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Isolation and characterization of Agung banana peel starch from East Java Indonesia

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Abstract

The utilization of banana peel resulting from the banana chips processing industry is still relatively low, as it is used only as an animal feed. The exploration of potential benefits of banana peel as a source of starch may significantly improve its economic values, since it can be used in various food and non-food industries and pharmaceutical industries. A preparation method to process starch from banana peel waste was investigated in this study. The raw material used was agung banana variety of Semeru obtained from Lumajang city, East Java, Indonesia as its peel is thick and more resistant to degradation process, and was characterized for its physical and chemical attributes. The properties of resulting starch processed from Agung banana peel Semeru variety met the specifications required. Its viscosity was higher and its gelatinization temperature was relatively lower than those of cassava starch. Based on the nature of viscosity, Agung banana peel starch has a great chance to be used as an ingredient in making a tablet dosage form, in particular as a tablet binder.

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Introduction

Banana (*Musa paradisiaca*) is an indigenous plant growing widely in the South East Asian region including Indonesia. Most parts of banana, including its flower, fruit, leaf, stem, and weevil, have been used by the community. However, banana peel is often considered as a waste material of this plant, being disposed as an organic waste or used as an animal feed for goat and cow. In fact, banana peel accounts for approximately one-third of the total weight of banana fruit. Banana peel is a rich source of starch (3%), crude protein (6-9%), crude fat (3.8-11%), total dietary fibre (43.2-49.7%), and polyunsaturated fatty acids, pectin, essential amino acid, and micronutrients (K, Ca, Mg) (Mohapatra *et al.*, 2010).

Starch is a polymer with the molecular formula of $(C_6H_{10}O_5)_n$. In general, starch is composed of two polymers of D-glucopyranose type namely amylose and amylopectin. Amylose is a linear polymer of glucopyranosyl whereas amylopectin is a chain polymer of glucopyranosyl. Starch contains about 20% amylose (water soluble part) and 80% amylopectin (water insoluble part). The properties of starch is determined by the size and proportion of each polymer molecule type (Swarbrick, 2007).

Banana used in this study to produce starch,



Figure 1. Agung banana Semeru variety from Lumajang: (A) one bunch of banana consists of 13 banana fruits; (B) the length of one banana fruit: ± 40 cm; and (C) the thickness of banana fruit: ± 0.5 cm.

was agung banana Semeru variety obtained from Lumajang city, East Java, Indonesia (Figure 1). Agung

banana belongs to horns banana group. Agung banana has many advantages, among others, sweeter taste, big fruit size with the weight of 10-20 kgs/bunch (19 cm in circumference and ± 40 cm in length), as well as thick banana fruit peel (± 0.5 cm) yielding higher stability of its compositions during storage (3-4 weeks) after fruit picking. In addition, agung banana can still be consumed although the color of banana peel has turned blackish as the fruit consistency is still relatively solid (Prahardini

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et al., 2010). The aim of this study was to prepare and characterize the starch processed from agung banana peel Semeru variety, since it can be used in pharmaceutical industries, especially in tablet dosage form.

Materials and Methods

Materials

The banana peel waste was obtained from banana chips processing industry in Lumajang, East Java, Indonesia; sodium metabisulphite was purchased from PT. Lestari, Surabaya, Indonesia, and distilled water.

Preparation of banana peel starch

The preparation method of banana peel starch used in this study was a modification of the preparation method of durian seeds starch (Soebagio *et al.*, 2009). The banana peel (Figure 1) was weighed and cleansed, cut into small pieces using a knife and then was homogenized with distilled water containing sodium metabisulphite (0.3%) at a proportion of banana peel:distilled water (1:2, w/v). The resulting homogenate was filtered, squeezed using a flannel filter cloth until dry, and collected into a container. Filtrate or the resulting suspension was kept for 24 h, yielding a complete starch sediment. The starch was collected by decantation and it was subsequently dried in an oven (40-60°C) for 24 h. The drying process took place until the moisture content of starch is within the specified range. The dried starch was grounded and screened through a 24-mesh sieve.

