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ANALYSIS OF CYANIDE ACID (HCN) CONTENT IN SHIP BAMBOO SHOOTS *(GIGANTOCHLOA SCHORTECHINII)* AS FOOD INGREDIENTS

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ABSTRACT

This study aims to determine the effect of different lengths of soaking on cyanide acid (HCN) content in ship bamboo shoots (*Gigantochloa schortechinii*) which are the main ingredient in making Lemea. Lemea is a specialty food of the Rejang tribe in Bengkulu Province where the main ingredient of Lemea is fermented chopped bamboo shoots (young bamboo). This study used a completely randomized design (CRD) with five repetitions and four treatment levels, namely. A = no treatment/control, B = 4 days soaking, C = 5 days soaking, D = 6 days soaking and E = 7 days soaking. The results of the analysis showed that different lengths of soaking time had an effect on reducing cyanide acid (HCN) content. The highest to lowest average cyanide acid (HCN) content was obtained from treatments A (70.066 ppm), B (53.759 ppm), C (42.374 ppm), D (29.739) and E (16.458 ppm). The best cyanide acid (HCN) content was obtained in the immersion treatment for 7 days with an average HCN content of 16.458 ppm, this result is highly recommended because the HCN content is safe for consumption according to FAO (Food and Agriculture Organization). Further research can be directed at testing the influence of other factors in order to obtain more comprehensive quality standards for Lemea.

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1. INTRODUCTION

Lemea is a specialty of the Rejang tribe in Bengkulu Province where the main ingredient of Lemea is chopped bamboo shoots (young bamboo) which are fermented, the ingredients of the Lemea mixture vary in each region, some use river fish, red chili, bay leaves, and lemongrass. The type of bamboo used in making Lemea is ship bamboo (*Gigantochloa schortechinii*) (Marcellina *et al.,* 2023). In Indonesia, *Gigantochloa schortechinii* can be found in areas with a humid tropical climate and high rainfall, such as on the islands of Sumatra (especially Bengkulu and Jambi provinces), Java, Kalimantan, and Sulawesi (Silalahi & Sutapa, 2024). This type of bamboo often grows in low to medium altitudes, in areas that have fertile soil and sufficient water flow. The spread of *Gigantochloa schortechinii* in Indonesia is supported by the habits of local people who cultivate it for the needs of building materials, making household furniture, and handicrafts. This ship bamboo is characterized by large, straight, and sturdy stems. Its presence in nature is

ecologically beneficial as it is able to maintain soil stability and prevent erosion, especially in areas with steep topography.

The process of making Lemea applies the principles of biotechnology, especially conventional biotechnology, namely fermentation. The making of Lemea relies on the activity of natural microorganisms to change the chemical composition of bamboo shoots, improve taste, and reduce toxic content, such as cyanide in bamboo shoots.The main principle in biotechnology is the utilization of microorganisms to produce biological changes in bamboo shoots. In making Lemea, microorganisms, such as lactic acid bacteria (LAB) found in natural fermentation, play an active role in the fermentation process. Lactic acid bacteria (LAB) break down the complex carbohydrates in bamboo shoots into lactic acid, giving rise to the distinctive sour fermented flavor and aroma as well as changes in the texture of Lemea. Despite being an integral part of the local culinary tradition, Lemea processing poses challenges related to the potential content of harmful compounds, such as cyanide, which are naturally present in bamboo shoots.

