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Extraction and enzymatic depolymerization of gum from *Artocarpus heterophyllus* Lam. seeds

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Abstract

The aim of this study was to compare the extraction of jackfruit seed gum by aqueous ethanol and water systems. The results showed that the highest extraction of crude polysaccharide from jackfruit seed gum was achieved with aqueous 50% EtOH. In contrast, water extraction at 50°C produced the greatest total sugar content in the form of non-reducing sugar represented by oligosaccharide. The enzymatic depolymerization of the crude polysaccharide was hydrolyzed by α-galactosidase. The purified oligosaccharide was subjected to weak anion exchange chromatography and determined its intrinsic viscosity. The viscosity-average molecular weight was calculated to be 1.551 x 10^6g. The results of this study suggest that jackfruit seed gum might be utilized as a functional food ingredient to supplement food products.

Introduction

Nowadays, natural polysaccharides from plants have evoked considerable interest due to the possibility of using them in both food and non-food industries. Due to their unique physicochemical properties, non-toxicity and the low cost of water-soluble polysaccharides, they have been used as a replacement for synthetic polymers in food and pharmaceutical products. Polysaccharide hydrocolloids are mostly found in the parts of plants such as rhizomes, roots, tubers and seed endosperms which act as energy reserves. These natural colloids include guar gum from the endosperm of plant seeds (Dakia *et al*., 2008), galactomannan from *Artocarpus integrifolia* (Garros-Rosa *et al*., 2006), tamarind seed gum (Khounvilay and Sittikijyothin, 2012), agar (Pereira-Pacheco *et al.*, 2007), konjac (Du *et al*., 2004), and chitosan (Phillips, 2012).

The jackfruit, Artocarpus heterophyllus Lam., is a tropical fruit. It is mostly found in South-East Asia, particularly in India, Malaysia, Indonesia, the Philippines and Thailand. The Artocarpus species is traditionally used for food and as a folk medicine for the treatment of diarrhea, diabetes, malarial fever, tapeworm infection and other ailments. Based on its botanical structure, 8-15% of the fruit weight is represented by the seeds which are enclosed in a brown spermoderm. The spermoderm covers the white cotyledon which has high starch and protein contents (Singh *et al*., 1991; Prakash *et al*., 2009). The seeds are sometimes cooked in Thai cuisine (Tongdang, 2008). However because of their high polysaccharide content the seeds may have other commercial applications. The seeds which are enclosed in fleshy endosperm cells, have a thick cell wall which stores polysaccharides. These include pure mannans, galactomannans, glucomannans, xyloglucans and galactans (Appukuttan and Basu, 1987; Bacic *et al*., 1988; Carpita and McCann, 2000). The storage polysaccharide in seeds are mainly contained guar galactomannan. According to the swelling property of guar galactomannan, it can absorb a large quantity of water which interacted with the hydrophilic portion of the galactomannan. Moreover, the storage polysaccharide serves a food source for the germinating seeds (Appukuttan and Basu, 1987). In the other hand, these natural polymers have many different applications in industries such as thickening, emulsifying, gelling, flocculating and film forming (Singh *et al*., 2000; Singh, Tiwari, Tripanthi and Sanghi, 2005; Mikkonoen and Tenkanen, 2012).

The galactomannans are neutral and water-soluble polysaccharides consisting of a linear mannose with β (1,4) linkages which randomly branch into α (1,6) linkage galactose units. They belong to the Leguminoseae family. The commercial galactomannans are available from locust bean gum (LBG), guar gum (GG) and tara gum (TG). Guar galactomannan with different ratios of mannose and galactose are employed in different industrial applications (Cheng *et al*., 2002). Chemical and

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enzymatic methods are employed to depolymerize the polysaccharide to prepare compounds with different molecular weights for industrial purposes. There are difficulties associated with the use of chemical hydrolysis, because the process needs to be controlled by varying the treatment time and the concentration of acid used (Dutta *et al.*, 2011). The reaction proceeds randomly and produces a large amount of free monomer and by-products. On the other hand, enzymatic hydrolysis using, for instance, mannosidase and/or galactosidase, involves shorter treatment times with milder conditions and can be used more selectively to produce specific glycosidic bonds (Vishu and Tharanathan, 2004). It has been reported in the medical literature that partially hydrolyzed guar gum. The soluble dietary fiber is effective in reducing the incidence of diarrhea and symptoms of irritable bowel syndrome. Furthermore, it also stimulates the production of increased amounts of bifidobacterium in the gut (Slavin and Greenberg, 2003).