Characterization of banana peel starch

The banana peel starch was evaluated qualitative for its sensory properties, microscopic and macroscopic observations, shape, pH, moisture content, gelatinization temperature, melting point, repose angle, Carr's index, Hausner ratio, density, viscosity, loss on drying, white color index, ash content, and amylose content.

Qualitative test and sensory properties of banana peel starch

The presence of starch was identified, as indicated by the appearance of bluish violet color upon the addition of iodine (Ministry of Health Republic Indonesia, 1995). In the sensory test, 1 g of starch was weighed and its shape, color, odor, and taste were subsequently observed (Ministry of Health Republic Indonesia, 1995).

Microscopic, macroscopic and shape observations

One hundred milligrams of starch was placed onto the object glass. Two drops of distilled water was added onto the sample, and then was observed under a microscope with a magnitude of 400x for the hyllus and lamellae of banana peel starch (Ministry of Health Republic Indonesia, 1995). For macroscopic evaluation, 100 g of starch was weighed and screened sequentially through 20-, 40-, 60-, and 80-mesh sieves. The size of starch particle was determined based on the weight proportion of starch collected on each mesh screen sieve (Ministry of Health Republic Indonesia, 1995). The particle shape of banana peel starch was observed using a scanning electron microscope (SEM), with the magnitudes of 1000x.

pH measurement

Five grams of starch was mixed with 25 ml of CO₂-free distilled water for 1 min and kept for 15 min. The pH value was subsequently measured using pH meter. In general, the pH of starch varies between 4 and 7 (Rowe *et al.*, 2009).

Testing of moisture content of banana peel starch powder

Three grams of starch powder was put into a moisture analyzer, and then kept for a while. The measurement was started and just terminated upon the reading of its moisture content. The moisture content of starch should be approximately 20% (Rowe *et al.*, 2009).

Observation of gelatinization temperature and melting point

Differential scanning calorimetry (DSC) was used for the determination of thermal properties including gelatinization temperature and melting point. About 5 mg of sample was put into an aluminium cylinder with a diameter of 5 mm, closed with an aluminium plate, and then placed into a DSC apparatus. The measurement was performed, commencing from 30°C till 300°C. Endothermic and exothermic processes occurring in the sample throughout the measurement were recorded, to determine the gelatinization temperature and melting point of banana peel starch.

Determination of the repose angle of banana peel starch

One hundred grams of powder was put into a funnel, in which its bottom part was closed with a piece of paper. If free flowing of the powder occurs, it will form a cone and the angle of repose can be determined. The lower the repose angle, the better the flow of the powder (Banker & Anderson, 1986).

The angle of repose (α) can be calculated using the Eq. (1):

$$\operatorname{tg} \alpha = \frac{\text{cone height (h) (cm)}}{\text{cone radius (r) (cm)}} \quad (1)$$

Determine of density, Carr's index, and Hausner ratio (The United tate Pharmacopeial Convention, 2014)

An empty measuring cylinder (100 ml) was weighed (W_1), then the sample was filled into it to 100 ml (V_0) and the total weight was determined (W_2). The measuring cylinder filled with sample was placed onto a motorized tapping apparatus. The start button was activated to tap the measuring cylinder for 500 times. The volume of sample was subsequently measured (V_1). The bulk density and tapped density were determined using Eqs. (2) dan (3) respectively, while the respective Carr's index and Hausner ratio were calculated using Eqs. (4) and (5):

$$\text{Bulk density } (\rho_{\text{bulk}}) = \frac{W_2 - W_1}{V_1} \quad (2)$$

$$\text{Tapped density } (\rho_{\text{tapped}}) = \frac{W_2 - W_1}{V_2} \quad (3)$$

$$\text{Carr's index (\%)} = \left(1 - \frac{\text{bulk density}}{\text{tapped density}}\right) \times 100\% \quad (4)$$

$$\text{Hausner ratio} = \frac{\rho_{\text{tapped}}}{\rho_{\text{bulk}}} \quad (5)$$

Determination of loss on drying

One gram of starch was weighed and put into a tared pre-heated (105°C for 30 min) weighing bottle with its lid, and then placed into an oven (105°C) and dried until a constant weight is achieved.