Cyanides are a group of compounds containing a cyano group (C=N) and are present in various forms (Kjeidsen 1999, Luque-Almagro *et al.,* 2011). Naturally in the environment, cyanide can be divided into free cyanide, simple cyanide, cyanide complexes, and cyanide-derived compounds (Smith & Mudder, 1991). Free cyanide is the determinant of the toxicity of cyanide compounds which can be defined as the molecular form of HCN and CN ions of cyanide liberated through the dissolution and dissociation process of cyanide compounds. These two species are in equilibrium with each other which depends on pH so that the concentration of HCN and CN is influenced by pH. At pH below 7 all cyanide is in the form of HCN (Pitoi, 2015). Cyanide can be very dangerous and deadly if abused with improper dosage, so it is included in the list of ten poisons that are deadly when consumed by humans (Syahda & Antoni, 2024). The level of cyanide acid that can cause poisoning and even death ranges from 50 mg - 100 mg, and if consumed continuously, it will trigger serious health problems, such as neuropathy and cretinism (Kurniati et al., 2015). Based on the WHO/FAO statement, the safety level of cyanide consumption is 40 ppm, and WHO also states that the total cyanide content allowed in processed products is ≥ 10 ppm while in food the allowed cyanide level is a maximum of 10 ppm (Sulistinah *et al.,* 2014).

Cyanide in bamboo shoots refers to the presence of toxic compounds called cyanogenic glycosides. These compounds are stored in an inactive form in plant tissues and will break down into hydrogen cyanide (HCN) when the plant tissues are damaged, such as when the bamboo shoots are cut or cooked. Fresh bamboo shoots contain about 100-1000 mg of HCN per kilogram of bamboo shoot weight. This is high compared to the recommended safe limit for consumption, which is less than 1 mg of HCN per kilogram of body weight per day. Cyanide is a toxic compound that can cause poisoning if consumed in excessive amounts (Sulfiani & Taufiq, 2022). This toxic substance can interfere with respiratory function in humans because it inhibits the enzyme cytochrome oxidase in mitochondria, thus preventing cells from using oxygen effectively. Twetongyere and Katonhole (2002) state that cyanide is a poison that can inhibit the work of respiratory enzymes resulting in respiratory disorders that can cause illness and death. When consumed Hydrogen Cyanide (HCN) is very quickly absorbed by the digestive system and enters the bloodstream. Based on medical studies, it is known that cyanide can interfere with health, especially the respiratory system, because oxygen in the blood is bound by these toxic compounds (Pratiwi *et al.,* 2023). The toxicity of cyanide is determined by its type and concentration (Smith & Mudder, 1991). Cyanide toxicity in the form of hydrogen cyanide (HCN) can cause death,

disorders of the respiratory system, heart, digestive system, circulatory system, and nervous system are also the main targets of cyanide. Prolonged exposure to high concentrations of HCN can stimulate the central nervous system followed by depression, convulsions, paralysis and death. HCN can be rapidly absorbed into the body and carried into the plasma. In most cases, consumed bamboo shoots are harmless if properly prepared.

Therefore, an appropriate processing method is needed to reduce cyanide levels in bamboo shoots to make them safe for consumption. One method that is known to be effective in reducing cyanide levels is the soaking method in line with Rusli *et al.* (2019) reducing or reducing cyanide levels can be done by washing or soaking because cyanide acid will dissolve and be wasted with water and because cyanide acid has soluble and volatile properties. 50% of cyanide acid can be removed by soaking and boiling. 50% of cyanide acid can be removed by soaking and boiling (Nurhidayanti *et al.,* 2021). Soaking using water allows water-soluble cyanide to be removed from the bamboo shoots, so that the toxicity levels are reduced. However, although soaking is effective in reducing cyanide levels, the appropriate soaking time still needs to be studied to ensure a balance between maximum detoxification and maintaining the quality of raw materials. Based on this phenomenon and the potential of traditional Lemea food in Lebong Regency, research was conducted on the optimal soaking time variation for cyanide detoxification in bamboo shoots. The time factor is crucial, as too short a soaking may not be effective enough to reduce cyanide levels to safe levels. Therefore, it is necessary to conduct in-depth research to determine the most efficient soaking time and safe for consumption.

2. METHOD

This research was conducted in December 2024 at the Lemea production house in Daneu Village, Lebong Regency. Furthermore, the samples were analyzed for cyanide acid (HCN) levels at the Basic Biology Laboratory, Basic Science Building, Faculty of Mathematics and Natural Sciences, Bengkulu University. The tools used in this research are plastic jars, basins, glasses, machetes, scales, liters, duct tape, markers, and label paper. The materials used in this study were ship bamboo shoots (Gigantochloa schortechinii), water, and chemicals for cyanide acid (HCN) analysis.

