

Fabrication of Native and Enzymatically Modified Durian Seed (*Durio zibethinus* Murr.) Starch

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ABSTRACT

Durian seed (*Durio zibethinus* Murr.) has a high starch content (46.2%) and thus can be used as a new source of starch for the raw materials of pharmaceutical and food industries. In this study, we fabricated native and enzymatically modified durian seed starch using a rough enzyme extract from *Saccharomycopsis fibuligera*. Wet grinding method was used for starch production. Physicochemical characterization of the starches was investigated by organoleptic, acidity-basicity, loss on drying, flow capability, compressibility, ash content and microbial limit. In addition, viscoamylograph had been done to clarify the viscosity properties of the starches. The result of starch production showed that the durian seed had a starch yield of 17.68%. Physicochemical characterization of the starch showed that the results of quality testing had fulfilled the Indonesian Pharmacopoeia 4th edition standards requirements, such as description, identification, acidity-basicity, loss on drying, ash content and microbial limit. In addition, viscoamylograph study showed that the enzymatically modified durian seed starch had a higher viscosity than the native durian seed starch. Interestingly, modification of the durian seed starch using a rough enzyme extract improved its flow capability and compressibility. These results suggest that the modified durian seed starch experienced an increase in viscosity, compressibility and flow capability compared to native durian starch.

Keywords: durian seed, starch, enzymatic modification

1. Introduction

Starch, a flour-like substance of carbohydrate with a glucose polymer, is one of the most useful biopolymer in the food and pharmaceutical industries as thickeners, gelling agents, texture modifiers and stabilizers [1, 2]. There are two types of starch commonly used in the pharmaceutical industry, namely, native starch and modified starch. Native starch is a starch without any changes in physical and chemical properties [3]. On the other hand, modified starch has a different characteristics compared to the original one, which can then be adjusted for certain purposes [4]. The desired characteristics of starch are a stable viscosity, a high durability, as well as a thickening capability towards mechanical and temperature changes [5].

In general, the modification methods of starch are divided into chemical, physical or enzymatic methods. Several enzymes are used in starch hydrolysis which subjected to break down its structure and to obtain the desired functional characteristics [6]. Enzymes hydrolyze the (1→4) or (1→6) bond between the α -D-glucopyranose unit in starch. The most commonly used enzymes to modify starch are α -amylase, β -amylase, glucoamylase, pullulanase, and isoamylase. These enzymes are isolated from mushrooms, yeast, bacteria, and plants [7].

Durian (*Durio zibethinus* Murr.), a tropical fruit originated from Southeast Asia with common name “king of fruit”, is one of the unique fruits in Southeast Asia due to its strong aroma and exclusive taste [8, 9]. In addition, 30-35% of the

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durian is edible, whereas one fourth of the seeds and the shell are usually as wastes [10]. Interestingly, durian seed has high starch content and potential as an alternative starch sources in pharmaceutical industry [11, 12]. In this study, to obtain modified durian seed with optimum functional characteristics, we fabricated and characterized enzymatically modified durian seed starch using a rough enzyme extract from *Saccharomycopsis fibuligera*.

2. Method

2.1. Materials

Durians were obtained from durian garden, Lampung, Indonesia. Yeast extract and sago starch were procured by Leiber, Jakarta, Indonesia. 70% ethanol, 80% ethanol, phenolphthalein 0.1%, glucose anhydrate, sodium hydroxide 0.1 N were bought from Brataco Chemical, Jakarta, Indonesia. All other reagents and chemicals were of analytical grade and used without any further purification.

2.2. Yeast production

The durian seed starch was produced using a wet grinding method [13]. 7.403 grams of durian seed were cleaned from its outer coat and epidermis, followed by cut into small pieces and then crushed using a blender with distilled water. The material was strained using a flannel cloth into a container until the residue no longer produced any liquid. Then, the suspension was precipitated for 24-48 hours until a clear supernatant obtained. The supernatant was then decanted. The starch precipitate was dried in a drying rack at a temperature of 35-40°C for 24 h. The powders were sifted using a 100 mesh sifter.

2.3. Physicochemical properties of native durian seed starch

Examination of the durian seed starch including: organoleptic, solubility, acidity-basicity, loss on drying, microbial limit, pH, real density and bulk density, pure density, flow capability, gelatinization temperature and viscosity, amylose

and amylopectin content, degree of whiteness, and proximate analysis [14].