Determination of ash content

Sample was heated at the temperature as such that the organic compound and its derivatives were destroyed and evaporated, yielding mineral elements and anorganic compound residues. The ash content should be less than 1%. The ash content was calculated using Eq. (6):

$$\text{Ash content} = \frac{\text{berat (Arus+adu)} - \text{berat (Arus kosong)}}{\text{berat sampel}} \times 100\% \quad (6)$$

Determination of amylose content

The amylose content of banana peel starch was measured using the procedure of Juliano (1971) with minor modification. Pure amylose (40 mg) was put into a test tube containing 1 ml of ethanol 95% and 12 ml of sodium hydroxyde 1N. The test tube was heated in a boiling water for 10 min yielding a gel and then it was cooled. The resulting gel (1, 2, 3, 4, and 5 ml) was placed into five 10 ml-volumetric flasks, added with 0.2, 0.4, 0.6, 0.8, and 1 ml of acetic acid

1N respectively. Two milliliters of iodine solution and a sufficient volume of distilled water were then added into each volumetric flask up to 100 ml. The resulting mixture was shaken until homogeneous and kept for 20 min. The intensity of blue color appeared in the mixture was measured using a UV-VIS spectrophotometer at a maximum wavelength of 620 nm. A linear calibration curve between absorbance values and amylose concentrations was constructed.

Procedure of sample determination, 100 mg of sample was put into a test tube and 1 ml of ethanol 95% and 12 ml of sodium hydroxyde 1N. The test tube was heated in a boiling water for 10 min yielding a gel and then it was cooled. The resulting gel (5 ml) was placed into a 100 ml-volumetric flask and then added with 1 ml of acetic acid 1N. Two milliliters of iodine solution and a sufficient volume of distilled water were then added into the volumetric flask up to 100 ml. The resulting mixture was shaken until homogeneous and kept for 20 min. The intensity of blue color appeared in the sample was measured using a UV-VIS spectrophotometer at a maximum wavelength of 625 nm. The amylose content was obtained by the intrapolation of absorbance value of the sample to the linear calibration curve, using Eq. (7):

$$\text{Amylose content} = A \times F_p \times 100\% \quad (7)$$

where A is the amylose content (mg/ml) and F_p is the dilution factor.

Determination of white color index

The determination of white color index was performed quantitatively using chromatometer apparatus (color reader). This evaluation aimed to determine the white color index of banana peel starch compared with BaSO_4 (barium sulphate), yielding three measuring parameters namely L, a, and b. The value of L indicates the clarity level of the sample. The clearer the sample, the L value becomes closer to 100. On the contrary, if the sample is more turbid, the L value becomes closer to 0. The value of a indicates the measurement of chromatic red-green mixture color, while the value of b indicates the measurement of chromatic yellow-blue mixture color (Hutching, 1999).

Statistical analysis

Analysis of the samples were completed in triplicate and standard deviations were evaluated. The results were compared with Tukey's test to a 5% level of significance) using analysis of variance (ANOVA).

