

Comparison of the Physicochemical and Pharmacopoeial Properties of Starches Obtained from *Artocarpus odoratissimus* Blanco, *Nephelium lappaceum* L., and *Mangifera indica* L. seeds with Corn Starch

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ABSTRACT

Objective. This study was conducted to characterize and compare the physicochemical and pharmacopoeial properties of starches isolated from the seeds of *Artocarpus odoratissimus* Blanco (*marang*), *Nephelium lappaceum* L. (*rambutan*), and unripe green *Mangifera indica* L. (mango) with corn starch, as possible sources of pharmaceutical grade starch.

Methods. The starch from the seeds of these fruits was isolated and characterized through their physicochemical (organoleptic characteristics, percent yield, amylose-amylopectin ratio, bulk density, tapped density, compressibility index, Hausner ratio, angle of repose, solubility, swelling power, and viscosity) and pharmacopoeial properties (identification test, pH, loss on drying, and limit of iron). Morphology of the granules was also assessed.

Results. The physicochemical properties showed that amylose content of the seed starches was significantly lower ($p=0.001$) and amylopectin content significantly higher ($p=0.001$) than the native high amylose corn starch. The lower values of bulk and tapped densities, and high values in compressibility index and Hausner ratio of the seed starches compared to corn starch may be due to their smaller particles. The results of the pharmacopoeial characterization showed compliance with the United States Pharmacopeia's (USP) acceptable limits, except for the pH of *marang* seeds.

Conclusion. The starches isolated from the fruit seeds have unique properties, but only *rambutan* seed starch has the most desirable physicochemical and pharmacopoeial properties that is comparable with corn starch. *Rambutan* seeds could be utilized as a source of starch for pharmaceutical applications.

Key Words: Mango, *marang*, *rambutan*, seeds, starch

INTRODUCTION

Starch is a polysaccharide mixture of amylose and amylopectin, which accounts for more than 50% of human carbohydrate intake.¹ It is mainly found, in large quantities, in staple foods, such as potatoes, wheat, corn, rice, and cassava.² In the food industry, it is usually used as thickener, extender, emulsion stabilizer, and binder, while in the pharmaceutical industry, it is commonly utilized as tablet disintegrant, binder, glidant, and diluent.³ It is also manufactured for non-food applications such as in papermaking and bookbinding, as substitute for talcum body

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powder, for laundering clothes, for synthesizing bioplastics, and others.⁴

The wide application of starch in different industries requires the search for additional sources. Various plant parts have been explored as alternative sources of starch, including seeds.^{3,5} In previous study, mango (*Mangifera indica* L.) kernels constitute approximately 58% starch.⁶ Although there are limited information on the starch content of *rambutan* (*Nephelium lappaceum* L.) seeds, its amylose content was found to be comparable with commercial potato and corn starches.⁷ On the other hand, there have been no published literature on the exact amount of starch in *marang* (*Artocarpus odoratissimus* Blanco) seeds. With the increased annual production of these fruit crops for food and export purposes, the volume of the generated fruit waste materials has also increased. Producing huge amount of discards with no economic value implies more spending for their disposal. When not managed efficiently, accumulated wastes may contribute as loci for disease vectors. Through upcycling, these wastes can be converted into useful materials which can reduce environmental pollution and increase economic value.

In this study, the starches from the seeds of *rambutan*, *marang* and unripe green mango were extracted, purified and evaluated for their physicochemical and pharmacopeial properties.

MATERIALS AND METHODS

Materials

Marang, *rambutan* and unripe green mango fruits were obtained from local wet markets in Manila. The fruits were authenticated by the Plant Division of the National Museum– Philippines. All other reagents used were of analytical grade except for practical grade high amylose corn starch (CS) (Sigma, S4180).

Isolation of starch⁸

Marang (MS) and *rambutan* (RS) seeds were cleaned with water to remove the seed coat. The unripe green mango seeds (GMS) were washed, dried for two days and the seed kernel was manually separated from the seed coat. The seeds were cut into small pieces, allowed to soak for 24 h in 0.1% sodium metabisulfite solution then immersed in 85°C 80% ethanol solution for 16 h to remove monosaccharides and oligosaccharides. Starch was extracted by grinding the soaked samples at minimum speed in a regular food blender to produce a smooth paste. The paste was allowed to settle and the supernatant liquid was removed. The residue was treated with 0.1 M NaOH and was left to sit for 18 h at room temperature. The top layer was removed and the remains were washed with 0.1 M HCl to neutralize the alkalinity and then washed several times using distilled water until the residue became light in color. The residue was filtered under vacuum filter to remove residual water, spread on a tray and

dried in an oven at 60°C. The dried powder was crushed and passed through a 200 mm mesh sieve. The powder was stored in a plastic container at 4°C until analyzed.