From the reports of Appukuttan and Basu (1987), it can confirm that the storage polysaccharides in jackfruit seeds mainly compose of guar galactomannan. The attractive interest is to find the other natural source of galactomannan beyond *Leguminoseae*. The seed endosperm of *Artocarpus heterophyllus* Lam., Moraceae family, largely located in Southern part of Thailand. Although both fruits and seeds of jackfruit serve as the diet which is the nutritious food. Some consumers are till considered the seeds as wastes. To increasing value added product as food ingredient is worth for seeds. Therefore, the purpose of this study aim to investigate the optimal conditions for the extraction of polysaccharide from jackfruit seed gum using aqueous ethanol and water due to being water-soluble galactomannan. It based on the highest non-reducing sugar content produced in water-soluble extracts. Non-reducing sugars are generally found in guar extracts with a lower molecular weight. The extracted guar was then depolymerized using α-galactosidase to prepare it as a food ingredient. The intrinsic viscosity of galactomannan sample was determined by the Huggins' and Kraemer's equations. The rapid determination of MW was then allowed.

Materials and Methods

Preparation of samples

Seeds of the jackfruit variety, *Artocarpus heterophyllus* Lam., known locally as Thong Sud Jai, were obtained from fruit purchased from a local market in Hatyai, Songkhla, Thailand. The fruits selected were ripe, of uniform size and without defects. The seeds from the fruits were soaked in water overnight to allow the outer seed coats to be easily removed. The seeds were then sliced into pieces and dried in a hot air oven at 50°C for two days. The dried seeds were ground into a powder with a blender, which was then kept in a desiccator until used.

Chemical composition of the seed

Determination of the ash, moisture, fat and protein content of the jackfruit seeds was conducted following the standard method of AOAC (AOAC, 2000).

Isolation of the polysaccharide

Samples (200 g) of the powdered jackfruit seeds were extracted with two solvent systems, aqueous ethanol (500 mL) and water (500 mL). Aqueous solutions with concentrations of 50%, 75% and 95% ethanol were used for ethanol extraction, with ambient temperatures of 50°C and 70°C for water extraction. The aqueous mixtures were stirred continuously for 3 hours. The extraction was repeated twice. The supernatant was separated from the extraction mixture by centrifugation and the crude polysaccharide was precipitated by aqueous 95% ethanol. The precipient was collected and dried by lyophilization. The crude polysaccharide was kept at -20°C before further analysis.

Determination of the total sugar content and reducing sugars content

The total sugar content was determined following the method of Dubois *et al.* (1956). A quantity of 100 µL of polysaccharide was dissolved in water (0.5 mL) and mixed with aqueous 5% phenol (0.5 mL). Concentrated H_2SO_4 (2.5 mL) was then added to the solution, which was then allowed to stand in the dark at room temperature for 30 minutes. The absorbance was measured at a wavelength of 492 nm. The standard curve was measured in a standard solution (mannose) with a concentration of 1-50 µg/mL.

The reducing sugar content was determined using a DNS reagent according to the method of Miller (1972). A quantity of 0.176 M of DNS reagent (3,5-Dinitrosalicylic acid) was dissolved in 2.67 M of NaOH and 300 g of sodium potassium tartrate gradually added. The DNS solution was adjusted to a volume of 1 L and stored in a brown bottle until used. The polysaccharide solution (0.5 mL) was added to the DNS solution (0.5 mL) in a screw-cap test tube and boiled in a water bath for 10 minutes. The sample tube was then cooled in an ice bath for 10 minutes to complete the reaction and 5mL of distilled water was added to the sample solution. Then the absorbance at λ_{max} 540 nm was monitored. The calibration curve

was created using concentrations of glucose in the range 1-50 µg/mL as the standard (Miller, 1972). The non-reducing sugar content in the water extracted sample and the aqueous ethanol extracted sample were then compared.

