Characteristics of chemical properties of fruit flour of mangrove (Bruguiera gymnorrhiza Lamk.) with lower cyanide content

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Abstract. This study aimed to determine the best method for reducing HCN levels of mangrove (Bruguiera gymnorrhiza Lamk.) flour and analyze mangrove flour’s chemical properties. This study used a completely randomized design with a treatment level, namely boiling with water for 30 minutes at 100°C, immersion in distilled water, immersion in 0.8 M NaOH solution, immersion in KOH solution 0.8 M, immersion in 0.8 M Na₂CO₃ solution, and immersion in 0.8 M Na₂HPO₄ solution. All immersion treatments were carried out at room temperature for 24 hours. The experimental parameters included HCN, ash, moisture, lipid, carbohydrate and protein contents. The research data were analyzed using analysis of variance and continued with the Tukey test. The results showed that the treatments that were tried to reduce HCN levels could also be used for mangrove fruit flour. The boiling treatment that is usually carried out by the community in Tual, Southeast Maluku is more effective when compared to other treatments in an effort to reduce HCN levels. There were also differences in the chemical characteristics of the mangrove flour with the various techniques that were tried. Boiling treatment for 30 minutes showed the highest reduction (84.14%) with HCN levels of 1.95 mg/kg. The characteristics of mangroves with the lowest levels of HCN were: 14.17% ash content, 12.08% moisture content, 0.51% lipid content, 67.76% carbohydrate content, and 3.64% protein content.

1. Introduction
Mangrove (Bruguiera sp.), also known as “Lindur” or “Tancang” in Central Java [1], thrives in river areas and river estuaries along the muddy coast with low salinity. Mangrove ecosystems provide protection for the beach, stabilize the soil and serve as a habitat for various fauna [2] and has economic benefits [1]. There are several mangrove species, but the one commonly used as a local food source is B. gymnorrhiza Lamk. Almost all parts of mangroves can be used, such as “Pepagan” (stem bark), leaves [2] and fruit [1,3]. That mangroves are suitable for processing fruit into flour because of their high carbohydrate content which can also be processed into other food products [3].

Mangrove fruit has not been fully utilized because some people still do not know better processing techniques. Mangrove trees that are two years old are already productive [3]. The people in the Tual area, Southeast Maluku Regency, have processed mangroves and produced flour, which is then processed into traditional food ingredients. Mangroves are used as a food source during famine [2].

The research conducted by IPB in collaboration with the East Nusa Tenggara Food Security Agency showed that mangroves' energy content was 371 calories/100 g, higher than rice (360 calories/100 g) and corn (307 calories/100 g). Mangroves' chemical composition includes a water content of 73.76%, a lipid content of 1.25%, a protein of 1.13%, and an ash content of 0.34%. The carbohydrate content of
mangrove fruit was 85.1 g/100 g, higher than rice (78.9 g/100 g) and corn (63.6 g/100 g) [4]. Mangroves' nutritional content is complete enough to be processed into cakes and cakes mixed with coconut spices [5].

The limiting factor for mangrove fruit consumption is anti-nutritional content, namely tannins and hydrogen cyanide (HCN). Some other plants that contain HCN include cassava [6–8], sorghum [9], koro benguk [10], koro nuts [11] and flax [12]. Those containing tannins include betel nut [13]. HCN is a dangerous compound if ingested in doses of 0.5-3.5 mg/kg body weight because it can kill humans [14].

HCN formation activities can be eliminated or reduced through cooking, boiling, or soaking. The soaking method is generally used to remove or reduce the antinutrient content. The immersion medium can be water, salt or alkaline solutions [10]. The treatments that have been carried out to reduce HCN levels are boiling and soaking cassava [7,8], soaking koro benguk in Na₂HCO₃ solution [10], soaking koro nuts [11], as well as for blanching and soaking in calcium solution [15]. As for mangroves, they are immersed in a solution of husk ash. Soaking mangrove fruit in 30% husk ash solution for 36 hours succeeded in reducing the HCN level to 3.36 mg/kg [16].