Physicochemical characterization of starch

Percent yield

The amount of starch yield on fresh basis from the fruit wastes was calculated using Equation 1.

$$\% \text{ Starch yield, fresh basis} = \frac{W_s}{W_m} \times 100 \quad (1)$$

Where W_s is the weight of extracted starch and W_m is the weight of the fruit waste material used.

Organoleptic characteristics

Organoleptic characteristics were evaluated using the attributes of texture, odor, and color of the starches.

Amylose-amylopectin ratio⁹

Defatting of the sample was conducted before the analysis by soaking in petroleum ether for 24 h and drying at 60°C for 5 h. A 10 mg sample was dissolved in 3 mL 1 M NaOH and placed in a water bath set at 60°C for 5 min, followed by sonication for 25 min. A 1 M HCl was added to make the solution neutral and the final solution was diluted to 10 mL with distilled water. A 0.2 mL of the solution was transferred to a test tube, and mixed with 0.2 mL distilled water and 1.6 mL iodine solution. The color was allowed to develop in the dark for 30 min, and the absorbance was measured using UV-Vis spectrophotometer (Hitachi UH5300, Japan) at 466, 535 and 650 nm for amylopectin, and 535, 620 and 730 nm for amylose.

Bulk and tapped density¹⁰

The bulk density, D_{bulk} , and tapped density, D_{tap} , were calculated using Equations (2) and (3) based on the USP:

$$D_{\text{bulk}} = \frac{W}{V_0} \quad (2)$$

$$D_{\text{tap}} = \frac{W}{V_1} \quad (3)$$

Where W is the weight of starch, and V_0 and V_1 are the volumes of the bulk and tapped starch, respectively.

Compressibility index and Hausner ratio¹⁰

Compressibility index and Hausner ratio were calculated from bulk and tapped densities using Equations (4) and (5), respectively:

$$\text{Compressibility index} = \frac{D_{\text{tap}} - D_{\text{bulk}}}{D_{\text{tap}}} \times 100 \quad (4)$$

$$\text{Hausner ratio} = \frac{D_{\text{tap}}}{D_{\text{bulk}}} \quad (5)$$

Angle of repose¹⁰

Angle of repose was carried out using a 2 cm powder funnel with its tip affixed above a clean white paper. The powders were allowed to flow through the funnel until the apex of the cone touched the tip of the funnel. The angle of repose was calculated by measuring the height and base of the powder cone using Equation (6) below:

$$\tan \alpha = \frac{\text{height of the cone of powder}}{0.5 \times \text{base}} \quad (6)$$

Morphology

Morphology of the samples was obtained using a scanning electron microscope (SEM) (JEOL JSM-5310, USA) combined with an Oxford cathode-luminescence system. Images of the samples sputtered with gold coatings were obtained at 15 kW accelerating voltage and magnifications of 1000, 3500 and 5000 times.

Solubility and swelling power¹¹

A 100 mg sample was placed in a glass tube and weighed. A 10 mL water was added and heated from 50 to 95°C, with 5 min interval, in a water bath for 30 min. It was then cooled to room temperature and centrifuged at 3200 rpm for 10 min. The supernatant was decanted and transferred carefully to a pre-weighed aluminum dish followed by drying at 100°C for 4 h and weighed until constant. Solubility was calculated using Equation (8). The weight of the wet residue in tube was recorded and the swelling power was also calculated based on Equation (9) below:

$$\% \text{ Solubility} = \frac{\text{Weight}_{\text{solid content of supernatant}}}{\text{Weight}_{\text{sample}}} \times 100 \quad (8)$$

$$\text{Swelling power} = \frac{\text{Weight}_{\text{wet sediment in tube}} - \text{Weight}_{\text{sample in tube}}}{\text{Weight}_{\text{sample}}} \quad (9)$$

Viscosity

Viscosity of the starch sample was evaluated using Brookfield digital viscometer (LVT Brookfield viscometer, USA).