This study used a completely randomized design (CRD) with treatments (A, B, C, D and E). Each treatment was repeated 5 times. When, A is No treatment/control, B is Ship bamboo shoots (*Gigantochloa schortechinii*) 200 g + 300 ml water + soaked for 4 days, C is Ship bamboo shoots (*Gigantochloa schortechinii*) 200 g + 300 ml water + soaked for 5 days, D is Ship bamboo shoots (*Gigantochloa schortechinii*) 200 g + 300 ml water + soaked for 6 days, E is Ship bamboo shoots (*Gigantochloa schortechinii*) 200 g + 300 ml water + soaked for 7 days .

Research Implementation

Tool Sterilization

All tools used were first cleaned and washed with detergent, then rinsed with clean water and then dried, then sprayed with 96% alcohol shortly before use.

Soaking Process

1. The bamboo shoots of ship bamboo (*Gigantochloa schortechinii*) were first cleaned and then chopped and put into a basin and soaked for 1 day to remove the sap on the bamboo shoots. The bamboo shoots were washed after 1 day of soaking and drained.
2. Ship bamboo shoots (*Gigantochloa schortechinii*) were given each treatment, namely treatment without soaking, soaking for 4 days, 5 days, 6 days and 7 days with a lot of material for each treatment of 300 grams.
3. Ship bamboo shoots (*Gigantochloa schortechinii*) were put into a jar and then soaked with soaking treatment for 4 days, 5 days, 6 days and 7 days using 300 ml of sterile water.
4. The vents on the jars were closed using duct tape to create an airless (anaerobic) environment. The jars were padded to avoid direct exposure to floor temperature.

Ship bamboo shoots (*Gigantochloa schortechinii*) that have been soaked with the soaking time of each treatment are then analyzed for HCN content. The schematic research procedure can be seen in Figure 1.

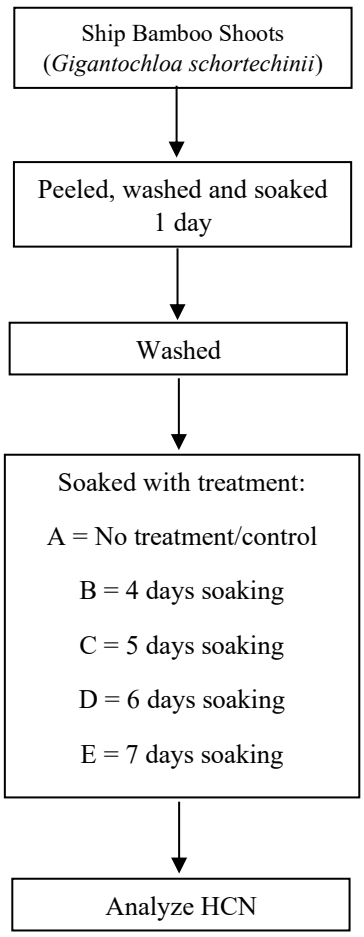

Figure 1. Schematic of Research Procedure

HCN Content Analysis Procedure

Each sample that has reached the treatment limit is then analyzed. Analysis of cyanide acid (HCN) content was carried out using the following procedure:

1. Weighed as much as 20 grams of ship bamboo shoot samples (Gigantochloa schortechinii) which were then added to 100 ml of distilled water in an erlenmeyer and allowed to stand for 2 hours.
2. Another 100 ml of distilled water was added and steam distilled. The distillate was collected in an erlenmeyer that had been filled with 20 ml of 2.5% NaOH.
3. After distillation (accommodated in erlenmeyer) reached a volume of 150 ml, the distillation process was stopped. The distillate was then added 5 ml of 5% KI and 8 ml of NH4OH. The distillate mixture was titrated with 0.02 N AgNO3 solution until turbidity occurred.
4. Then calculated the cyanide acid content formula:

$$\text{HCN} = \frac{ml\ AgNO3\ X\ 0{,}54}{Material\ Weight}\ X\ 1000\ mg/kg \quad (1)$$