Decreasing HCN levels in plant tissues such as cassava can be done by boiling and soaking, and immersion in sodium bicarbonate (NaHCO₃). Boiling and soaking can cause cyanide is easily dissolved in water, and its boiling point is 29°C [8]. Immersion in sodium bicarbonate, alkaline, makes it easier to break down cyanide [7,10]. Besides, it can also reduce the hydrolysis of cyanogenic glucoside compounds, namely linamarin. Hydrolysis of linamarine with linamarase produces acetone cyanohydrin and glucose. The cyanohydrin acetone, in turn, has HCN and acetone [17].

Thus, alkaline treatment can reduce the HCN content in a food ingredient. However, so far, the use of alkaline solutions such as NaOH, KOH, Na₂CO₃ has not been carried out to reduce mangroves' HCN levels. When compared to the percentage decrease between the use of the alkaline solution and husk ash, it appears that the alkaline solution gives a higher percentage of HCN reduction (range 24-70%) [7,10] than rub or husk ash (15%) [18]. Thus, the alkaline solution is maximal in reducing the HCN levels of mangroves.

The processing of mangrove fruit into flour with low HCN content is a semi-finished product that can extend its shelf life and be processed further. The processing semi-finished products are one way of preserving crops, especially for commodities with high water content, such as various tubers and fruit [19]. Flour has a low water content with a composition of specific chemical properties. It is more flexible in being applied to various types of food preparations so that it is hoped that it will be easier to introduce to the public. The procedure for making flour is very diverse, differentiated by the properties and chemical components of foodstuffs. However, in broad terms, they can be grouped into two, namely: 1) foodstuffs that do not turn brown quickly when peeled (cereal group); and 2) foodstuffs that promptly turn brown (a group of various tubers and fruits that are rich in carbohydrates) [19]. From this group, mangroves are included in the foodstuff group that turns brown quickly.

In addition to the low HCN content of mangrove flour, it is also necessary to know the chemical properties of flour, such as moisture, ash, protein, lipid, and carbohydrate content. The mangrove fruit flour with low HCN met the requirements as a food ingredient [16]. Thus, research is needed to obtain an appropriate soaking method with an alkaline solution and has the best effectiveness in reducing HCN levels of mangroves to a safe limit for consumption and characterizing the chemical properties of the mangrove flour it produces. This study’s objectives were to determine the best method to reduce mangrove fruit flour's cyanide content and describe its chemical properties.

2. Methods
Mangrove fruit used in this study originated from the Tual, Southeast Maluku district, Indonesia. Fresh mangroves fruit are stored properly and then prepared in the laboratory.

2.1. Preparation of fruit flour of mangrove
All steps were carried out at room temperature, which ranged from 28 to 33°C. The freshly harvested mangrove fruits were peeled, washed with water and then was cut into pieces with a size of 1 cm. Mangrove pellets were boiled with water for 30 minutes, soaked in distilled water for 24 hours; soaked in 0.8 M NaOH (Merck, Germany) solution for 24 hours; soaked in 0.8 M KOH (Merck, Germany)
solution for 24 hours; soaked in 0.8 M Na$_2$CO$_3$ (Merck, Germany) for 24 hours; and soaked in 0.8 M Na$_2$HPO$_4$ (Merck, Germany) for 24 hours. Furthermore, mangrove pellets were dried using a cabinet dryer at 40°C for 48 hours.

2.2. Total cyanide content
The total cyanide content of mangrove fruit flour was determined by the method of Askhurahman [6]. Five g of mangrove flour is put into a 250 mL measuring flask, then 250 mL of distilled water is added, then shaken, and after that, the distilled water is added again until it is precisely 250 mL, then shaken again. Next, take 1 mL and mix it with 1 mL of 0.1 N NaOH and 5 mL of 0.5% picric acid at pH 11 in a test tube. Then boil for 30 minutes and vortex. The results are then read with a spectrophotometer UV-Vis (Shimadzu, Japan) at a wavelength of 480 nm.