Pharmacopoeial characterization¹⁰

Identification

A 2% sample solution was boiled for 1 min. A 1 mL paste was transferred to a tube and added with 0.5 mL iodine solution. A positive result of orange-red to dark blue color, which disappeared on heating, was noted.

pH measurements

A 5 g starch sample was agitated continuously at a moderate speed with 25 mL of freshly boiled water for 1 min. The solution was allowed to stand for 15 min and the pH was determined.

Loss on drying (LOD)

A 1 g sample was dried at 130°C ± 2°C for 1.5 h in a weighing dish, placed in a desiccator for 30 min to cool and then weighed. LOD was calculated based on Equation (7) below:

$$\% \text{ LOD} = \frac{\text{Weight}_{\text{initial (dish+sample)}} - \text{Weight}_{\text{final (dish+sample)}}}{\text{Weight}_{\text{sample}}} \times 100 \quad (7)$$

Limit of iron

A mixture of 1.5 g sample and 15 mL 2 N HCl was filtered, and a 10 mL filtrate was transferred to a test tube. The filtrate was added with 2 mL citric acid solution (2:10) and 0.1 mL thioglycolic acid. A 10 M ammonium hydroxide was added to make the solution basic to litmus paper and diluted to 20 mL with water. The sample solution was observed after 5 min and the intensity of pink color change was noted after 5 min.

Statistical analysis

The tests were done in triplicate and the data generated were reported as mean ± standard deviation (SD). The data were analyzed by analysis of variance (ANOVA) followed by Tukey HSD post hoc test to compare the differences between the isolated starches and corn starch using Statistical Package for Social Sciences (SPSS) 23.0 software. Probabilities were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

The physicochemical and pharmacopoeial properties of the starch isolated from MS, RS, and GMS were compared with that of CS. The percent yield of the starch from MS, RS, and GMS were 63.78%, 56.06% and 29.91%, respectively. The GMS yield was similar to a previous study,¹² which is above 20%. The samples were off-white in color, have fine texture with characteristic odor, except for GMS which was odorless.

The data for the physicochemical properties for the three samples are shown in Table 1. Amylose-amylopectin ratio determines the potential utilization of starch, but it is the amylose content that largely influence the functional properties of starch.¹³ The amylose contents of MS, RS and GMS were significantly lower ($p=0.001$) and amylopectin content significantly higher ($p=0.001$) than the native high amylose of CS used. The amylose content of CS is close to the value of 67.8% reported from previous study.¹⁴ Variability on the sources and conditions may have influenced the amylose-amylopectin content of the isolated starch samples.¹⁵

Density measurements of starch are important for functional properties like powder flow and compactibility.¹⁶ The tapped and bulk densities of the samples were significantly lower ($p < 0.05$) than CS. The lower bulk and tapped densities exhibited by the isolated starches suggest

Table 1. Physicochemical properties of the starch samples \pm standard deviations

Property	Isolated Starch Samples			
	MS	RS	GMS	CS
Amylose	10.06 \pm 0.86*	11.14 \pm 1.79*	18.91 \pm 3.44*	65.37 \pm 4.49
Amylopectin	77.76 \pm 7.78*	87.73 \pm 5.93*	78.51 \pm 9.21*	30.16 \pm 4.04
Bulk density (g cm ⁻³)	0.34 \pm 0.01*	0.32 \pm 0.01*	0.31 \pm 0.01*	0.47 \pm 0.02
Tapped density (g cm ⁻³)	0.48 \pm 0.01*	0.46 \pm 0.01*	0.42 \pm 0.01*	0.61 \pm 0.01
Compressibility Index (%)	27.92 \pm 0.90*	29.61 \pm 0.57*	30.64 \pm 0.77*	22.93 \pm 1.95
Hausner ratio	1.39 \pm 0.02*	1.42 \pm 0.01*	1.44 \pm 0.02*	1.30 \pm 0.03
Angle of repose (°)	38.63 \pm 0.72	43.64 \pm 0.79*	44.26 \pm 0.90*	39.06 \pm 1.20
Solubility (%)	5.53 \pm 0.67	5.81 \pm 0.99	6.80 \pm 1.04*	3.70 \pm 0.89
Swelling power (g/g)	9.52 \pm 0.33*	6.45 \pm 0.22	14.56 \pm 0.75*	6.59 \pm 0.43
Viscosity (cP)	138.33 \pm 26.50*	34.17 \pm 3.82*	1040.00 \pm 43.59*	331.67 \pm 12.58

MS – Marang seed starch; RS – Rambutan seed starch; GMS – Unripe green mango seed starch; CS – Corn starch.

