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Sequential Process to Valorisation of Rambutan Seed Waste by Supercritical CO₂-Ethanol and Subcritical Water Extractions

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This work demonstrates the extraction of crude lipid and crude polysaccharide from rambutan ($Nephelium\ lappaceum\ Linn.$) seed using supercritical CO₂ with ethanol as co-solvent (SCE) and subcritical water (SCW) extractions, respectively. As dried rambutan seeds contain 32.48 \pm 1.07 g of extractable lipid/100 g dried sample, pre-extraction of 16.65% of the lipid content by a screw pressing machine improved the efficiency of SCE extraction. Moreover, the addition of ethanol as a co-solvent resulted in a fivefold improvement in phenolic compound extraction. The crude lipid extract had a total phenolic content of 163.81 \pm 0.47 mg GAE/100 g extract. The SCW extraction was investigated by a Box-Behnken design using extraction yield and total sugar content as responses. The optimal condition was found at temperature range of 150–180°C and extraction time in the range of 15–60 min where both responses were maximized. The solid residue discharged from subcritical water extractor was identified as hydrochar because it contained rich oxygen functional groups. This zero-waste process produced 5.2 kg of SCCO₂-extracted lipid, 16.2 kg of crude polysaccharide, and 37.7 kg of hydrochar from 100 kg of fresh rambutan seed.

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 (Jahurul et al., 2020; Sirisompong et al., 2011). Rambutan seed lipids contain 33.35–46.64 g/100 g fat of oleic acid and 26.03–34.36 g/100 g fat of arachidic acid as the major fatty acids (Afzaal et al., 2023; Jahurul et al., 2020). Bioactive compounds, e.g., tannins, saponins, alkaloids, and flavonoids, are found in rambutan seed (Solís-Fuentes et al., 2020). The extraction and purification of seed polysaccharides by subcritical water extraction (SCW) have been recently summarized elsewhere (Muthusamy et al., 2021). Increasing the temperature enhances the heat and mass transfer during the extraction process, reduces the dielectric constant and viscosity of water, and induces the dissociation of hydronium ions (Zhang et al., 2020). However, SCW carries the risk of the thermal degradation of active compounds when performed at higher temperatures. Although the protein and carbohydrate in rambutan seeds are valuable, there are relatively few studies of these compounds obtained by SCW because of the high lipid content of the rambutan seeds. Defatting the seed with *n*-hexane or ethanol prior to aqueous and/or ethanolic extractions of carbohydrate is necessary (Jahurul et al., 2020; Wichienchot et al., 2011).

Because rambutan seeds generated environmental problems in the factory that donated them as sample, this research aimed to valorise rambutan seed waste as feedstock for production of crude lipids and polysaccharides. This work introduces a defatting method for rambutan seed lipid that uses mechanical and supercritical fluid extractions (SFE). High value lipids from *Cucurbita pepo* L. seeds, *Serenoa repens* L. fruits, and coffee silverskin were extracted by SFE as zero-waste and green approaches to valorisation of vegetable wastes (Marzorati et al., 2022). A screw press machine was employed to partially extract lipids and to enhance the efficiency of SFE. Mechanical extraction is a chemical-free method for extracting seed oil such as hemp seed (Crimaldi et al., 2017). Previously, this approach was successfully demonstrated with *Moringa oleifera* seed (Ngamprasertsith et al., 2021). Consequently, defatted seeds were subject to SCW to obtain crude polysaccharides (Sakdasri et al., 2022). The effects of temperature, extraction time, and liquid-to-solid (L/S) ratio on crude polysaccharide extraction yield and total carbohydrate were investigated by response surface methodology. This research hypothesized that supercritical CO₂-ethanol extraction can remove phenolic compounds—antimicrobial agents from dried rambutan seed. Recently, a review article reported nutritional, pharmaceutical, and functional aspects of rambutan from industrial perspective (Afzaal et al., 2023); however, utilization of crude polysaccharides extracted from rambutan seed as prebiotic was not reported in the literature.

2. Methodology

2.1 Raw material and chemicals

Rambutan seeds were supplied by Pissanumhon Food Products Company Limited, Chumphon Province, Thailand. The sample was cleaned under running tap water without residual flesh or integument removal prior to drying under forced air circulation at 60°C for 8 h. The dried seed was stored at 4°C until extraction. All chemical reagents used in the proximate analysis and fatty acid profile determination were supplied by SAC Sci-Eng, Ltd., Thailand. Analytical grade dimethyl sulfoxide (DMSO), L-ascorbic acid, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazl (DPPH) were purchased from Sigma-Aldrich, Inc. Carbon dioxide (99.8%) was supplied by Linde Co, Ltd (Thailand). Anhydrous ethanol (99.5%) and hydrated ethanol (95.5%) were purchased from RCI LabScan, Thailand.