Enzyme activity assay (α-galactosidase activity assay)

The assay of enzyme activity was carried out according to the method of Viana *et al.* (2005). The α-galactosidase enzyme activity was assayed using a substrate of PNP-αGal. The unit enzyme activity was assessed based on the concentration of enzyme in McIlvaine buffer (0.1 M citric acid + 0.2 M Na_2HPO_4, with the pH adjusted with 0.1 M HCl) released per µmol of *p*-nitrophenol per minute at 37°C. The pH and temperature of the α-galactosidase activity was optimized.

Optimization of pH and temperature for α-galactosidase activity

A quantity of 2 mM of PNP-αGal (250 µL) was dissolved in 0.1 M citrate phosphate buffer (650 µL) pH 4-6 at 40-80°C. The solution was stirred thoroughly at 37°C for 5 minutes. A quantity of 0.09 unit/mL of α-galactosidase enzyme (100 µL) was then added. The reaction was completed with 0.1M Na_2CO_3 (1 mL). The absorbance at a wavelength of 405 nm was recorded by a spectrophotometer.

Hydrolysis of crude polysaccharide byα-galactosidase enzyme

The crude polysaccharide was prepared as a 5% solution (w/v) in sodium acetate, pH 5.0 (5 mL). It was then kept for further analysis. The 5% polysaccharide solution was diluted into 1% in sodium acetate buffer. The hydrolysis of the 1% crude polysaccharide with 1.09 U α-galactosidase (20 µL) was allowed to stand at 37°C for 5 minutes. The hydrolyzed polysaccharide was sampled at intervals of 5 minutes. The hydrolysis was completed by the addition of Aq. 0.5 M Na_2CO_3 (250 µL). The quantity of galactose monosaccharide released was determined by the DNS reagent method. After the hydrolyzed polysaccharide was obtained, the supernatant was separated by centrifugation at 3500 rpm and dialyzed with distilled water. Thereafter, the oligosaccharide was purified using a weak basic anion exchange resin. The loaded oligosaccharide was eluted by 0.1-0.5 M NaCl. The oligosaccharide fraction was tested by the phenol-sulfuric method.

Intrinsic viscosity

The average molecular weight of the oligosaccharide (MW) was determined by using the measurement of a capillary Ubbelohde viscometer. After filtration using a membrane filter 0.45 µm of the oligosaccharide solution was prepared to a concentration of 0.005-0.08 mg/mL. The intrinsic viscosity was determined by using an Ubbelohde viscometer in a water bath at a controlled temperature of 25°C. The measurement was determined following the Mark-Houwink-Sakurada (MHS) equation (Doublier and Launay, 1981):

$$[\eta] = K \times MW^a$$

Hence, at 25°C in distilled water, $K = 3.04 \times 10^{-4}$; $a = 0.747$ (Cheng, Brown, & Prudhome, 2002; Shobha *et al*., 2005), $[\eta]$ = viscosity of solution at dilute solution.

Statistical analysis

Comparison of the contents which extracted by aqueous EtOH and water solvent was based on Duncan's multiple range test. P-values < 0.05 were considered significant. The triplicate determinations were used in the analysis to estimate measurement variances.The data were represented as mean ± SD.

Results and Discussion

Chemical composition of jackfruit seed flour

On a dry weight basis, jackfruit seeds flour comprises mainly carbohydrate (74.39±0.07%) and protein (13.07±0.62%) as shown in Table 1. The carbohydrate content reported by Tulyathan et al. (2002) was higher at 82.25% for seeds without the brown spermoderm, whereas that reported by Singh *et al.* (1991) was 74%. Moreover, the protein content reported by Tulyathan *et al.* (12.25%) was less than that found in the present study (%Nx6.25). It has been noted that carbohydrate and protein content may differ according to the variety of jackfruit, the size of the seeds and the environmental conditions under which the jackfruit was grown. However in view of the high carbohydrate content, jackfruit seed flour has the potential to be used as a thickening and gelling agent in the food industry with its uses depending upon the viscosity produced by the varying properties of the flour.

