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Proximate and Toxicity Analysis and The Utilization of Durian Seed Flour (*Durio Zibethinus* Merr)

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Abstract: To preserve the environment and ensure food availability and sustainability, this research aims to utilize the abundant durian seeds to be a safe-to-eat and nutritious flour, which could produce many products such as crackers and other non-gluten products, which are in demand recently. This research is consisted of two steps: (1) making the durian seed flour in 3 ways (T1, T2, and T3) and subjected into proximate and severe toxicity tests to find the best way of making it, and; (2) processing the T3 flour into crackers, which subjected to proximate and sensory tests. The research concludes that T3 is the best flour due to its nutritious content and has the LD50 score of the toxicity test to be less than 1000 ppm. The produced crackers consist of 1.82% water, 13.77% protein, 1.73% fat, 4.36% ash, 89.09% carbohydrate, and 2.15% crude fiber. The cracker's sensory test results of color, taste, and crispness are 6.18 + 0.09, 6.12 + 1.03, and 6.31 + 1.01, which means panelists like these non-gluten crackers.

Keywords: Durio; flour; toxicity; crackers; non-gluten.

1. Introduction

As the world population is expected to reach 9 billion by 2040, there will also be food availability limitations [1]. Food scarcity would not only be related to the foodstuff's bioactive compound content as the agent of anti-inflammation, anti-hyper-cholesterol, anti-diabetics, et cetera, but also its availability and sustainability [2]. Moreover, the Covid-19 pandemic diminished people's economic condition and purchasing power [3], so innovation is needed to create edible and nutritious foodstuffs from affordable, easy-to-obtain, and safe ingredients.

On the other side, despite the consumption of durian fruit reaching 242,000 tons/year due to its nutritious and delicious taste [4], only a third portion of this fruit is edible. The rest of the fruit is the seeds (count as 20-25% of the fruit) and skin [5]. Generally, the skin and seeds are mostly dumped, and only a tiny part is recycled as cattle food and bioethanol [6]. From durian seeds and skin, the seeds can be utilized as foodstuffs due to their content of 46.2% carbohydrate, 2.5% protein, 0.2% fat, and 51.1% water[7]. According to [8], its carbohydrate content is higher compared to other frequently consumed carbohydrates such as cassava (36.8%) and sweet potato (20%). This high carbohydrate content could be utilized further as foodstuffs such as flour, which could be the base of processed food such as cookies, bread, and snacks such as crackers, et cetera. Cracker is one of the snacks favored by people of all ages.

However, the raw durian seeds cannot be eaten raw due to the content of the toxic substance of cyclopropene fat acid in the form of mucus, which is dangerous if consumed at more than 10 ppm [9]. The seed also contains oxalate. The cyclopropene fat acid could act as a sedative. Still, it could affect the body mechanism, such as the inability to dissolve fat which could increase body fat and reduce appetite [10]. The cyclopropene fat acid is handled by sulfation, by flowing sulfate substance into fat, or through high heating to detach the cyclopropene group [11]. The use of sulfate substances should be avoided due to their harmful health effects. Therefore, high heating is the preferred method to remove the toxic compound in this research because it is natural, accessible, and affordable [7].

The making of durian seed flour has been conducted through various methods, such as keeping durian seeds for eight months before being made into flours [12], fermentation [13], and vacuum method [14], which require time and using expensive equipment, so it is still less applicable and has not subjected to toxicity test. Based on the methods mentioned above, this research is conducted to determine the correct method to produce nutritious and safe-to-eat (through toxicity test) durian seed flour, which later could be utilized as the ingredients for making snacks such as non-gluten crackers and adding variations of non-gluten products that are being in great demand recently.

2. Materials and Methods

This research consists of two stages: (1) the early stage to look for the best method of making durian seed flour (T), reviewing the lowest toxicity level and its nutrition content; and (2) the utilization of the best T as a product to determine the exact T concentration during cracker making to achieve the best chemical and organoleptic quality.

2.1. The making process and proximate test of durian seed flour (T).

T's making process starts by cleaning from its remaining flesh, washing, and sorting. The selected seed the processed further into the flour using three different treatments: (1) Treatment 1 (T1 flour), from thinly-sliced and unpeeled fresh durian seed, which then sun-dried until dry (its water content reached 8%) and later dry-blended and sifted with an 80-mesh sieve; (2) Treatment 2 (T2 flour), from fresh durian seed that being roasted and peeled before being thinly sliced and sun-dried to achieve 8% water content and later being dry-blended and sifted with an 80-mesh sieve; and Treatment 3 (T3 flour), from the fresh durian seeds being boiled at 100^{o}C for 15 minutes before being peeled, thinly sliced, and sun-dried to achieve 8% water content and later to be dry-blended and sifted with an 80-mesh sieve.

