Extraction and Characterization of Starch From Mango Seeds

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Abstract. The mango (Mangifera indica) seed starch were extracted via distillation, alkaline, sedimentation and centrifugation method. The effects of extraction methods on its morphology, structural and physicochemical properties extracted were comparatively studied. Morphological characteristics like shape and size of the starch granules, display significant differences under scanning electron microscope (SEM). The extracted mango seed starch granules showed oval and irregular or cuboidal-shaped with smooth surface despite of different extraction method. Distillation method yields smallest (13.80 ± 2.90 µm) starch granules in size, followed by alkaline method (15.83 ± 3.65 µm), sedimentation method (15.97 ± 4.44 µm) and centrifugation method (19.91 ± 6.82 µm). X-ray diffraction pattern whereas, showed the isolated mango seed starch is an A-type starch, hence, corroborated to suggest the morphology of the starch granules by their size. Moreover, starch isolated via alkali method had highest starch yield of 91.72 % than starch isolated via distillation (81.87 %), sedimentation (74.82 %) and centrifugation (74.56 %), suggest that mango seed as one of the potential sources of starch.

1. Introduction

Starch is greatly in abundance and the main source of carbohydrate constituent for most plants [1]. The growing demand for starch in many applications including food, pharmaceutical, paper, adhesive and textile has rising attention in discovering new sources of this type of polysaccharide from other plants or fruits [2]. Many attempts have been focused on the use of starch obtained from various plant sources such as rice, wheat, corn and potato [3]. Yet, limited work available on the extraction of starch from fruit by-products such as seeds. Therefore, in this study, native starch is obtained from mango seed.

Mango (Mangifera indica) is a popular tropical fruit that is widely grown and cultivated in many tropical regions of the world including Malaysia, Thailand, and India [4]. Typically, in Malaysia, the mango seeds are discarded as wastes after the fruits are eaten. Thus, due to the availability and be a reliable source of starch, this type of agricultural waste could be used as value-added products [5]. Hence, with appropriate extraction method (via distillation, alkaline, sedimentation and centrifugation), this study is aiming to evaluate the morphological properties of starch extracted from mango seeds.

2. Experimental study
2.1 Sample collection and seed treatment

Ripe mangoes Lily cultivar seeds were collected from the local market. The seeds were washed with tap water to remove their impurities and dried in the oven at 70 °C overnight. Then, the dried seed’s kernels were separated manually using a kitchen knife to recover the seeds and dried in the oven at 50 °C for 24 hours. The brown layer (seed coats) were removed manually to obtain the cotyledons that were used to prepare flour by grinding using a laboratory grinder. The obtained flour was sieved through sieve (500-50 µm mesh), packed and sealed in a plastic bag, then stored in a refrigerator (< 5 °C) until further subjected to starch extraction.

2.2 Extraction of mango seed starch via distillation method

The mango seed starch was isolated according to the procedure described by Noor et al. [5] with minor modifications. Firstly, 5 g of mango seed flour was added to 100 ml of distilled water with continuous stirring for 6 hours at room temperature. Next, a cloth bag (about 200 µm mesh) was used for filtering the slurry and the remaining residues were washed with distilled water for three times. The filtrate was mixed and precipitated overnight at 4 °C. Finally, the obtained starch was filtered and dried in the oven at 40 °C for 24 hours. The starch was ground with a mortar and pestle, packed in a sealed plastic bag and kept at room temperature until further use.

2.3 Extraction of mango seed starch via alkaline method

The mango seed starch was isolated using procedure mentioned by Noor et al. [5] with some modifications. Firstly, 5 g of mango seed flour was added to 0.5% concentration of sodium hydroxide (NaOH) with continuous stirring for 6 hours at room temperature. Next, the slurry was filtered using a cloth bag (about 200 µm mesh) and the remaining residues were washed with distilled water for three times. Then, the filtrate was mixed and precipitated overnight at 4 °C. Finally, the obtained starch was filtered and dried in the oven at 40 °C for 24 hours. The starch was ground with a mortar and pestle, packed in a sealed plastic bag and kept at room temperature until further use.

2.4 Extraction of mango seed starch via sedimentation method

The mango seed starch was isolated using Oates and Powell [6] method with minor modifications. Firstly, 50 g of mango seeds were steeped in water overnight, washed and ground in a kitchen blender. Next, the slurry was filtered through a cloth bag (about 200 µm mesh) and the filtrate was reserved to sediment the starch. Then, the starch was re-slurried in distilled water and sedimented three times. The final product was sedimented in 0.1 M sodium chloride (NaCl) and 1/10 volume of toluene for 3 hours. Finally, the obtained starch was thoroughly washed with distilled water and dried in the oven at 50 °C for 24 hours. The starch was ground with a mortar and pestle, packed in a sealed plastic bag and kept at room temperature until further use.

2.5 Extraction of mango seed starch via centrifugation method

The mango seed starch was isolated using Hassan et al. [4] method with some modifications. Firstly, 50 g of mango seeds were steeped in 0.16% aqueous solution of sodium hydrosulfide hydrate at 50 °C for 24 hours. Next, the solution was decanted and the seeds were ground in kitchen blender. The ground slurry was filtered through a cloth bag (about 200 µm mesh), washed thoroughly with distilled water and re-slurried in distilled water for 1 hour. Then, the supernatant was decanted from the filtrate and the settled starch layer was re-suspended in distilled water. Finally, the starch was centrifuged at 5000 rpm for 30 minutes and dried in the oven at 50 °C for 6 hours. The starch was ground with a mortar and pestle, packed in a sealed plastic bag and kept at room temperature until further use.
2.6 Scanning electron microscopy (SEM) analysis

The morphology of starch granules was observed using a scanning electron microscope (JEOL, Model JSM 5600 SEM). The samples were in powder form and sputter coated with palladium (Polaron SC515) and observed under the SEM at an accelerating voltage of 7 kV.