The efficacy of extraction of polysaccharide by aqueous EtOH and water solvent

Due to the solubility of galactomannan in water, It is a crucial key to find the solvent for extraction. Therefore, the experiment is investigated the efficacy of the methods of extraction with the two different selected solvents, aqueous EtOH and water. The results showed that the highest extraction of crude

Table 1. Chemical composition of jackfruit seed flour on a dry weight basis

Parameter	%content of jackfruit seed flour		
	This study	Tulyathan *et al.* (2002)	Singh *et al.* (1991)
Moisture	7.56 ± 0.07	8.57 ± 0.25	5.10
Crude protein (%Nx6.25)	13.07 ± 0.62	12.25 ± 0.21	18.88
Crude lipid	0.78 ± 0.02	0.99 ± 0.08	2.20
Ash	4.20 ± 0.02	3.92 ± 0.03	3.60
Total carbohydrate	74.39 ± 0.07	82.25	74

1). All the values found in the present study and in Tulyathan *et al.* (2002) were based on triplicate measurements.
2). The seeds used in Tulyathan *et al.* were without the brown spermoderm.

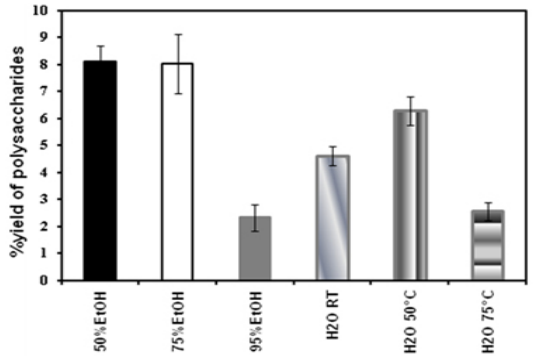

Figure 1. The efficiency of extraction by various ratios of ethanol and water solvent systems for jackfruit seed gum.

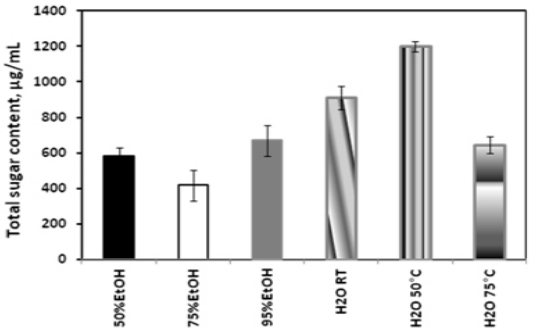

Figure 2. Total sugar content (µg/mL) of crude polysaccharides produced by solvent systems consisting of aqueous EtOH and water at different temperatures.

polysaccharide was yielded by the aqueous 50%EtOH solvent (8.10±0.58%), followed by the aqueous 75%EtOH solvent (8.04±1.10%) followed by water at 50°C (6.30±0.54%), (Figure1). This suggests that suitable aqueous EtOH hydrophilic solvents used to extract crude polysaccharides should not have an ethanol content exceeding 50%. Higher ratios of water to EtOH are less efficient in the extraction of crude polysaccharide.

A total sugar analysis was employed to measure the total soluble polysaccharide using the phenol-sulfuric method. The result of the total sugar analysis showed that water extraction at 50°C produced the greatest total sugar content (1,200.897±29.38 µg/mL) when compared with the other solvent systems studied. This suggests that water is the best solvent to dissolve hydrophilic sugars. However, it was revealed that the temperature under which extraction occurs, affects the extraction of the sugar content. The

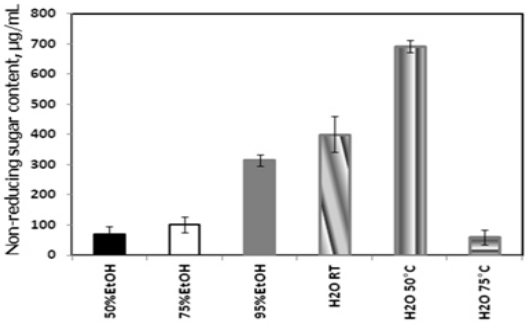

Figure 3. Non-reducing sugar content (µg/mL) of crude polysaccharides produced by solvent systems consisting of aqueous EtOH and water at different temperatures.