2.4. Production of rough enzyme extracted from *Saccharomycopsis fibuligera*

S. fibuligera was grown on a slanted agar medium composed of 6% sucrose and 1.5% bacto-agar in 10% bean sprout extract, continued with an inoculum containing 1% sago starch and 1% yeast extract for 48 hours. Afterwards, the inoculum was moved into a production media which has the same composition as the inoculum, it formed 10% of the production medium. This fermentation was done at a shaking rate of 180 rpm for 72 hours. The enzyme was then strained using straining paper in cold conditions. The result was a rough enzyme extract (containing α -amylase and glucoamylase). The rough enzyme extract was added by ammonium sulfate salt at concentration of 25% (v/v) [15].

2.5. Hydrolysis of raw durian seed starch

Optimization of the starch hydrolysis used various enzyme concentrations in selected times at optimum enzyme temperature (50°C). A 5% of starch suspension was produced in a reaction mixture containing 10 ml of enzymes with various concentration (100, 200, and 300 ppm). The mixture was hydrolyzed at 2, 4, and 6 h. A sample of the hydrolyzed result was then centrifuged at 10,000 rpm for 15 minutes and its supernatant were withdrawn. The absorbance was measured at a wavelength of 420 nm [16].

2.6. Determination of the total amount of reducing sugars

The reducing sugar for the supernatant was determined using the Schales method. Briefly, as much as 200 μ L of the hydrolysis solution was placed in a closed test tube, followed by addition of 800 μ L $K_3Fe(CN)_6$, then incubated for 10 minutes. A total measurement of the reducing sugar was also done towards the dilution of the hydrolysis result samples for 5, 8, and 10 times. A standard glucose curve was made from the measurement of glucose absorbance with different concentrations

Table 1. Physicochemical properties of durian seed starch

Examination	Literature data	Durian starch seed
Organoleptic	A very soft, white powder	Soft powder, brownish-white.
Solubility	Does not dissolve at all in cold water or ethanol.	Does not dissolve at all in cold water or ethanol.
Identification:		
A. Heating until boiling point for 1 minute a suspension of 1 g of starch in 50 ml of water, then cooling.	A dilute starch solution is formed.	A dilute starch solution was formed.
B. Mixing 1 ml of the starch solution obtained from identification A with 0.05 ml iodine 0.005 M	A blue color appears which disappears when heated and reappears when cooled.	A blue color appeared which disappeared when heated and reappeared when cooled.
Acidity	Not more than 2.0 ml is needed	1.7 ml was needed
Loss on drying	Not more than 15.0%	13.04 %
Ash content	Not more than 0.6%	0.17 %
Microbial impurities limit	no <i>Escherichia coli</i>	no <i>Escherichia coli</i>

Table 2. Characterization of durian seed starch

Characteristics	Durian Seed Starch
pH \pm SD	4.26 \pm 0.02
Pure density \pm SD	2.325 \pm 0.57
Water content	87.98%
Ash content	0.17%
Protein content	3.75%
Fat content	17.51%
Carbohydrate content (rough estimate)	78.57%
Rough fiber content	11.62%

(100, 200, 300, 400, and 500 ppm), which was given the same treatment as the determination of the total reducing sugar [10, 17].

3. Results and Discussion

3.1. Starch Production

The starch yield obtained was 17.68% with a brownish-white color. During washing and drying, some starch was decreased.

3.2. Physicochemical properties of native durian seed starch

Examination on the starch quality according to the monograph in the Indonesian Pharmacopoeia 4th edition had been done including solubility, acidity, loss on drying, ash content, and microbial impurities limit compared to that of cassava starch. The results of the examination of starch quality can be seen in Table 1. The test results showed that the

durian seed starch fulfilled the requirements as stated in the Indonesian Pharmacopoeia 4th edition. Other quality examinations such as pH, pure density, water content, protein content, and fat content were also conducted and the results can be seen on Table 2.

3.3. Production of rough enzyme extracted from *Saccharomycopsis fibuligera*

The enzyme produced from 5 g of yeast extract and 5 g of starch in a 500 ml aquadest solution was 351 ml (70.2 %). By testing the enzyme activity with the Goyal et.al method, it was obtained that the activity of the extracted enzyme was 312.33 U/ml [18].

