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Proceeding

Polysaccharide extraction of defatted rambutan seed by hot water and subcritical water extractions

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Abstract: Rambutan seeds (RS) are industrial waste often generated in the canned fruit industry. The aim of this study is to extraction polysaccharide from defatted rambutan seed or crude polysaccharides (POLS-DRS) by subcritical water. The defatted seed powder (DRS) was extracted by a hot water extraction (HWE) at 100°C as a reference condition. Subcritical water extraction (SWE) was performed at 120–140°C and initial pressure of 2 MPa. The sample to water ratio of 1:10 (w/w) and extraction time of 15-60 min were performed for both methods. The results show that the gravimetric extraction yields of 53.01 g/100 g DRS and 7.71–41.70 g/100 g DRS were obtained from HWE and SWE, respectively. Besides, HWE provided total sugar of 30.75 g/100 g POLS-DRS, while SWE generated the total sugar in the range of 27.00–49.76 g/100 POLS-DRS. Antioxidant activities of POLS-DRS were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The 40 mg of POLS-DRS which obtained from 120°C and 60 min provided the highest DPPH activity of 82.93% inhibition. The POLS-DRS were suitable for growing microorganisms because they had a high sugar content and a low total phenolic content. The prebiotic activity assay will be measured in the future studies.

Keywords: Rambutan seed; Polysaccharides; Subcritical water extraction; Bioactive compound

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1. Introduction

Rambutan (*Nephelium lappaceum* Linn.) is a native seasonal fruit in southeast Asia. It can be consumed as fresh fruit or processed into various food products. The production capacity of rambutan in Thailand is approximately 0.3–0.4 million tons per year. It is reported that annually, an average of 1900 tons of rambutan seed is wasted [1, 2].

The extraction and purification of seed polysaccharides by hot water extraction (HWE) and subcritical water extraction (SWE) have been recently summarized elsewhere [3]. HWE is performed below 100°C, whereas SWE is performed between 100°C and 374°C under pressures of 0.1–22.1 MPa. Increasing the temperature enhances the heat and mass transfer during the extraction process, reduces the dielectric constant (polarity) and

viscosity of water, and induces the dissociation of hydronium ions [4]. However, SWE carries the risk of the thermal degradation of active compounds when performed at higher temperatures. Although the protein and carbohydrate compounds in rambutan seed are valuable, there are relatively few studies of these compounds obtained by HWE and SWE because of the high lipid content of the seeds. Defatting the seed with *n*-hexane prior to aqueous and/or ethanolic extractions of carbohydrate is necessary [2]. For example, a study on the polysaccharide extraction of the selected plants from southern Thailand reported problems with the extraction of rambutan seed. The aqueous and/or ethanolic extractions of RS were impaired by the formation of a fat layer on the surface [5]. The aim of this work was to produce a crude polysaccharide extract containing the highest total sugar with low phenolic compound content by subcritical water extraction.

2. Materials and Methods

2.1. Rambutan Seed Preparation

Rambutan seed waste supplied by Pissanumhon Food Products Company Limited, Chumphon province, Thailand. Feedstock was cleaned under running tap water prior to drying under hot air circulation at 60°C for 8 h. Dried rambutans seeds were pre-extracted fat by a screw press machine at feed rate of 7 kg/h. A kilogram of screw pressed cake subsequentially extracted by supercritical CO_2-ethanol extraction at 30 MPa and temperature 50±5°C for a static extraction time of 90 min. Proximate analysis of DRS was conducted by AOAC standard methods.