2.7 X-ray diffractometry (XRD) analysis

The structural properties of starch samples were examined using a wide-angle x-ray diffractometry (Rigaku, Model Ultima IV diffractometer). The samples were in powder form and scanned using Cu-Kα radiation (wavelength (λ) = 1.54056 Å) at 40 kV and 40 mA. The scanning region of the angles (2θ) was from 4° to 70° at a scanning speed of 0.02°/min.

2.8 Starch yield determination

The percentage yield of isolated starches was determined using equation (1) according to Noor et al. [5]:

\[
\text{Starch Yield (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\%
\]  

(1)

3. Results and discussion

3.1 Morphological properties

Table 1 shows the morphology of starch granules. The micrographs of four starch samples revealed the presence of starch granules at ×500 magnification, from small to large and oval to irregular or cuboidal shape with smooth surface. There is a little significant change was observed among the starch granules with different extraction method. Distillation and centrifugation method had majority of starch granules with oval shape, whereas sedimentation method had more irregular shape, but to a lesser extent than that of alkaline method. The average diameter of the starch granules ranges from 13.80 µm to 19.91 µm. The range of starch granules in size for distillation method (< 18 µm), followed by alkaline method (< 23 µm), sedimentation method (< 25 µm) and centrifugation method (< 37 µm). This indicates that the different starch extraction method had little impact on the granule shape and size of mango seed starch. The results revealed are consistent with those observed by Kaur et al. [7] for mango kernel starch granules with 15 µm to 21 µm in size.
Table 1. Micrographs of mango seed starch granules and size

| Extraction method | SEM micrographs (magnification × 500) | Starch granule size (µm) |
|-------------------|--------------------------------------|--------------------------|
|                   |                                      | Range                    |
| Distillation      |                                      | 7.98 - 17.14             |
|                   |                                      | 13.80 ± 2.90             |
| Alkaline          |                                      | 10.20 - 22.30            |
|                   |                                      | 15.83 ± 3.65             |
| Sedimentation     |                                      | 9.35 - 24.59             |
|                   |                                      | 15.97 ± 4.44             |
| Centrifugation    |                                      | 14.72 - 36.48            |
|                   |                                      | 19.91 ± 6.82             |

3.2 X-ray diffraction analysis

The X-ray diffractograms of the four mango seed starch samples are presented in Figure 1. The broad x-ray diffraction peak pattern indicates the mango seed starch is in amorphous state similar to the jackfruit seed starch reported by Tulyathan et al. [8]. Furthermore, the mango seed starch exhibited strong diffraction peaks in Bragg angles 2θ at 18° and 23°. These diffraction patterns fit the A-type starch which is in agreement with tamarind kernel starch reported by Kaur and Singh [9]. The different starch extraction method had no influence on the type x-ray diffraction pattern; however, the crystallite size varies among them. The crystallite size was calculated using Scherrer equation [10], as presented in Table 2, shows significant reduction in crystallite size with respective diffraction peaks positioned at 2θ from centrifugation, sedimentation, alkaline and distillation method.
Figure 1. X-ray diffraction pattern of mango seed starch, (a) distillation, (b) alkaline, (c) sedimentation, and (d) centrifugation method

Table 2. XRD data for mango seed starch

| Extraction Method | 2θ (degree) | Crystallite size (nm) |
|-------------------|-------------|-----------------------|
| Distillation      | 17.50       | 11.60                 |
|                   | 22.87       | 33.00                 |
|                   | 18.02       | 13.80                 |
| Alkaline          | 23.35       | 33.00                 |
|                   | 17.23       | 16.5                  |
| Sedimentation     | 23.26       | 29.00                 |
|                   | 17.90       | 14.90                 |
| Centrifugation    | 22.93       | 27.50                 |

3.3 Starch yield

Figure 2 shows the percentage of starch yield of the mango seed with distillation, alkaline, sedimentation and centrifugation method were 81.87, 91.72, 74.82 and 74.56 %, respectively. These values were higher than that found by Noor et al. [5] who observed the yield of jackfruit seed starch was 50.69 % to 84.48 %, and yield of rice starch was 45.70 % to 65.00 % [12]. The high starch yield of the mango seeds compared to other sources of starch might be due to the granular size which allows the starch granule to be extracted easily [13]. Hence, the results suggest that the extracted mango seed as one of the potential sources of starch.
4. Conclusion

This study has shown that, the starch was prepared from mango seed and extracted via different extraction methods which are distillation, alkaline, sedimentation and centrifugation method. The results showed significant differences in their properties. Investigation on morphology of starch granules revealed oval to irregular shape with small to large granule size. Moreover, the mango seeds revealed an A-type starch pattern and high starch yield. The information obtained in this work could be very useful for the mango seed as new source of starch. However, there are need to carry out more detailed investigation to characterize other properties, particularly proximate composition and physicochemical properties essential to applications as a potential biopolymer.

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