Characterization and Heavy Metal Determination of Starch Obtained from Rambutan (*Nephelium lappaceum*) Seed

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Abstract: The needs of excipients in pharmaceutical industry and raw materials in food industry such as starch, elevate the local plants to be utilized as an alternative source. Rambutan seed has the potentiality to be used as the one of the starch sources. The aim of the study was to produce starch and do the characterization included the heavy metal determination of starch obtained from rambutan seed. The starch was obtained by grinding the rambutan seed with water in ratio of 1:5. The characterization parameters were the organoleptic and microscopic features; yield value; moisture content; viscosity; and FT-IR spectra. The heavy metals measured were lead (Pb), copper (Cu) and zinc (Zn) by using Atomic Absorption Spectrophotometer instrument. The results showed that the rambutan seed starch had granular shape and gave 23.5% of yield value, 4.24 ± 0.78% of water content, 270.83 ± 7.22 cP of viscosity (in 5% solution), acceptable concentration of the existed heavy metal. It can be concluded that the rambutan seed can be suggested to be utilized as an alternative source for the industry to produce standardized starch.

1 INTRODUCTION

Starch either as pharmaceutical excipient or raw material is mostly required in pharmaceutical and food industries (Mohammed, 2017). However, the availability of the commercial starch in the market is not enough to fulfil the requirement. The starch producing industries is very limited compared to the industries that need starch as raw material. Cassava, potato, and corn are the most common sources to produce starch. Starch is the most abundant carbohydrate reserve in plants and it is found in leaves, flowers, fruits, seeds, different types of stems and roots (Smith, 2001). Indonesia is well-known to have abundant natural sources which have not been utilized properly. Therefore, it is needed to explore and manage the natural sources to overcome the nation problem. The utilization of plants, especially fruits that rich in carbohydrate is one of the ways that can be implemented.

Rambutan (*Nephelium lappaceum* L.) is a tropical fruit belonging to the Sapindaceae family. The fruits are ovoid, with a red or yellow pericarp covered with soft hairs that vary in colour from green, yellow and red (Suganthi and Josephine, 2016). Rambutan fruit contains fat 35%, ash 2% and vitamin C 4%. The seeds were abundant in fats (38.9%), protein (12.4%) and carbohydrates (48%). The seeds have traces of an alkaloid, sugar, starch and ash (Harahap et al., 2012). Knowing the high amount of carbohydrate in rambutan seed, it is a potent source to make the seed as the alternative starch source since the usage of the seed is not very much applied. However, it is needed to confirm that the starch obtained from rambutan seed is fulfil the requirement as pharmaceutical excipient. Therefore, the purpose of this research was to characterize the starch and determine the heavy metal content of the starch obtained from the rambutan seed.

2 MATERIALS AND METHODS

2.1 Materials

The materials used were rambutan seed obtained from rambutan fruit available from the local market in Binjai - North Sumatera (Indonesia), distilled water, 65% nitric acid (Smart Lab, Jakarta-Indonesia), demineralized water (Bratachem, Medan-Indonesia).

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2.2 Methods

The methods conducted in this study were rambutan seed preparation, starch preparation, characterization of the rambutan seed starch included organoleptic and identification; yield value; moisture content; viscosity; FT-IR spectra and heavy metal determination.

2.2.1 Rambutan Seed Preparation

The skin of rambutan fruits were peeled off and the fleshes were removed to obtain rambutan seed. The seeds were washed, dried and put in the refrigerator (LG) for further experiment.

2.2.2 Starch Preparation

The rambutan seed were ground with the addition of distilled water in the ratio of 1:5. The mixture was filter to obtain starch suspension. The suspension were left until the starch was sedimented at the bottom of the container. The water part was removed and the starch was dried in the oven (Dynamica) and ground, then filtered using shieve no. 80. The yield value was calculated based on the obtained starch (b) with the amount of the rambutan seed (a) as stated in the equation (1).

$$yield\ value = \frac{b}{a}\ x\ 100\% \qquad (1)$$

2.2.3 Organoleptic and Identification

The organoleptic parameters evaluated were colour, taste and odour. The identification of starch was included the color test with lugol solution, the mucilage formation and the microscopic characterization (The United State of Pharmacopoeia, 2005). The microscopic characterization was conducted using distilled water as the mounting agent (Selvam, 2013). The starch was evaluated using microscope (Boeico, Germany) with 40x magnification.

2.2.4 Moisture Content Determination

Moisture content determination was done by using gravimetry methods. Some amount of sample was weighed and dried in the oven at 105^0C for 3 hours and weighted until constant weight was obtained (Ningsih et al, 2010). The moisture content was determined in triplicate. The moisture content was calculated by comparing the initial weight of the starch (w1) with the constant weight of the starch obtained after dried in the oven (w2) as stated in the equation (2).

$$moisture\ content\ = \frac{w1-w2}{w1}\ x\ 100\% \qquad (2)$$

2.2.5 Viscosity Determination

The rambutan seed starch was made into 5% suspension in water, and then heated until boiled. The viscosity at this state was measured, and then continued left the mucilage to be cool (approximately 40^oC) for the next measurement.The viscosity of rambutan seed starch was determined by Brookfield Viscometer. The determination of the viscosity was performed in triplicate for each state of temperature.

2.2.6 FT-IR Analysis

The starch of rambutan seed was analysed for the functional groups using Fourier Transform Infra Red Spectrophotometer (Prestige 21, Shimadzu, Japan). Each spectra was recorded at a resolution of 4 cm^{-1} in a range of 500-4000 cm^{-1}. The obtained spectra was compared with the potato starch as the official starch in United State of Pharmacopoeia.

