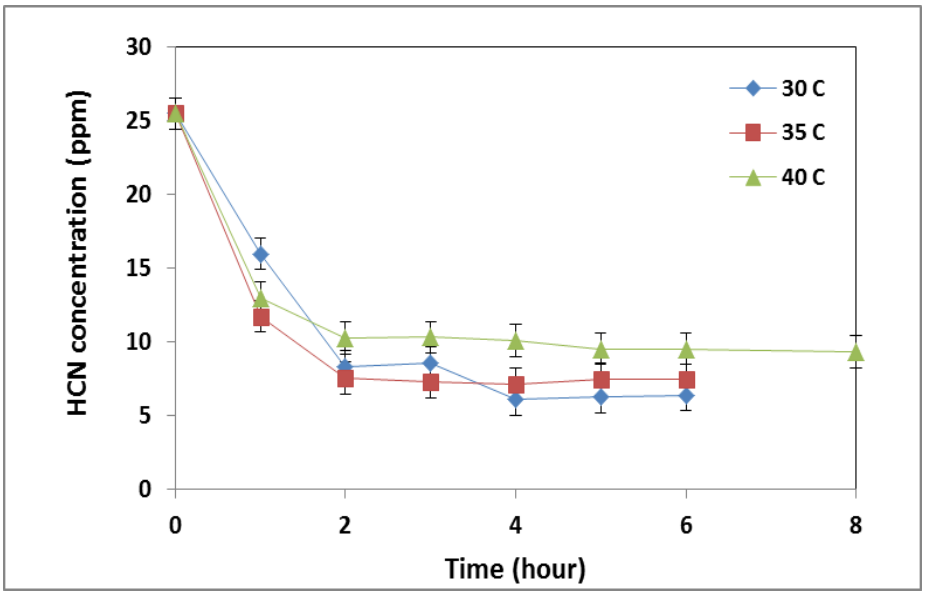

Fig. 4: Effects of temperature on the reduction of cyanogenic content during cassava fermentation

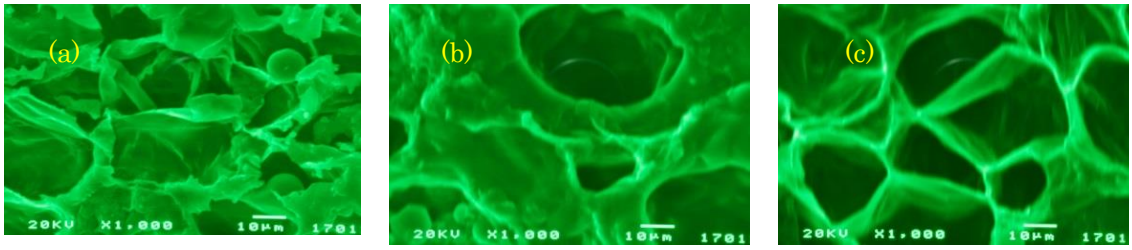

Fig. 5: SEM profile of chipped cassava fermented at different temperature (a. 30°C, b. 35°C, c. 40°C)

all the three evaluated temperature: 30, 35, and 40°C were comparable.

The morphology analysis performed by SEM somehow gives different trend (Figure 5). At the same magnification the cassava chips fermented at higher temperature were observed to have bigger holes, the morphology was more damaged. This results confirmed that the reduction of cyanogenic content was not the only process occured during the fermentation. This other process could be the hydrolysis of starch and cellulosic materials, resulting in cassava chips with shorter polysaccharide chains.

Indeed, the cellulosic and the lactic acid bacteria were reported to play an important role in cassava fermentation (Achi and Akomas 2006). The cellulosic bacteria excretes cellulolytic enzymes that catalyses the hydrolysis of cellulosic materials, giving shorter polysaccharide chains. The lactic acid bacteria produces lactic acid which may also help the hydrolysis of polysaccharide. These bacteria are mesophilic whose optimum growth temperature ranges from 35-45°C. Thus, at higher fermentation temperature the bacteria grows faster, produces more lactic acid, and gives higher enzyme activities. Overall, higher fermentation temperature results in faster hydrolysis