2.2. Subcritical Water Extraction and Biological Activity Assay

The SWE was performed at a constant ratio of 20 g sample to 200 ml of DI water (1:10 w/v). Extraction conditions were conducted by using the central composite design as shown in Table 1. In this work, SWE was compared to HWE at 100°C for 60 minutes. The extractor (Parr company, Series 4625, 500 mL working volume) was charged by DRS sample and pressurized by nitrogen to 2 MPa. After extraction time was achieved, the extractor was quenched in an ice-water bath to room temperature. The 100 ml of extracts were filtered by paper filter, centrifuged, and mixed with 95% ethanol at sample to ethanol ratio of 1:4 (v/v). The mixture was incubated overnight at 4°C before subjected to centrifugation at 8000 rpm for 20 minutes at 4°C to separate the precipitated POLS-DRS. The POLS-DRS were dried in a hot air oven at 40°C for 24 hours. POLS-DRS yield was calculated from the weight of dried precipitate divided by the weight of DRS. The POLS-DRS was ground for further bioactive analysis. The total sugar content was determined by the phenol–sulfuric acid method using D-glucose as a standard. Antioxidant activities and total phenolic content (TPC) of POLS-DRS were measured by DPPH radical scavenging assay at substrate concentration of 40 mg/ml [6].

Table 1. Experimental conditions of POLS-DRS.

No.	Extraction	DRS (g):DI water (ml)	Temperature (°C)	Time (min)
1.	HWE	20:200	100	60
2.	SWE	20:200	120	15
3.	SWE	20:200	120	30
4.	SWE	20:200	120	60
5.	SWE	20:200	140	15
6.	SWE	20:200	140	30
7.	SWE	20:200	140	60

2.3. Statistical analysis

Statistical analysis results of all data are reported in term of average ± S.D. Total variation, present of data was estimated by one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) used for determining significance ($p \leq 0.05$) by SPSS program version 22.

3. Results and Discussion

3.1. Proximate analysis of DRS

The proximate analysis of DRS showed the moisture content of 2.57±0.07 %(w/w), carbohydrates content of 72.31±0.08 %(w/w), fat content of 8.07±0.32 %(w/w), protein content of 8.95±0.21 %(w/w), crude fiber content of 6.19±0.11 %(w/w), and ash content of 1.91±0.01 %(w/w). In our previous work, RS had 29.00±0.18 %(w/w) of fat and 42.96±0.15 %(w/w) of carbohydrate [7]. It was clear that screw-press and supercritical CO_2-ethanol extractions effectively removed fat from RS and enhanced carbohydrate in DRS.

3.2. Effects of temperature and time on POLS-DRS yield

POLS-DRS yields obtained from various conditions were illustrated in Figure 1. The HWE generated the highest POLS-DRS yield of 51.01 g/100 g DRS because the extracted polysaccharides were water-soluble starch [8]. At temperature of 120°C, the maximum POLS-DRS yield of 41.71 g/100 g DRS was produced at 30 min. Whereas, SWE conducted at 140°C gave the maximum POLS-DRS yield of 36.72 g/100 g DRS at 15 min. At escalated temperature, subcritical water partially hydrolyzed polysaccharides to oligosaccharides [9]. Hence, it was certain that temperature and time are a crucial parameter for SWE.