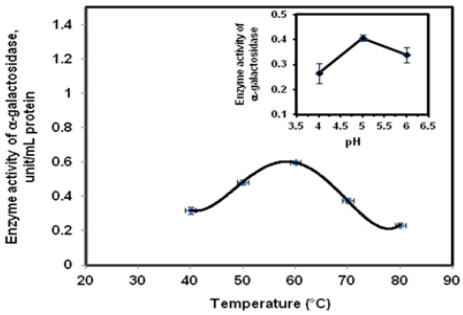

Figure 4. The effect of temperature and pH to enzyme activity of α-galactosidase in hydrolyzing PNP-αGal substrate in 0.1M aqueous citrate phosphate buffer.

optimal temperature was found to be 50°C (Figure 2). The proportion of reducing monosaccharides and disaccharides to crude polysaccharides was analyzed by the reducing sugar method and determined by a DNS reagent. The non-reducing sugar content was calculated by subtracting the reducing sugar content from the total sugar content. The non-reducing sugar represents low molecular weight oligosaccharide which can be obtained from water extraction, and water extraction at 50°C provided a greater non-reducing sugar content (691.579±20.08 µg/mL) than the other systems studied (Figure 3). The hydroxylic group in the oligosaccharide structure might contribute to their accessibility to extraction by water.

Hydrolysis of jackfruit seed gum by α-galactosidase enzyme

Hydrolysis using the artificial substrate PNP-αGal by α-galactosidase was performed to determine the optimum pH and temperature for enzyme kinetics. The result showed that the optimum pH and temperature for the activity of α-galactosidase were a pH of 5.0 (Figure 4) and a temperature of 60°C (Figure 4). The characteristics of purified α-Gal I (α-galactosidase I) from *Streptomyces griseoloalbus* have been reported in the literature. Purified α-Gal I was noted by Anisha *et al.* (2009) to be active between pH values of 5.0 and 7.0 for 2 hours. The temperature range within which it was active was 50 to 65°C for 2 hours. The enzyme kinetics of α-Gal I from *S. griseoloalbus* were also

studied to determine the hydrolysis of the substrate PNP-αGal. It was found that the Michaelis constant (Km) was equal to 0.79±0.1M and the V_{max} value was equal to 693.4±0.05 μmol $min^{-1}mg^{-1}$. Although the characteristics of α-galactosidase enzyme has studied yet the effect of pH and temperature, the K_m and V_{max} values of kinetic parameter were obtained from the comparison with the α-Gal I from *S. griseoloalbus.*

In this study, polysaccharides from jackfruit seed gum were hydrolyzed by α-galactosidase. The maximum rate of degradation was achieved at 30 minutes after which it gradually declined (data not shown). Manzanares *et al.* (1998) reported that the degradation of PNP-αGal did not occur in accordance with Michaelis-Menten kinetics. When the concentration of the substrate was higher than 1.26 mM, the reaction was inhibited by the release of D-galactose. It seems that D-galactose acts as a competitive inhibitor for α-galactosidase activity (Manzanares *et al.*, 1998).

Intrinsic viscosity

Enzymatic depolymerization is a method of decreasing the intrinsic viscosity of polysaccharides by reducing the chain length and molecular weight of the seed gum (Mudgil *et al.*, 2012). D-galactose depolymerized with α-galactosidase produces branch chain residues which link with the main mannan chain. In this study, the relative viscosity (η_r) of the purified oligosaccharide was determined by measuring its viscosity over time in a solution at a low concentration flowing through a Ubbelohde viscosmeter and comparing it to that of the solvent. The intrinsic viscosity (η) was extrapolated by linear graphs of Kraemer ($\ln \eta_{r/c}$) and Huggins ($\eta_{sp/c}$) plots. The specific viscosity, η_{sp} is equal to ($\eta_r - 1$). In this study, the intrinsic viscosity was 12.8 dL/g. According to the Mark-Houwink-Sakurada equation, the viscosity-average molecular weight (MW_v) was $1.551x10^6$ g. The values of a and K were 0.747 and 3.04×10^{-4}, respectively. It were the same as those used by Cheng (2002) for the commercial guar galactomannan. These values are closed to those used by Robinson *et al.* (1982), where a and K were 0.72 and $3.8x10^{-4}$ g, respectively with a polydispersity ($M_w/M_n < 3$). The difference is relevant for the distribution of the polydispersion ratio.

Conclusions

Hot water extraction of jackfruit seed produced the most non-reducing sugar represented by crude polysaccharide. Depolymerization of the polysaccharide by α-galactosidase produced low viscosity oligosaccharide which when purified may have the potential to be utilized as a soluble dietary fiber supplement.

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