2.3. Ash and moisture contents
Standard AOAC methods [20] were used to measure moisture and ash. Moisture content (%, w/w) was assayed by weight loss on drying at 105°C for five h in a hot air oven (Memmert, Germany).

The ash (%, w/w) was determined by incinerating known sample weights in a muffle furnace (Vulcan A-550 Ney, USA). Starch was burned in a furnace overnight (12 h) at 550°C and cooled down in a desiccator.

2.4. Lipid content
Approximately 5 g of homogenate was weighted and dried in oven 105°C for 2 hours. Removed from the oven, and samples were cooled to room temperature. Cotton has covered the sample and put it into extraction tubes. Dried and weighted extraction balloons were attached to the extraction device. Petroleum ether (Merck, Germany) used as a solvent added into tubes. Extraction completed approximately in 4-5 hours. Balloons were dried in an oven for about 45 min. After cooling to room temperature in a desiccator, balloons were weighted.

2.5. Carbohydrate content
Carbohydrate content was determined by the method of Anthrone. Carbohydrates are dehydrated by conc. H$_2$SO$_4$ (Merck, Germany) to form furfural. The reagent's active form is anthranol, the enol tautomer of Anthrone, which reacts by condensing with the carbohydrate furfural derivative to give a green colour in dilute and blue colour in concentrated solutions, which is determined calorimetrically. The blue-green solution shows an absorption maximum at 620 nm, using spectrophotometer UV-Vis 1800 (Shimadzu, Japan).

2.6. Protein content
The sample was weighed as much as 0.5 g, added with 2 g of selenium (Merck, Germany) and 25 mL of concentrated H$_2$SO$_4$ (Merck, Germany), then put in a 100 mL Kjeldahl flask. The destruction process is carried out until the solution becomes clear greenish. After cooling, then placed in a 100 mL volumetric flask and diluted with distilled water to the boundary mark. As much as 5 mL of 30% NaOH was put into a distillation flask, then distilled for 5 minutes. The distillate was accommodated as much as 10 mL with 2% boric acid (Merck, Germany) and BCG-MR (Merck, Germany) indicator. The amount of reacted boric acid with ammonia could be identified by 0.01 HCl (Merck, Germany) titration. A change solution color marked the end of the titration from blue to pink. Protein content was calculated by the following formula as mentioned in the previous method [21].

2.7. Statistical analysis
Data were statistically analyzed by analysis of variance test procedure, and significant differences were identified by Tukey’s test ($p < 0.05$) using Minitab 18 software (Minitab, Inc.).
3. Results and Discussion

3.1. HCN levels for mangrove flour

The results showed that the HCN content of the mangrove flour was 12.3 mg/kg. The levels of HCN in these mangroves are still very high so that if consumed directly without any processing, there is concern that it can cause poisoning. This study showed that HCN levels' value was lower than that of the previous study [16] 31.68 mg/kg for direct powdering, but thorough soaking using a bleach solution yields 12.96 mg/kg.

After boiling or soaking treatment, the HCN levels of mangrove flour decreased to 1.95-3.22 mg/kg or reduced by 73.82–84.14 mg/kg (Table 1). The results of the analysis of variance showed that the immersion treatment had a significant effect on HCN levels ($p < 0.05$). The highest average HCN level of mangrove fruit flour was 3.22 mg/kg. It was indicated by the immersion treatment with KOH 0.8 M. Based on the Tukey test ($\alpha = 0.05$), it was significant differences from the immersion treatment with distilled water for 24 hours (2.18 mg/kg), immersion treatment with 0.8 M NaOH (3.07 mg/kg) and immersion treatment with 0.8 M Na$_2$CO$_3$ (2.82 mg/kg).