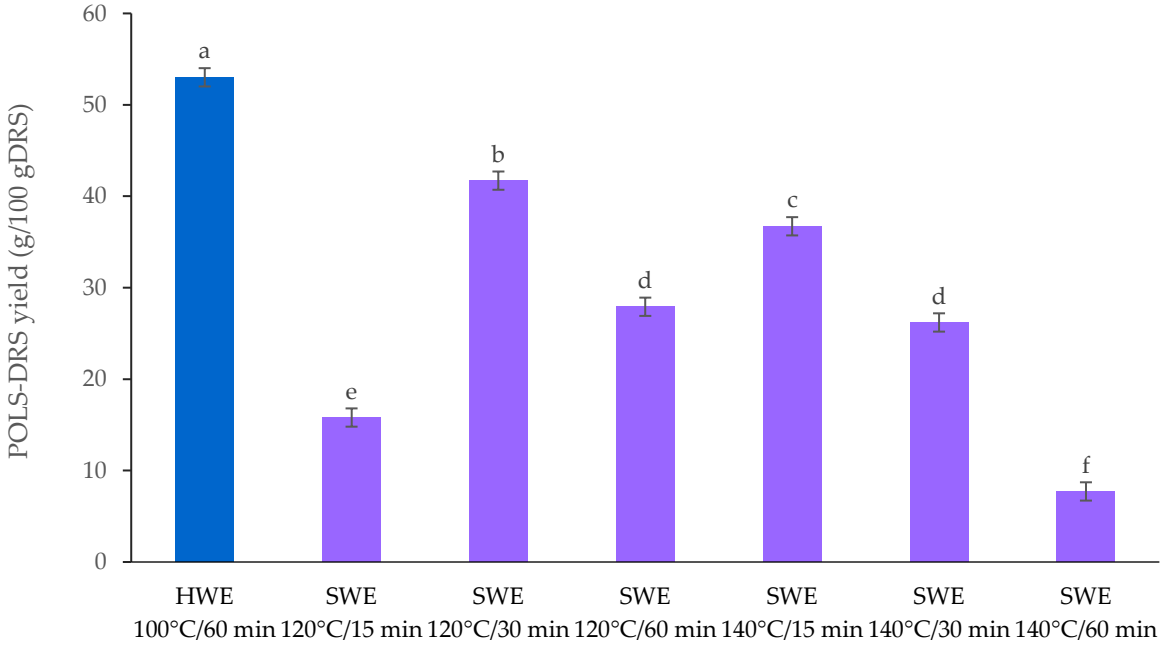

Figure 1. POLS-DRS yields obtained from HWE and SWE extractions. Mean values with different superscript letters in each column are significantly different ($p \leq 0.05$, DMRT).

3.3. Total sugar, DPPH activity, and pH of POLS-DRS solution

According to Table 2, SWE at temperature of 120°C and extraction time of 60 min, the highest total sugar content of 49.76 g/100g POLS-DRS and the DPPH scavenging activities of 82.93 % inhibition at 40 mg POLS-DRS /ml were observed, while a lower TPC of 28.78 mg gallic acid equivalent (GAE)/ 100g POLS-DRS was shown. The total sugar and DPPH activity of POLS-DRS obtained from SWE were higher than that obtained from HWE. TPC were found in low amounts for all samples, except the sample produced from 140°C and

60 min. The pH value of POLS-DRS solutions aligned within range of 6.55-7.15 which is suitable for microbiological growth. The monosaccharide profile of POLS-DRS will be analyzed in further study.

Table 2 Total sugar, TPC (mg/100g POLS-DRS), DPPH (%inhibition) and pH of POLS-DRS from virous conditions

Extraction	Time (min)	Total sugar (g/100g POLS-DRS)	TPC (mg GEA/100g POLS-DRS)	DPPH (%inhibition)	pH
HWE 100 °C	60	30.751±1.168 ^{e}	12.396±0.628 ^{g}	51.251±1.947 ^{f}	6.78±0.03 ^{a}
SWE 120 °C	15	35.949±2.158 ^{c}	24.137±0.558 ^{f}	59.916±3.597 ^{c}	6.55±0.07 ^{a}
	30	27.004±0.510 ^{f}	25.833±1.241 ^{e}	45.004±0.851 ^{g}	6.78±0.02 ^{a}
	60	49.757±1.158 ^{a}	28.775±0.455 ^{d}	82.928±1.930 ^{a}	6.64±0.03 ^{a}
SWE 140 °C	15	34.560±2.294 ^{d}	54.019±0.805 ^{c}	57.601±3.825 ^{e}	7.02±0.02 ^{a}
	30	35.002±1.655 ^{cd}	72.292±1.419 ^{b}	58.337±2.758 ^{d}	7.15±0.07 ^{a}
	60	38.012±1.020 ^{b}	448.407±22.554 ^{a}	63.354±1.701 ^{b}	6.97±0.05 ^{a}

Mean values with different superscript letters in each column are significantly different ($p \leq 0.05$, DMRT).

5. Conclusions

POLS-DRS were extracted from DRS using HWE and SWE. Under the optimum extraction, a temperature of 120°C and an extraction time of 60 min, SWE resulted in a yield of 27.91 g/100 g DRS, while total sugar and DPPH higher than that of the HWE. To use POLS-DRS as prebiotic, higher total sugar, lower amounts of TPC and neutral pH were expected. Except the sample obtained from 140°C and 60 min, all samples are suitable for testing of prebiotic activity. After extraction time of 30 min at 140°C, the exponentially increasing of TPC informed that the degradation of lignin and tannin took place at this condition.

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