3. RESULTS AND DISCUSSION

The results of the analysis of variance showed that the length of immersion in water had a very significant effect (P<0.01) on the cyanide acid (HCN) content of bamboo shoots. In general, HCN content decreased as the length of soaking increased. In treatment A (without soaking), the highest HCN content was recorded at 70.066 ppm. Soaking for 4 days (treatment B) reduced the HCN content to 53.759 ppm, then decreased further in treatment C (5 days) to 42.374 ppm. In treatment D (6 days), HCN levels were recorded at 29.739 ppm, and the lowest levels were found in treatment E (7 days), at 16.458 ppm. These results indicate that soaking in water is an effective method to gradually reduce HCN levels in bamboo shoots.

Based Figure 2 on the results of the study, soaking time in water proved to significantly reduce the cyanide acid (HCN) content of bamboo shoots. Soaking is an important method in the detoxification process of toxic compounds in food, especially in bamboo shoots which naturally contain high amounts of HCN. High HCN levels are very dangerous to human health as they have the potential to cause toxic effects, even in low concentrations. Therefore, efforts to reduce HCN levels in foodstuffs such as bamboo shoots are very important. This study makes an important contribution to the development of a simple yet effective method for safe food processing. In addition, the method of soaking in water is also easily applied by the community at large, especially in traditional food processing. Sample A produced the highest HCN level, which amounted to 70.066 ppm. This value indicates that without any pre-processing such as soaking, bamboo shoots contain toxic compounds that could potentially endanger human health. This figure is far above the safe consumption limit set by FAO, which is <50 ppm. With such levels, untreated fresh bamboo shoots have a high risk of causing poisoning if consumed. This emphasizes the importance of pretreatment before bamboo shoots are consumed or used as processed food ingredients. The public needs to be educated about the dangers of HCN and the need for proper

processing. This knowledge is important to prevent cases of poisoning due to consumption of raw and untreated bamboo shoots.

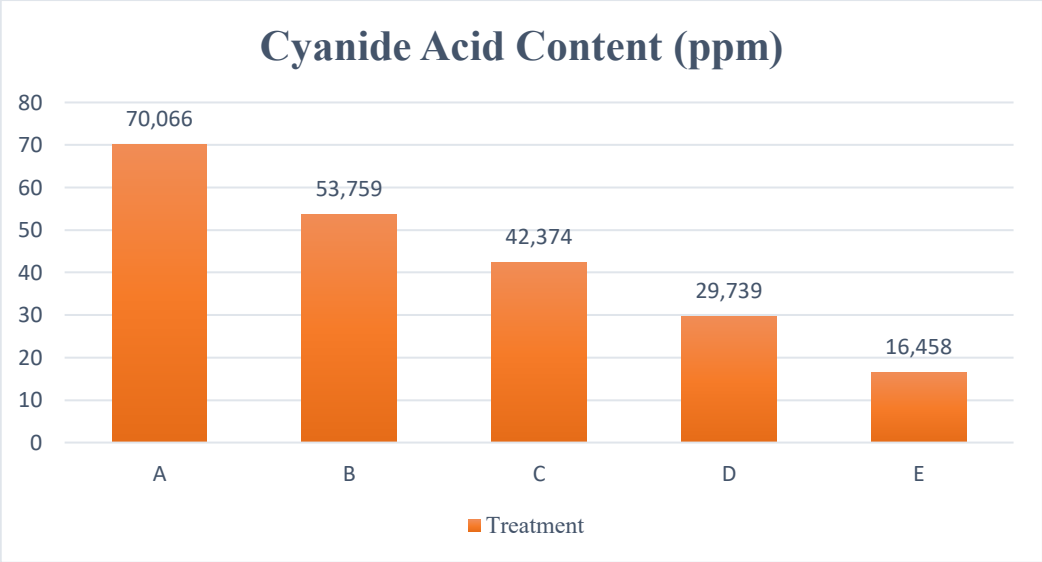

Figure 2. Average Cyanide Acid Content in Ship Bamboo (Gigantochloa Schortechinii) Bamboo Shoot Soaking Using Different Soaking Durations (A = No Treatment/Control, B = 4 Days Soaking, C = 5 Days Soaking, D = 6 Days Soaking and E = 7 Days Soaking).