3.4. Hydrolysis of durian seed starch

From the linear regression curve of the absorbance of the standard glucose solution, the linear equation $y = 0.001x - 0.002$ was obtained,

with $R^2 = 0.999$. The results of the absorbance measurements of the optimization sample was used to determine the reducing sugar based on the linear equation of the standard glucose curve. The optimization showed that in order to hydrolyze 5% of durian seed starch, the optimum time and concentration of enzymes required is 6 hours and 200 U/ml. The yield value of reducing sugars was similar to the hydrolysis experiment published by Purnomo *et al* [16].

3.5. Physicochemical properties of modified durian seed starch

Determination of the total reducing sugars showed that the native starch had been successfully modified. The modified durian seed starch had a total amount of 148.79 $\mu\text{g/ml}$ of reducing sugars. This value is smaller than that from the optimization results, which was as much as 578.33 $\mu\text{g/ml}$.

The results of density, compressibility (Table 3), flow capability, and angle of repose testing (Table 4) showed that there were an increase in the flow properties of modified starch.

Gelation temperature is the temperature at which the starch granules burst because of the addition of water and heat. From the Brabender viscoamylograph testing, it is known that the gelatinization temperature of native durian seed is 91.5 °C. Durian seed starch does not have a peak temperature, thus it also does not have a peak viscosity value. After gelatinization, the starch's viscosity usually increases due to the lack of water, which acts as a lubricant between the expanding granules. With an increase in temperature and stirring, the viscosity value of durian seed starch is 10 BU (Brabender Units). After stirring, the viscosity of the durian seed starch increased to 35

BU. When the temperature was lowered and with stirring, the viscosity value of the durian seed starch increased to 120 BU. The increase in viscosity when the temperature was lowered was affected by the retrograding capabilities of the starch. The viscosity value of the durian seed starch remained constant by stirring without any changes in temperature. This shows that the viscosity stability of durian seed starch was at 50°C.

Table 3. Density and Compressibility Testing of Native and Modified Starch

	Native starch	Modified starch
Average bulk density (g/ml) \pm SD	0.370 ± 0.008	0.45 ± 0.012
Average tapped density (g/ml) \pm SD	0.500 ± 0.025	0.60 ± 0.021
Average pure density (g/ml) \pm SD	2.325 ± 0.565	-
Average compressibility (%) \pm SD	26.85 ± 2.45	23.02 ± 4.12

Table 4. Flow Properties of Native and Modified Starches

No.	Angle of Repose (degrees) \pm SD		Flow Capability (g/s) \pm SD	
	Without vibration	With vibration	Without vibration	With vibration
Native starch	-	-	-	-
Modified starch	24.07 ± 1.04	23.75 ± 0.76	13.14 ± 1.72	16.14 ± 2.51

Modified starch has lower gelation temperature compared to native starch, however, modified starch has a higher viscosity compared to native starch. This results showed that modified starch has more viscous compared to native starch. The temperature needed to form a gel is low but the viscosity is high.

Table 5. Flow Properties of Native and Modified Starches

	Gel Temp (°C)	Peak Temp (°C)	Peak Viscosity (BU)	Viscosity 93°C (BU)	Viscosity 93°C/20' (BU)	Viscosity 50°C (BU)	Viscosity 50°C/20' (BU)
Native starch	91.5	-	-	10	35	120	120
Modified starch	88.5	-	-	50	90	220	220

Table 6. Characterization of durian seed starch

	Amylose content
Native starch	38.89
Modified starch	30.78

The results of amylose content testing (Table 6) showed that modified starch had a lower amylose content than native starch. The amylose content affects the viscosity of the gel to be formed. The lower the amylose content, the viscous the gel.

Table 7. Degree of Whiteness testing

	Degree of Whiteness (%)
Native starch	97.93
Modified starch	84.36

The results of the degree of whiteness testing (Table 7) showed that native starch has a higher degree of whiteness compared to modified starch. Native starch has a 97.93% degree of whiteness and modified starch has an 84.36% degree of whiteness. The water used in the extraction process, the duration of precipitation and the temperature of the drying process affects the degree of whiteness of native starch. The reduction in the degree of whiteness of the modified starch can be attributed to the addition of the enzyme and the heating during hydrolysis.

4. Conclusion

We successfully fabricated native and enzymatically modified durian seed starch using a crude enzyme extracted from *Saccharomycopsis fibuligera*. Physicochemical characterization of the starch showed that the results of quality testing had fulfilled the Indonesian Pharmacopoeia 4th edition standards requirements, such as description, identification, acidity, loss on drying, ash content and microbial limit. It can be concluded that the modified durian seed starch experienced an increase in viscosity, compressibility and flow capability compared to native durian starch.

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