Table 1. Characteristics of banana peel starch

Types of testing	Results	Specifications
Qualitative test	Bluish violet color	Bluish violet color
Physical and chemical properties:		
a. Sensory		
Form	Powder	Powder
Color	Brownish white	White
Odor	Odorless	Odorless
Taste	Tasteless	Tasteless
b. Microscopic evaluation		
	Hyllus and lamellae (+)	Hyllus and lamellae (+)
c. Macroscopic evaluation		
	4.28 ± 0.22 µm	2-100 µm
d. Viscosity (5% gel)		
	3703 ± 280.23 cps	Com (300-1000 cps)* Potato (1000-5000 cps)* Tapioca (500-1500 cps)* Wheat (200-500 cps)*
e. pH		
	6.15 ± 0.35	4 - 7
f. Moisture content		
	6.06 ± 0.83%	< 20%
g. Angle of repose		
	No free flow (very poor)	> 55° (very poor)**
h. Carr's index		
	25.89 ± 2.67%	25 - 31% (poor)**
i. Hausnerratio		
	1.35 ± 0.04	1.35 - 1.45 (poor)**
j. Loss on drying		
	10.62 ± 0.01%	< 15%
k. Ash content		
	0.98 ± 0.27%	< 1%
l. Amylose content		
	39.57 ± 1.65%	17-39%
m. White color index		
	55.96 ± 3.95°	> 95°

Results

Characterization of banana peel starch

The resulting starch in this study are as shown in Table 1. Macroscopic evaluation was performed by determining the particle size of banana peel starch powder using sieving method through 20-, 40-, 60-, and 80-mesh sieves. The particle size (dg) of banana peel starch powder was 4.28 ± 0.22 µm. In comparison to large granules, the smaller starch granules has lower resistance to heating process and exposure to water (Singh *et al.*, 2010).

Banana peel starch powder was mixed with distilled water yielding a suspension. The pH of this mixture was determined using pH meter, and was found to be 6.15 ± 0.35. This pH value met the required specification. In general, a starch suspension in water has a pH of 4 - 7 (Rowe *et al.*, 2009).

The moisture content of starch powder may determine its quality and stability. Starch powder with high moisture content is more prone to bacterial growth, compared with that with low moisture content. The moisture content of starch should be less than 20% (Ministry of Health Republic Indonesia, 1995). The moisture content of banana peel starch powder was found to be 6.06 ± 0.83%.

The viscosity values of banana peel starch gel and cassava starch gel were determined using Brookfield

viscometer. It was found that the gel of banana peel starch was about 2-fold more viscous than cassava starch gel.

Testing of loss on drying is performed to measure the loss of water upon heating process. The result showed that the loss on drying of banana peel starch was 10.92 ± 0.01%, meeting the required specifications (Table 1). In general, the loss on drying of starch is less than 15% (Rowe *et al.*, 2009).

The white color index of banana peel starch did not meet the required specifications namely 95° (white color). The color of resulting banana peel starch was brownish white with the white color index of 55.96 ± 3.95° (Table 1).

Gelatinization temperature and melting point

The DSC thermogram showed the first endothermic peak (Figure 3), reflecting the gelatinization of starch. The initial, peak, and end gelatinization temperatures of banana peel starch were observed to be 48.06, 52.37, and 59.75°C respectively, with the enthalpy of 6.6535 J/g. Similarly, the respective initial, peak, and end gelatinization temperatures of cassava starch were 51.67, 54.40, and 62.13°C, with the corresponding enthalpy of 5.685 J/g. In addition, the second endothermic peak correlated with the melting point of starch, namely 102.45-104.04°C for banana peel starch and 105.47°C for cassava starch.

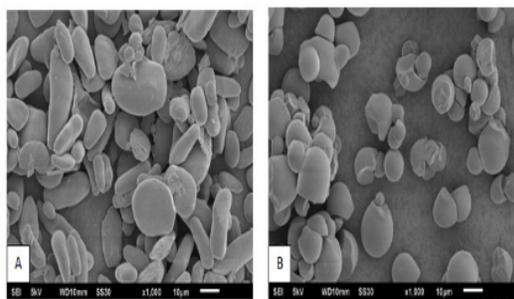


Figure 2. The particle shape of starch using SEM: (A) banana peel starch, (B) cassava starch, with the magnitude of 1000x.