In the 4 day immersion (treatment B), HCN levels decreased significantly to 53.759 ppm. This indicates that HCN compounds began to dissolve and break down into water during the soaking process. Although it has not reached the safe limit according to FAO, this decrease shows that soaking for several days can gradually reduce toxicity levels. This process can be the first step in traditional fermentation procedures. In addition, physical changes also began to appear in the bamboo shoots, such as a softer texture and a changing aroma. This could be an early indicator of chemical reactions in the plant tissue. Thus, the 4-day soaking, although not yet safe enough, has shown the initial potential of this method. This decrease was even more evident in treatment C (5 days), where HCN levels decreased to 42.374 ppm. This means that an additional day can increase the effectiveness of detoxification. At this point, the HCN level was already below the safe threshold according to FAO. Thus, bamboo shoots that have been soaked for five days have met the safety standards for consumption. However, to ensure maximum safety, longer soaking is required to reduce possible variations in HCN content between bamboo shoots. Longer soaking will also improve the sensory quality of the bamboo shoots. This study shows that traditional community methods are in line with modern scientific principles in food processing.

In treatment D (6 days), the HCN content was recorded at 29.739 ppm. This shows that a longer soaking process results in a more significant reduction in HCN levels. With this level, bamboo shoots are not only safe, but have also experienced a reduction in toxicity by more than 50% from the initial condition. This change reflects the effectiveness of the soaking process as a sustainable detoxification method. Soaking for 7 days (treatment E) showed the best results with HCN levels of only 16.458 ppm. This value is far below the dangerous threshold and can be considered safe for consumption. Even in the context of international standards, this value indicates that the bamboo shoots are free from toxic risks. Therefore, a soaking time of 7 days can

be considered as the optimal time for detoxification of bamboo shoots. This 7-day soaking time is also in line with the traditional fermentation practices of Lemea-making communities. Thus, the results of this study not only confirmed the local practice but also reinforced it with a strong scientific basis.

The consistent decrease in HCN levels from day to day shows that the water soaking process is not only effective but also efficient in reducing the toxicity gradually. The decrease is linear and stable, indicating that the method can be replicated with predictable results. This efficiency is a distinct advantage over chemical or other high-tech methods that are not always available in rural areas. With this approach, communities can empower local foodstuffs to be safer. Cyanide is known to have physical properties easily soluble in water, soaking with water can break down or decompose HCN from cyanogenic glycoside bonds, so that a lot of HCN is dissolved and carried away by water. Haris *et al.,* (2023) have conducted research with the case of cyanide detoxification in bamboo shoots and gadung tubers using four soaking media. Based on statistical tests, the significance value of $p = 0.000$ with a value of ($p < 0.05$) indicates that the concentration of media and soaking time affects its ability to detoxify cyanide.

Diffusion and osmosis processes occur during immersion in water. Diffusion during soaking occurs by dissolving the remaining substances in the bamboo shoots. This is indicated by the condition of the water which changes color or bubbles. Osmosis occurs with water whose low solution content will move into bamboo shoots with high solution content so that the bamboo shoots will contain high water content. The decrease in cyanide levels in bamboo shoots does not only occur through the process of dissolving into water, but is also influenced by the fermentation process which produces cyanide-degrading enzymes, such as the enzyme linamarase. This enzyme has the ability to break down glycoside compounds that produce cyanide (Adeleke *et al.,* 2017). Although the fermentation process is carried out in a closed container, enzymatic activity and fermentative microbes still take place optimally, so that they can break down cyanide compounds without relying on the HCN gas vaporization process.