All flours produced were then subjected to proximate analysis, which consisted of water content analysis through the oven method, protein analysis through the Kjeldahl method, fat analysis through the Soxhlet method, ash analysis through the dry ashing method, and carbohydrate analysis through the difference method [15].

2.2. Toxicity test of durian seed flour.

The toxicity test aims to know which kind of flour is the safest to consume, as shown by the lowest mortality level of Artemia salina Leach larvae. The acute toxicity test would be conducted through the Brine Shrimp Lethality Test (BSLT), which encompasses all kinds of the T, larvae preparation, sample preparation, acute toxicity test (LD50), and data analysis [16].

2.2.1. Extraction of durian seed flour.

The extraction is conducted based on research by [17] on each flour (T) being made using the maceration method, in which 100 grams of T is inserted into a 1000 mL beaker glass and poured by 200 mL of equates stirred for 5 minutes. After 24 hours, the liquid was then filtered by Whatman paper No. 42. The resulting filtrate was then moved into another glass. The same treatment is then repeated until the filtrate is moderately clear. All gathered filtrates are then put into an evaporator flask whose weight is known. The evaporation is conducted at 45°C until thickened. Afterward, the content is weighted (ET1, ET2, and ET3) and stored in a desiccator until subjected to a toxicity test.

2.2.2. Preparation of *A. salina* Leach larvae.

The *A. salina* Leach larvae eggs are hatched by soaking the eggs in artificial seawater (made by one liter of water mixed with 20 grams of non-iodized salt, then filtered and aerated) for two days. After hatching, the larvae are ready for an acute toxicity test [18].

2.2.3. Flour (T) extract preparation.

The preparation is conducted by making the 2000 ppm stock solution by dissolving the 40 mg of T extract into 20 mL of artificial seawater and then diluting it with the concentrations of 100, 0, 25, 12.5, and 0 ppm (0 ppm as the control without the addition of T extract) [19].

2.2.4. Toxicity test.

6 ml of each test solution with the concentration of 100, 50, 25, and 12.5 ppm is gathered by pipette and inserted into the test tube with ten prepped larvae on each tube. Each concentration is subjected to two-time repetition and compared to the control solution. The first observation is conducted every hour for the next six hours before continuing to the second observation, conducted on the 12th, 18th, and 24th hours by counting the dead larvae [19]. The LC50 calculation is obtained by the mortality percentage data, which is then transferred to the concentration log [20].

2.2. Utilization of the best flour as a cracker's ingredient.

The best flour is then utilized to reduce wheat flour usage in making free gluten crackers [21]. The test parameters are proximate [15]. Organoleptic test with the Hedonic (likeness) method [22], which encompasses color, taste, and crispness with seven likeness scales: very dislike (1), dislike (2), rather dislike (3), neutral (4), rather like (5), like (6), and very like (7). The data result of the organoleptic test is then analyzed using SPSS version 26.

3. Results and Discussion

3.1. Proximate analysis result of durian seed flour.

The different flour-making processes of the fresh durian seeds could result in the different nutrition content of each T (see Table 1).

Table 1. Proximate analysis result of the durian seed flour (T) through different treatment.

Parameter	T content		
	T1	**T2**	**T3**
Water content (%)	9.15	6.97	8.71
Crude protein (%)	6.31	6.72	6.12
Fat (%)	0.42	0.67	0.74
Ash (%)	4.75	3.14	4.36
Carbohydrate (%)	79.37	82.50	80.07
Dry content (%)	90.23	94.79	92.16
Crude fiber (%)	1.91	2.21	1.73

[1] Note: the data result is the average number of three repetitions.

Table 1 saw the water content of all durian seed flour treatments is below 10%, with the lowest in T2 treatment because this treatment includes a roasting method, so more water evaporates than T1 and T3. According to [23], the flour would be more durable if its water content was less than 15%. Overall, the roasting and boiling treatment tends to increase the flour's nutrition content, which follows the research conducted by [24], which stated that roasting could increase an ingredient's nutrition content. In T3, the nutrition content is lower than in T2, except for fat and ash. This result is due to the peeling and boiling in T3 treatment, which is following [25], who said that the skin peeling, boiling, and soaking process could lower the nutritional content of an ingredient.

3.2. Acute toxicity of durian seed flour extract.

The result of the Artemia salina Leach larvae's mortality rate after being given the T extract treatment in ranges of doses within 24 hours of the observation period can be seen in Table 2 below.

Table 2. The mortality rate of *Artemia salina* Leach larvae after being given the T extract treatment for 24 hours.

Time (hours)	**Extract concentration (ppm)**														
	TI					**T2**					**T3**				
	0	12.5	25	50	100	0	12.5	25	50	100	0	12.5	25	50	100
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
6	0	0	1	1	2	0	0	0	0	0	0	0	0	0	0
12	0	0	2	3	5	0	0	0	0	1	0	0	0	0	0
18	0	1	4	7	10	0	0	1	1	1	0	0	0	0	0
24	0	5	8	10	10	0	0	3	6	8	0	0	0	0	1
% M	0	50	80	100	100	0	0	30	60	80	0	0	0	0	10
LC_{50}	7.234					15.016					1000				

[2] Note: %M= mortality percentage.