The lower the gelatinization temperature of starch, the shorter the gelatinization time of starch, which may in turn affect the efficiency of the product.

Diffraction pattern

XRD analyses of banana peel and cassava starches exhibited broadly comparable diffraction patterns (Figure 4). Both samples were available in semi-crystalline form and tend to be an amorphous phase.

Discussion

Identification of banana peel starch was performed using qualitative test, indicated by the appearance of bluish violet color upon the addition of iodine solution. The color appeared due to the linkages formed between amylose and amylopectin upon the addition of iodine. Amylose bonded with iodine resulted in blue color whereas amylopectin bonded with iodine formed bluish violet color.

The sensory properties of banana peel starch evaluated in this study were form, color, odor and taste, as shown in Table 1. Based on the sensory evaluation, banana peel starch powder met the required specifications with respect to its form, taste, and odor. However, the color of resulting banana peel starch has yet to be improved for it still appeared as brownish white powder. This might be due to an oxidation process occurring during the preparation of starch.

Microscopic observation, including the starch composition and identification of the shape and location of hyllus and lamellae in banana peel starch, was done using a microscope apparatus. Microscopically, hyllus and lamellae are specific characteristics of starch (Ministry of Health Republic Indonesia, 1995). Hyllus is an initial point of the formation of starch, while lamellae are smooth lines surrounding the hyllus. Based on the microscopic

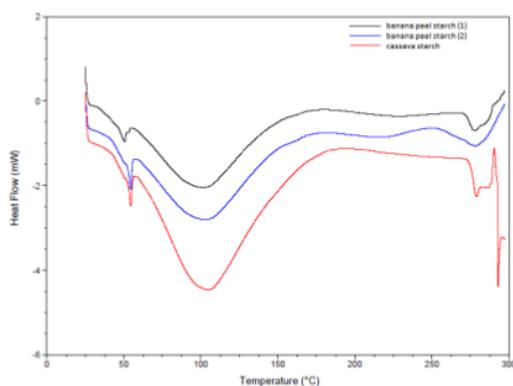


Figure 3. DSC profiles of banana peel and cassava starches.

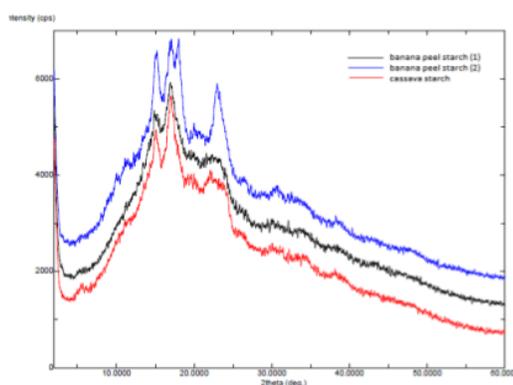


Figure 4. XRD patterns of banana peel and cassava starches.

evaluation, the resulting banana peel starch met the required specifications, indicated by the presence of hyllus and lamellae. The shape of hyllus in banana peel starch was a dot in a narrow edge, and located at the edge of starch, or it was located eccentrically.

The original shape of starch naturally is a small particle so-called granule. The shape and size of granule is unique and characteristic for each granule type where the green banana starch granules were elongated and cylindrical (Sukhija *et al.*, 2016). Thus it can be used as a tool for the identification of source of starch. The shapes of banana peel and cassava starches are shown in Figure 2. It was observed that banana peel starch had a single or separate granule with elongated and cylindrical shape whereas cassava starch showed a more rounded or spherical shape.

Evaluation of the physical quality of resulting starch powder included flowability properties testing. This was represented by the angle of repose, Carr's index, and Hausner ratio. The angle of repose is determined by measuring the slope or the angle of cone following the flowability of starch powder. Carr's index indicates the ability of powder to reduce

the volume by shrinking or squeezing itself upon the introduction of compression force. It is known that the Carr's index and Hausner ratio significantly influence the flowability of powder. The smaller the Carr's index and Hausner ratio of powder, the greater the flowability of powder.