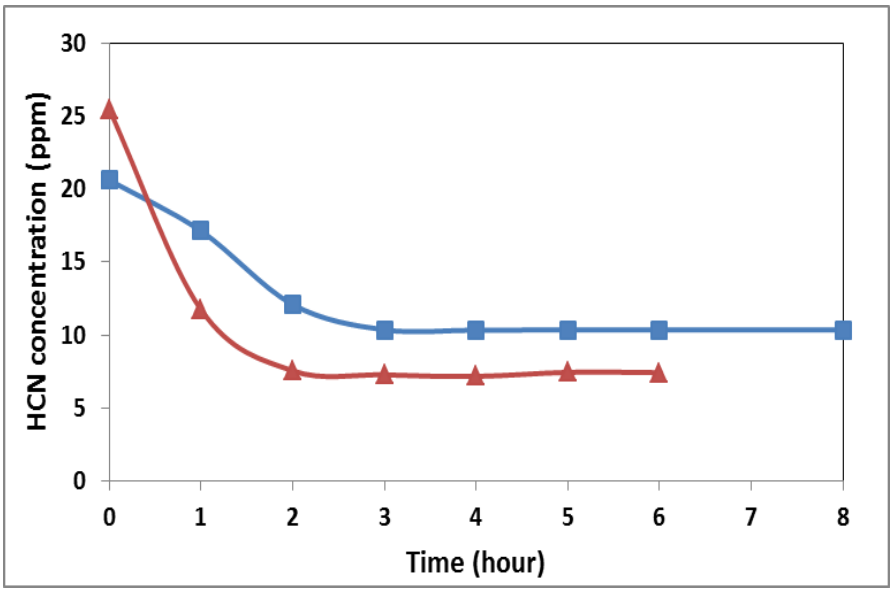

Fig. 6: Effects of scale on the reduction of cyanogenic content during cassava fermentation (triangles: small scale using 300 g cassava chips, squares: larger scale using 3750 g cassava chips)

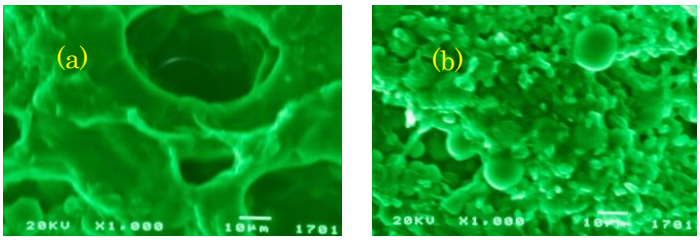

Fig. 7: SEM profile of chipped cassava fermented on different scale (a. Small scale using 300 g cassava chips, b. Larger scale using 3750 g cassava chips)

which was observed in the SEM results (Figure 5).

Effect of Scale

The fermentations were performed at 35°C using the same amount of liquid media and on two different scales, using 300 grams or 3750 grams of cassava chips. Followingly samples were taken from each fermentation and analysed for the cyanogenic content as well as morphology. The time profile of cyanogenic content of cassava chip throughout the fermentation process was presented in Figure 6 whereas the morphology was presented in Figure 7.

Figure 6 shows that the rate of reduction of cyanogenic compounds were faster at the small scale fermentation, despite the differences of the initial concentration. At the small scale the stable steady state concentration has been achieved on 2 hours of the fermentation. On the other hand, the stable steady state concentration was achieved on 3 hours of fermentation. This observation indicated that the fermentation was better at the small scale.

Similar trend was observed in the morphology analysis. SEM pictures of the cassava chips fermentated at different scale (Figure 7) showed that the morphology was more damaged at the small scale. This observation also indicated that the fermentation performed better at the small scale. Further research is required to obtained the optimize scale of fermentation.

CONCLUSION

This research showed that both the circulation of retting media and the higher fermentation temperature contributed to the improvement of cassava fermentation process. This was clearly shown by the more damaged of cassava structure when high temperature and media circulation were applied. The scale of fermentation also affected the cassava fermentation performance, as was clearly shown in acceleration in the cyanogenic content reduction as well as more damage morphology at the small scale. Future research in the optimization of fermentation scale is neccessary.

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