2.2.7 Heavy Metal Determination

The starch of rambutan seed was analysed for the presence of the Pb, Cu and Zn.The heavy metals were determined using atomic absorption spectrophotometer (Hitachi Z-2000) with wavelength of 283.3 nm for Pb, 324.8 nm for Cu and 213.9 nm for Zn. Samples were analyzed in triplicate.

3 RESULTS AND DISCUSSION

3.1 Yield Value of Starch

Based on the starch preparation, it was obtained 23.5% of yield value . The result indicated that rambutan seed could produce 23.5% starch from the amount of the seed.This amount is high enough as the seed of rambutan contains 48% of carbohydrate which means approximately 48.96% of the carbohydrate was isolated as starch.

3.2 Organoleptic and Identification Results

The organoleptic results showed that rambutan seed starch had yellowish white colour, bitter taste, and distinctive odour of rambutan. The identification of starch using lugol solution gave dark purple colour. It was fulfilled the required identification as stated in USP which starch will give orange-red to dark blue color (The United State of Pharmacopoeia, 2005).

The microscopic feature showed that the starch of rambutan seed had circular shape with hilum as seen in the Figure 1.

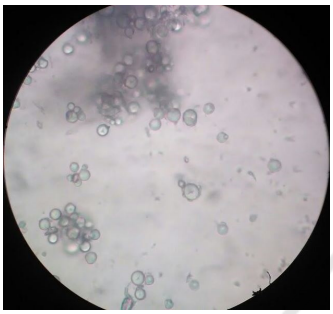

Figure 1: The microscopic characterizationof rambutan seed starch with 40 x magnification, most of the granules had concentric hilum (cross mark in the middle of granules)

This shape has similarity with the starch in common. It was confirmed that the sample produced was starch. The presence of starch is different according to the types of plant and the composition of starch for each part of the plant is not similar either. Starch can be found in the roots, rhizomes, tubers, stems, leaves, flowers, pollens, fruits and seeds as store of energy. Each part can has transitory or storage type of starch. The granule type of starch can be simple, compound or semi-compound (Selvam, 2013). From Figure 1, it can be seen that the rambutan seed starch had simple and compound granule type with concentric hilum position. Concentric hilum means that hilum located near to the middle of the granule.

3.3 Moisture Content and Viscosity Results

The moisture content of the starch was 4.24 ± 0.78%.The moisture content of the starch is one of the very important parameter that influences the quality of the starch. High moisture content will cause the stability of the starch become decrease due to the possibility of the growth of microorganisms. The starch granules usually have moisture content below than 10% (Copeland et al., 2009).

The viscosity value of the rambutan seed starch was 270.83 ± 7.22 cP. The temperature of a solution or suspension will influence the viscosity value (Haase et al., 1995). The increase of viscosity value of the starch is due to the gelatinization process (Sulaiman, 2011).

3.4 FT-IR Characterization

The FT-IR analysis of starch rambutan seed can be seen from the Figure 2. The spectra showed some functional groups. These functional groups included –C-H stretch, –O-H group and C-O-C asymmetric at 2924.09 cm-1, 3340.71 cm-1 and 1149.57 cm-1; -C-OH bending vibrations at 856.39 cm-1 and 570.93 cm-1 . These results were in accordance with the earlier reports of starch [Amini et al., 2014; Tongdeesontorn et al., 2011). FTIR is used to perform preliminary testing, especially to compare the functional group of a compound with another compound. A functional group of potato starch and rambutan seed starch as seen on the FTIR spectrum was appeared almost identical. It means that the rambutan seed starch had similar functional group with the potato starch.

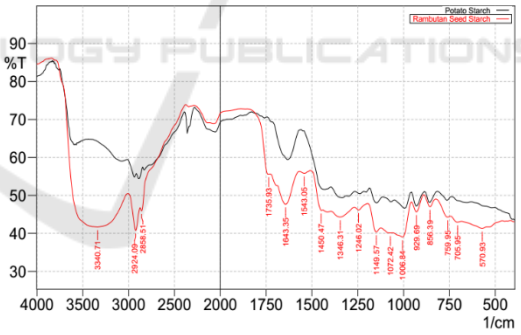

Figure 2: The spectra of starch obtained from the rambutan seed compared with potato startch

3.5 Heavy Metal Analysis Result

The heavy metal analysis of starch obtained from rambutan seed was conducted using Atomic Absorption spectrophotometer. The result can be seen in the Table 1.

Table 1: The heavy metal analysis of starch obtained from rambutan seed

Heavy metal	Concentration (ppm)
Pb	not detected
Cu	0.6228±0.37
Zn	0.6171±0.91

Normally, Pb is found in the sample that contaminated with pollution such as place which near to the factory or street. Lead in the starch of rambutan seed was not detected. This result might be due to the position of the seed is in the inner part of the fruit which not directly exposed to the environment. In the other hand, copper and zinc were detected in the starch obtained from the rambutan seed. The presence of the metals might be due the soil environment factor and the hyper accumulator characteristic of the plant. This hyper accumulator character could accumulate specific metals in the certain tissue of the plant (Irawanto et al., 2015; Knox et al., 2000).

4 CONCLUSION

It can be concluded that rambutan seed have big potency to become one of the alternative source of starch, however further development needed to increase the utility of the starch.

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