Based on the above three parameters, it was observed that banana peel starch powder had a poor flowability. The angle of repose of resulting banana peel starch could not be determined due to its very poor flow properties. In other words, banana peel starch could not pass through the funnel. The results of Carr's index and Hausner ratio are presented in Table 1. In general, starch has a relatively poor flow properties. Soebagio *et al.* (2009) have reported that banana peel starch has small particle size, and hence it demonstrates relatively poor flowability.

The ash content of banana peel starch is determined to measure the internal and external mineral contents, commencing from the initial process until the formation of starch. The presence of mineral content may cause a more rapid burning process leading to the formation of ash residue. This may diminish the quality and purity of resulting starch. The mineral content in the material may be composed of two types, namely water soluble mineral and water insoluble or slightly soluble mineral. Examples of water insoluble or slightly soluble minerals in banana peel starch are macro (calcium) and micro (iron and phosphorous). The ash content of agung banana peel starch met the required specifications (Table 1), namely less than 1%.

In general starch is composed of two main component types of D-glucopyranose polymer namely amylose (linear polymer) and amylopectin (chain polymer). Amylose may absorb water easily and demonstrates a very good swelling capacity. Amylopectin is more sticky and tends to form a gel when it is suspended in hot water. Thus amylopectin is generally used as a good binding agent. In general the amylose content in starch is less than amylopectin. Amylose plays a significant role in gelatinization process and contributes significantly in determining the starch characteristics in paste form. Starch with high amylose content exhibits a stronger hydrogen bonding due to the large number of linear chain in granules, hence it requires higher energy for gelatinization. On the contrary, amylopectin has a long branch chain, tends to form a gel. As shown in Table 1, the amylose content in banana peel starch was $39.57 \pm 1.65\%$.

Gelatinization is the swelling phenomenon of starch granules in water upon heating process. Banana peel starch is insoluble in cold water but it

swells in hot water. An increased temperature may increase the swelling degree of starch granules, resulting in an increased pressure among granules. The initial swelling is a reversible process, but as the temperature increases beyond a critical point, it becomes an irreversible process, hence this is so-called gelatinization. The critical point of this turning process is known as gelatinization temperature (Pomeranz, 1991). Gelatinization temperature correlates with granules compactibility, as well as amylose and amylopectin contents. The viscosity of starch gel starts to increase at initial gelatinization temperature, whereas the viscosity of starch gel reaches its maximum value at end gelatinization temperature. Gelatinization temperature is a complex phenomenon of physical properties of starch and it is influenced by several factors, among others, particle size of amylose and amylopectin, as well as the heating process of medium.

Gelatinization temperature is affected by the size of starch granules. The larger the size of starch granules, the lower the gelatinization temperature of starch granules. Large starch granules are able to readily absorb more water, hence the starch swells easier and has a lower gelatinization temperature. The banana peel starch granules had larger particle size than cassava starch, hence the banana peel starch granules exhibited lower gelatinization temperature.

Physically, starch granules are available in semi-crystalline form, composed of crystalline and amorphous units (Banks and Reewood, 1975). Crystalline unit is more resistant to strong acid and enzyme treatment. Amorphous form can absorb cold water up to 30% of its weight without damaging the whole structure of starch (Hodge and Osman, 1976). It is thought that amylopectin is a component responsible for crystalline properties of starch granules (Banks and Reewood, 1975).

Conclusion

Agung banana peel starch can be used as an alternative source of starch. Characterization of Agung banana peel starch indicated that it exhibited the characteristics of starch in general with the advantages of its higher viscosity compared with other starches, including cassava, corn, potato, tapioca, and wheat starches. In addition, Agung banana peel starch demonstrated lower gelatinization temperature than cassava starch.

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Conflict of Interest

Declared None

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Isolation and characterization of Agung banana peel starch from East Java Indonesia

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