The results showed that the boiling treatment for 30 minutes resulted in decreased HCN levels reaching 84.14%, higher than other treatments. A relatively similar effect was also demonstrated for yam tuber [15]. The blanching process of unpeeled yam tuber for 30 minutes combined with immersion in clean water reduced HCN levels to 4.12 mg/kg. This may be due to the higher softening of mangrove fruit tissue at high temperatures. The softer the fruit tissue, the easier the process of removing linamarin from the pulp. The immersion resulted in softening of the tissues and pores so that the transfer of material that could pass through the permeable membrane occurred [15].

The HCN compounds are volatile in the boiling, steaming, and other cooking processes [11]. Compared with immersion in alkaline conditions (pH 12), it can also cause softening of mangrove fruit tissue. Softening the tissue in foodstuffs such as fruit and tubers using an alkaline solution is known as lye peeling; the softening is lower. Also, HCN is water-soluble, so HCN that has been dissolved in water or alkaline solution will be wasted after the immersion is complete. Similar results are also shown by the researcher [7] on cassava's soaking treatment using an alkaline solution of NaHCO$_3$. The immersion resulted in a 74% reduction in HCN levels. The softer the fruit tissue, the easier the process of removing linamarin and lotaustralin from the fruit.

The decrease in HCN levels is caused by boiling or soaking the linamarin compound will be hydrolyzed (reacting with water) and form cyanide acid soluble in water. The hydrolysis of linamarin will form HCN, which is water-soluble and volatile [7]. This condition causes linamarin levels to be lowered through the immersion process. During the hydrolysis process carried out by $\beta$-glucosidase on cyanogenic glucosides, some of the sugar and hydroxynitrile will be partially separated or enzymatically into cyanide and a mixture of carbonyl (ketose and aldose) [22].

High boiling temperatures also spurred the decrease in HCN levels. In the boiling process, more HCN is dissolved than in the immersion process because it is easy for HCN to evaporate when the temperature rises (HCN boiling point 26.5°C). Also, at high temperatures, it can cause the inactivation of the linamerase enzyme in mangroves to reduce the hydrolysis of linamarin or stop the formation of HCN. Linamarin's hydrolysis with linamarase produces acetone cyanohydrin and glucose. Cyanohydrin acetone spontaneously at pH above 5 has HCN and acetone [17].

Immersion in 0.8 M Na$_2$HPO$_4$ for 24 hours showed a lower HCN levels reduction than immersion in Na$_2$CO$_3$, NaOH and KOH. This is because the pH value shown in the Na$_2$HPO$_4$ solution is around 6.6, which is the optimum pH condition for $\beta$-glucosidase enzyme activity. The more effective softening and the inactivation of linamarase can hydrolyze linamarin in alkaline conditions. The optimum activity of $\beta$-glucosidase and linamarase enzymes is pH 5-6 [23]. The linamarase enzyme's optimum pH isolated from cassava tubers is pH 6. Conditions above six can cause enzyme inactivation [6]. The protein denaturation can be caused by enzyme inactivation [23].
Table 1. Cyanide, moisture, ash, protein, lipid and carbohydrate contents of fruit flour of mangrove after treatments.

| Treatments                              | Cyanide content (mg/kg) | Moisture content (%) | Ash content (%) | Lipid Content (%) | Carbohydrate content (%) | Protein content (%) |
|-----------------------------------------|-------------------------|----------------------|-----------------|-------------------|--------------------------|---------------------|
| Boiling with water (30 min)             | 1.95 c                  | 12.08 a              | 14.17 a         | 0.51 bc           | 67.76 c                  | 3.64 bc             |
| Soaked in distilled water (24 hr)       | 2.19 bc                 | 6.62 e               | 12.95 b         | 0.54 b            | 74.31 ab                 | 4.08 ab             |
| Soaked in 0.8 M NaOH (24 hr)            | 3.07 a                  | 12.00 a              | 11.72 c         | 0.41 c            | 72.41 b                  | 2.78 d              |
| Soaked in 0.8 M KOH (24 hr)             | 3.22 a                  | 8.14 c               | 8.84 e          | 0.69 a            | 76.75 a                  | 3.83 abc            |
| Soaked in 0.8 M Na₂CO₃ (24 hr)          | 2.83 ab                 | 10.09 b              | 11.68 c         | 0.45 bc           | 73.09 b                  | 3.59 c              |
| Soaked in 0.8 M Na₂HPO₄ (24 hr)         | 2.01 bc                 | 7.68 d               | 10.09 d         | 0.75 bc           | 75.98 a                  | 4.24 a              |