* Significant difference with control (CS) at $p < 0.05$.

Table 2. Pharmacopoeial properties of the starch samples \pm standard deviations

Property	Isolated Starch Samples			
	MS	RS	GMS	CS
Identification	Dark blue	Dark blue	Dark blue	Dark blue
pH	3.53 \pm 0.01*	4.25 \pm 0.00*	4.23 \pm 0.01*	6.31 \pm 0.02
Loss on Drying (%)	7.11 \pm 0.27	5.00 \pm 0.14*	4.35 \pm 0.19*	8.59 \pm 1.25
Limit of Iron (ppm)	<10	<10	<10	<10

MS – Marang seed starch; RS – Rambutan seed starch; GMS – Unripe green mango seed starch; CS – Corn starch.

* Significant difference with control (CS) at $p < 0.05$.

that they are not as porous as corn starch because void spaces formed by larger powder particles are not permeated by smaller particles, which leads to less compression.¹⁷

Some of the fundamental methods for testing powder flow are compressibility index, Hausner ratio and angle of repose.¹⁰ The compressibility index describes the flow properties of powder as excellent (<10%), fair (16-20%), passable (21-25%) and poor (26-31%). Powder flowability is inversely proportional to its compressibility index.¹⁸ Similarly, Hausner ratio ranging from 1.26 to 1.34 is passable while a range of 1.35-1.45 indicates poor flow properties. The compressibility index and Hausner ratio of all the isolated starches were significantly higher than CS ($p=0.001$), and showed poor flow properties based on these tests. The angle of repose describes the flowability of starch powders relying on the resistance of interparticulate mobility within particles.¹⁹ The angle of repose is passable on the range of 41-45° and fair when it is 36-40°. MS was comparable ($p=0.939$) with CS while GMS and RS were significantly higher than CS ($p=0.001$). However, all the isolated starches displayed passable flow properties. This latter test is not fairly consistent with the results from compressibility index and Hausner ratio because of the non-numerical measurement of scales and the technique is different from the others.²⁰

The low values of bulk and tapped densities and high values in compressibility index and Hausner ratio of the isolated starches may suggest poor flow properties.²¹ This may be due to their particle size and shape. Starch in plants have characteristic sizes, shapes, and morphology. Scanning electron microscopy indicates the size distribution of a

potential excipient, which affect formulation properties such as drug release and flowability.²² The morphological structure of MS, RS, GMS and CS is shown on Figure I. CS granules are polygonal and smooth, consistent with previous literature.²³ The SEM images of the isolated starch samples were irregular in shape and with smaller particle size which could be responsible for their poor flowability.²⁴

The solubility of starch is dependent on the relative composition of amylose and amylopectin,²⁵ while the swelling power is influenced by the hydrogen bonding of starch with water when they are heated.^{26,27} MS ($p=0.140$) and RS ($p=0.083$) displayed comparable solubility with CS while GMS ($p=0.001$) displayed significantly higher solubility. For swelling power, MS and GMS were significantly higher ($p=0.001$) except for RS ($p=0.984$) which was comparable with CS. GMS displayed significantly higher final viscosity than CS ($p=0.001$) while both MS and RS demonstrated significantly lower ($p=0.001$) final viscosity. This may be due to high level of phosphorus in mango seed kernels which could be present as monoester phosphate.²⁸ This phosphate group is responsible for the paste viscosity and is connected with the amylopectin portion of starch. This influences also its solubility and swelling properties.¹³ The low viscosities of RS and MS may be due to the interaction of fats and proteins with starch.²⁹ RS have been reported to have higher fat content of up to 38.9%³⁰ than 6.2% of CS.³¹

The results of the pharmacopoeial characterization are indicated in Table 2. All samples passed the USP criteria of dark blue color in identification test. The pH of RS, GMS and CS, ranging from 4.23 to 6.31, were within the acceptable limits of 4.0 to 7.0 in the USP. However, MS