A toxicity test is conducted to know whether a certain ingredient is safe to consume, and the most common method used is the BSLT method [26]. This method uses Artemia salina Leach larvae as the bioindicator. The observation conducted counts the mortality percentage of the dead larvae as the toxicity analysis. The percentage is gathered from the quantitative comparison of dead larvae and test larvae. Further action is to calculate the LD50 to determine the toxicity concentration. LD50 (Lethal Dosage 50% of Responses) is the dose that could give a 50% mortality response from all people consuming it [27]. According to [28], if a certain extract has an LD50 score of less than 1000 ppm, the extract is deemed toxic.

Table 2 shows that the larvae's mortality and LD50 score that less than 1000 ppm occurred in T1 and T2 extract treatment. Based on the said results, the best flour is T3, which is considered safer to consume because it does not cause larvae death and contains good nutrition.

3.3. Proximate analysis result of snacks.

The chosen product in this durian seed flour utilization is crackers because it is commonly favored by people of all ages [29]. In this research's context, the product would be free-gluten crackers, a food diversification that provides processed food for people avoiding gluten consumption, such as autistic children [30]. The proximate analysis result of the free gluten crackers from durian seed flour is illustrated below in Table 3.

Table 3. Proximate analysis result of free gluten crackers.

No.	Parameter	Average value
1.	Water content (%)	1.82
2.	Crude protein (%)	13.77
3.	Fat (%)	1.73
4.	Ash (%)	2.77
5.	Carbohydrate (%)	89.09
6.	Crude fiber (%)	2.15

Crackers do not require eggs but a fermentation phase, which is different from biscuits that require eggs and skip the fermentation process. Crackers' making requires additional ingredients such as butter, sugar, skim milk, salt, sodium bicarbonate, water, shortening, and palm oil [31] to enhance the cracker's nutritional value.

The resulting crackers have low water content, which confirmed previous research that stated lower water content equals more crispness and durability of a product [32]. Crackers with durian seed flour as its ingredients contain more protein than crackers made in the research conducted by [33], which are made with wheat flour and the addition of *Hibiscus sabdariffa* extract.

3.4. Organoleptic test of crackers from durian seed flour.

Crackers usually contain wheat flour as the main ingredient, supported by other additional ingredients. Crackers have a savory taste and crispy texture, are thin-shaped, and are durable, so it favored by all people [34]. The organoleptic test conducted by 25 semi-trained panelists comprises flavor, crispness, and color. The result is shown in Table 4 below.

Table 4. Mean ± SD and P-value of crackers' Hedonic test.

No	Parameter	Mean ± SD	P-value
1.	Color	6,18 ± 0.09	0,192
2.	Flavor	6,12 ± 1, 03	0,189
3.	Crispness	6,31 ± 1,01	0,162

A sensory test is a method that depends on responses from all five senses. Color is the earliest scoring aspect before continuing to flavor, crispness, aroma, et cetera. This becomes more important if the product tested is new. If the color does not look attractive, it negatively affects other parameter scores. It is also possible that panelists would refuse to continue scoring.

On the contrary, if the panelists deem the color attractive, they will also be interested in scoring the flavor because it determines whether a product is being accepted. Another important sensory parameter of cracker products is crispness because it is one of the characteristics that makes them not too long to be chewed. This is following [35], which stated that crackers made with wheat flour have crispy characteristics and are not too long to be chewed. According to de [36], crispness is closely related to the product's water content. The lower the water content, the more crisp and breakable the product.

The sensory test result of the research shows color, flavor, and crispness scores are around 6, which means that panelists liked the product. Therefore, this non-gluten cracker is expected to fulfill the increasing non-gluten product demand due to health reasons.

4. Conclusions

Fresh durian seed could be utilized as a safe-to-eat and nutritious flour if processed correctly. This research determines that the correct method is T3, where the fresh durian seeds are, in chronological order, cleaned, washed, boiled, peeled, thinly sliced, sun-dried, dry-blended, and sifted. The flour contains 8.71% water, 6.12% protein, 0.74% fat, 4.36% ash, 80.07% carbohydrate, 92.16% dry ingredient, and 1.73% crude fiber. The toxicity result of T3 showed an LD50 score of 1000 ppm, which means that it is safe to consume. The T3 flour was then processed into crackers with nutritional values of 1.82% water, 13.77% protein, 1.73% fat, 4.36% ash, 89.09% carbohydrate, and 2.15% crude fiber. The cracker's sensory test results of color, taste, and crispness are 6.18 + 0.09, 6.12 + 1.03, and 6.31 + 1.01, which means panelists like these non-gluten crackers.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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