Note: Means in the same column without the same letter differ ($p < 0.05$) according to the Tukey test ($\alpha = 0.05$)

3.2. Ash content of mangrove flour

The results showed that the mangrove flour's ash content ranged from 14.17–10.09% (Table 1). The results of the analysis of variance showed that the treatment had a very significant effect on the ash content of mangrove fruit flour ($p < 0.01$). The highest average ash content was shown by mangroves boiled for 30 minutes (14.17%), while the lowest ash content was offered by mangroves immersed in 0.8 M KOH for 24 hours (8.84%). The Tukey test results ($\alpha = 0.05$) showed that the boiling treatment for 30 minutes was significantly different from the immersion treatment in distilled water for 24 hours, immersion treatment with 0.8 M NaOH and immersion treatment KOH solution 0.8 M for 24 hours.

Immersion treatment in several alkaline solutions reduced the ash content of mangrove fruit flour by a range of 8.84–11.72%. The dissolving of mineral molecules can cause a decrease in ash content during immersion into the immersion medium. The effect of pH treatment on ash content illustrates that a high pH (KOH 12.9) solution can reduce the ash content significantly compared to other treatments. This is because KOH can break the bonds of complex compounds, lignin, cellulose and pectin and compounds forming cell walls as part of the complex compounds that make up food minerals. The decomposition of these compounds becomes simpler, resulting in a decrease in ash content [24]. Compared with the ash content of some yam flour 3.78%, the ash content of the mangrove flour shows a relatively high value. The lower the ash content of the flour, the better it will be applied in the food industry.

3.3. Moisture content of mangrove flour

The results showed that the mangrove flour's moisture content ranged from 6.62–12.08% (Table 1). The results of the analysis of variance showed that the treatment had a very significant effect on the moisture content of mangrove fruit flour ($p < 0.01$). The results showed that the highest water content was obtained in the boiling treatment for 30 minutes (12.08%), while the lowest water content was obtained in the immersion treatment with distilled water for 24 hours (6.62%). The Tukey test results ($\alpha = 0.05$) showed that the boiling treatment for 30 minutes was not significantly different from the immersion treatment in 0.8 M NaOH for 24 hours but was significantly different from other treatments.

Boiling mangrove fruit for 30 minutes and immersing in 0.8 M NaOH for 24 hours causes the particles to become more porous when compared to other treatments, thereby increasing the ability to
absorb water. After drying, the water is trapped in the material. Immersion with a strong alkaline solution and its salts can cause changes in the starch granule structure, making it more porous so that it is easier to absorb water [14]. Meanwhile, immersion in distilled water without heating will not change the starch granule structure so that it provides the lowest water content compared to other treatments. The water content obtained in this study was still included in the water content of commercial flour (11-12%) [25].

3.4. Lipid content of mangrove flour
The results showed that the mangrove fruit flour's lipid content ranged from 0.41–0.75% (Table 1). The analysis results showed that the treatment significantly affected mangroves' lipid content (p < 0.01). The highest average lipid content was found in the immersion treatment in 0.8 M Na$_3$HPO$_4$ solution for 24 hours (0.75%). The Tukey test results ($\alpha = 0.05$) showed that the treatment was not significantly different from the immersion treatment in the 0.8 M KOH solution for 24 hours but was significantly different from other treatments.