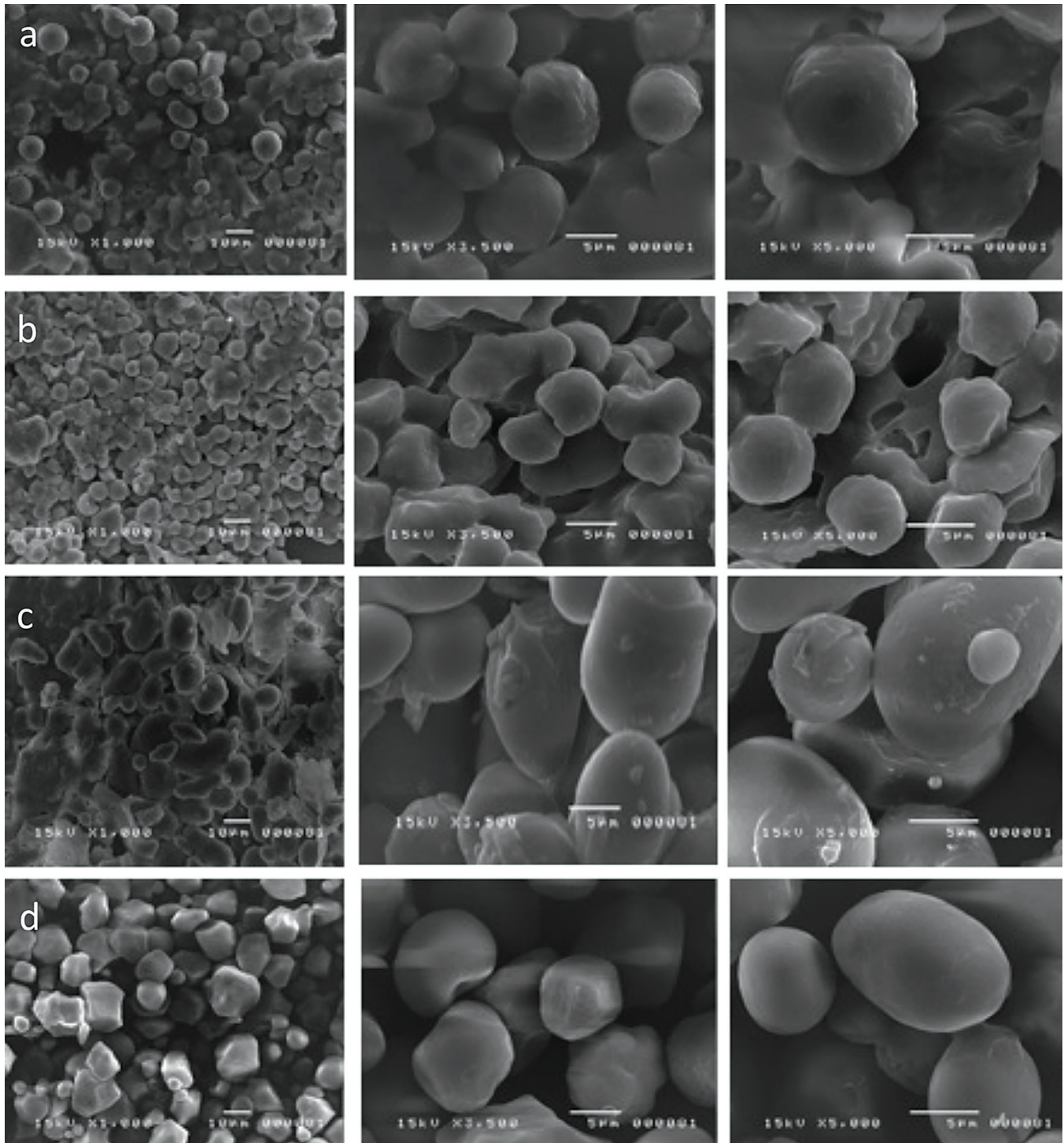


Figure 1. Micrographs of MS, RS, GMS and CS under the magnification of 1000, 3000 and 5000 times.

was acidic, with a pH of 3.53 which may be influenced by the insufficient washing with water during extraction.³² The samples were also within the limits of not more than 15% loss on drying as set by the USP. The test for limit of iron of the samples also complied with the acceptable criteria where the pink color is not more intense than the 10ppm standard iron.

CONCLUSION

The isolated starches from fruit by-products have unique properties which could be utilized as sources of starch in pharmaceutical applications. Among the starches, only *rambutan* seed starch was observed to have comparable and desirable physicochemical and pharmacopoeial

property with corn starch. It is recommended to optimize the *rambutan* starch extraction process, particularly the neutralization of the samples. Moisture content of the powders should also be monitored and controlled to improve flowability.

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Statement of Authorship

All authors have approved the final version submitted.

Author Disclosure

All authors have declared no conflict of interest.

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