The length of fermentation time is proven to have a significant influence on the effectiveness of cyanide compound detoxification (Hermanto & Fitriani, 2018). This effectiveness is strongly influenced by various environmental conditions during the fermentation process, including pH, temperature, limited oxygen levels, and the type of microorganisms that develop. In the context of the closed fermentation applied in this study, the anaerobic environment tends to favor the growth of certain microbes that play a role in the detoxification process, allowing for a significant reduction in cyanide levels. Traditionally, Lemea makers often carry out the fermentation process for 4 to 7 days based on hereditary experience, without any clear scientific reference regarding food safety, especially in relation to cyanide content in bamboo shoots. There are not many scientific references that validate the safety of these processed products. This study addresses this gap by providing supporting quantitative data. This will help in the development of safe and quality Lemea production standards. Traditional practices backed by scientific data will be more easily accepted by the wider community and the food industry.

This study provides evidence that soaking for 7 days is the optimal time to reduce HCN levels to safe levels, namely 16.458 mg/Kg. This finding is expected to provide new insights for the community, especially for traditional food processors, regarding the importance of determining the right length of fermentation time in reducing the content of natural toxins such as cyanide. Thus, the resulting products will be safer for consumption, while increasing awareness of better

and scientifically-based food processing practices. The results showed that the length of soaking had an effect on reducing HCN levels in ship bamboo shoots. The longer the soaking time, the lower the HCN levels detected. The decrease in HCN which is getting bigger along with the increase in soaking time is also supported by Oman *et al.,* (2022) who also examined the decrease in cyanide levels in yellow bamboo shoots (Bambusa vulgaris Schrad. Ex J.C) with the soaking method that the length of soaking is very effective in reducing HCN in bamboo shoots where the cyanide content obtained is 0 ppm with a percentage decrease of 100%.

In this study, soaking for 7 days resulted in the lowest HCN levels compared to other treatments. This finding indicates that the longer the soaking time, the more HCN dissolved in the water. Soaking for 7 days is recommended because the HCN content is safe for consumption at 16.458 ppm. According to FAO (Food and Agriculture Organization), the limit of safe consumption and harmless is less than 50 ppm, moderately toxic levels are 50-100 ppm and very dangerous at levels greater than 100 ppm. The importance of paying attention to fermentation time in traditional food processing, as well as proving that the local community's practices in processing lemea have a scientific basis. Furthermore, further research can be directed at testing other factors so that more comprehensive lemea quality standards can be obtained in terms of both safety and sensory quality.

4. CONCLUSION

This study proved that soaking in water can significantly reduce cyanide acid (HCN) levels in bamboo shoots. There was a sharp decrease in HCN levels from the treatment without soaking (A) with 70.066 ppm, to 16.458 ppm after soaking for 7 days (E). These results indicate that the soaking process contributes significantly to the detoxification of HCN from bamboo shoots. This decrease in HCN levels indicates that water immersion is an effective method to reduce HCN content in bamboo shoots. Cyanide acid is water soluble, so during the soaking process, HCN released from plant tissues can move to the soaking water. This process is supported by diffusion and osmosis mechanisms, which occur when soluble compounds from within the bamboo shoot tissue migrate to the external environment (water), as well as the entry of water into the tissue which can trigger the release of toxic compounds. The results showed that the longer the soaking time, the more effective the process of reducing HCN levels in ship bamboo shoots, especially treatment E (7 days) which was the most effective. This result is in line with Haris *et al.* (2023) and Oman *et al.* (2022) who stated that the length of soaking affects the success of cyanide detoxification. Based on FAO standards, HCN levels below 50 ppm are categorized as safe for consumption, so bamboo shoots that have been soaked for 7 days (16.458 ppm) can be considered suitable for consumption in terms of toxicological safety. Therefore, this simple soaking method can be a practical solution in processing bamboo shoots before consumption or processed food.

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