During the immersion process, the mangroves will be hydrolyzed due to contact with the solution, causing the lipid content to decrease due to oxidation. Other researcher showed that soaking and boiling treatments could significantly reduce lipid levels [24]. Treatment of 0.8 M NaOH and 0.8 M Na$_2$CO$_3$ solution for 24 hours reduced the lipid content lower than the other treatments. This is because the NaOH solution can break down lipid into water-soluble glycerol and fatty acids to not accumulate in the fruit. Compared with the immersion treatment using KOH with Na$_3$HPO$_4$, the value of lipid content is higher, which is because the KOH and Na$_3$HPO$_4$ solutions only damage the lipid globules and are not water-soluble but settle in the fruit. Hence, the results of the lipid analysis are high [14].

3.5. Carbohydrate content in mangrove flour
The results showed that the mangrove flour's carbohydrate content ranged from 67.76 to 76.75% (Table 1). The analysis results showed that the treatment significantly affected carbohydrate content (p < 0.01). The soaking treatment led to the highest average carbohydrate content with 0.8 M KOH 24 hours (76.75%). The Tukey test result ($\alpha = 0.05$) showed that the treatment was not significantly different from the immersion treatment using 0.8 M Na$_3$HPO$_4$ (75.98%) and distilled water for 24 hours (74.31%). Still, it was significantly different compared to other treatments.

The results showed that the boiling treatment showed the lowest value of carbohydrate content (67.76%). This may be due to damage to the starch granules during boiling. The starch in the fruit undergoes gelatinization at boiling temperature (± 100°C) can weaken the hydrogen bonds or breaks the glucoside bonds to cause the dispersion of glucose into the solution. Granule damage caused by immersion in distilled water and alkaline solutions is lower than boiling, indicating higher carbohydrate content. Previous research [26] also suggested that the alkaline solution can break the protein matrix's disulfide bonds that enclose the starch granules to free the starch granules or increase the separation between starch and the protein that wraps the starch granules. This resulted in higher levels of carbohydrates detected in the mangrove flour samples.

3.6. Protein content in mangrove flour
The results showed that the mangrove flour's carbohydrate content ranged from 2.78–4.24% (Table 1). The results of the analysis of diversity showed that the treatment had a very significant effect on protein content (p < 0.01). The immersion treatment with Na$_3$HPO$_4$ indicated the highest average protein content (4.24%). The Tukey test ($\alpha = 0.05$) results showed that the treatment was not significantly different from the immersion treatment with distilled water and 0.8 M KOH for 24 hours, but it was different from other treatments.

The protein content of mangrove flour in the boiling treatment was 3.64% using an alkaline solution, and the protein content decreased. This is because protein is more difficult to dissolve in acids than in alkaline conditions. The higher the concentration of immersion food, the more acidic the soaking solution is so that the protein does not dissolve easily and not much of it comes out, leaving starch granules. On the other hand, in alkaline Na$_3$HPO$_4$ immersion, the protein will form a cross-link with amyllose to quickly absorb water. The protein molecules diffuse, leaving the granules dissolving in a soaking solution that will be wasted during the mangroves' washing process. The higher the
concentration or strength of the alkaline solution will affect the protein content. The protein content of the ingredients soaked with Na$_2$HPO$_4$ is higher than the other solutions.

pH gives varying results on decreasing protein levels where NaOH solution's pH solution with a pH of 12.6 can reduce the largest protein levels. It can be left in mangrove fruit flour is very low. This is by the decrease in HCN levels in mangroves soaked with NaOH compared to KOH solution, both of which have high pH (KOH 12.9). Meanwhile, the Na$_2$CO$_3$ solution (pH 8.7) reduced mangrove flour's protein more than the immersion treatment with Na$_2$HPO$_4$ solution (pH 6.6). This is because, at high pH, there is damage to protein.

4. Conclusion

All treatments tested were able to reduce levels of HCN mangrove fruit flour. However, the fruit of mangrove treated with boiling water for 30 min showed the highest reduction in the cyanide content, which was 84.14% and were having the final content of 1.95 mg/kg cyanide content. Mangrove fruit flour characteristics with the lowest cyanide content had 4.17% ash, 12.08% moisture, 0.51% lipid, 67.76% carbohydrate, and 3.64% protein contents.

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