2.2 Feedstock and extracts characterization

Proximate analysis of rambutan seed was performed based on the Association of Official Analytical Chemists (AOAC international) standard methods (Horowitz & Latimer, 2006). The crude lipid contents were determined by a Soxhlet extractor using petroleum ether and anhydrous ethanol as the solvents. The DS was milled in a grinding machine (Spring Green Evolution Pte Ltd., Model PG500) before the analysis. The protein content was estimated using an automatic Kjeldahl analyser (MultiKjel, BÜCHI Labortechnik).

The extracted lipid samples were characterized in accordance with AOAC standard methods (Abdulkarim et al., 2005; Bhutada et al., 2016). The antioxidant activities were determined by following the DPPH and ABTS *in vitro* assays as described elsewhere (Alam et al., 2013; Govardhan Singh et al., 2013). The extracted lipids were dissolved in 5% dimethyl sulfoxide (DMSO) before measurement of the total phenolic content using a modified Folin–Ciocalteu colorimetric method. Gallic acid was used as the external standard, and total phenolic content was expressed in mg of gallic acid equivalent (GAE) per 100 g of dried extract.

For subcritical water extracts, the filtered supernatant was mixed with 99.5% ethanol and incubated overnight at 4° C (Dhahri et al., 2023). The precipitate was washed by cold 99.5% ethanol and dried overnight at room temperature (30° C \pm 5° C). The crude polysaccharide extraction yield (Y_1) was calculated from the weight of dried precipitate divided by the dried sample weight (SPC-SCCO₂). The total sugar content (Y_2) of the crude extract was determined by the phenol–sulfuric acid method using D-glucose as a standard (Nielsen, 2010; Sakdasri et al., 2022). Solid residue discharged from SCW extraction at optimal condition was dried overnight at 30° C \pm 5°C before analysed by the Fourier Transform Infrared Spectrometer (Perkin Elmer, Spectrum One).

2.3 Extraction procedures

2.3.1 Lipid extraction by mechanical and supercritical CO₂-ethanol extraction

A single-screw press was employed for the pre-extraction of 100 kg of whole dried rambutan seed without any size reduction step. Further details on the mechanical extraction are described in a previous work (Ngamprasertsith et al., 2021). The crude extracted lipid was filtered through a filter cloth and allowed to settle overnight to partially remove heavy solid impurities. Screw press cake (SPC) was then processed in a SCCO₂ batch extractor. The extractor and separator volumes were 10 L and 200 mL, respectively. The maximum CO₂ flow rate and working pressure were 200 L/h and 20.0 MPa, respectively. First, 1 kg of seed cake was soaked in 2500 mL of 95.5% ethanol for 90 min prior to SCCO₂ extraction. Then, the extraction chamber was pressurized to 15.0 MPa by SCCO₂ and heated to 50°C ± 5°C. The extraction was maintained for 90 min in static mode,

then it was depressurized to collect the sample. The CO₂ consumption was measured from the weight loss of the CO₂ cylinder. Because of the low working pressure (15.0 MPa) in the pilot-scale batch extractor, SCCO₂ extraction without using ethanol as a co-solvent could not be attempted. To investigate the effects of co-solvent, a laboratory-scale extractor was employed to extract rambutan seed oil at 50°C and 30.0 MPa for 90 min. The sample weight was 20 g with an SCCO₂ flow rate of 20 g/min per batch. Further details of a laboratory-scale extractor are presented in a previous work (Ngamprasertsith et al., 2021).

2.3.2 Crude carbohydrate extraction by subcritical water

A high-pressure batch reactor (Parr Company, Series 4625, 500 mL working volume) was charged with 30 g of defatted sample. Deionized water was filled into the reactor to make up the volume to 200 ml. The reactor was purged and pressurized by nitrogen prior to increasing the temperature. All extraction conditions followed the Box-Behnken design. After the extraction was completed, the product was centrifuged at 4400 rpm for 20 min to separate the solid residue. The supernatant was filtered and cold-percolated with 99.5% ethanol. The remaining solid was dried at 60°C for 12 h before weighing to permit the mass balance of overall process.

2.4 Experimental Design and statistical analysis

The Box-Behnken design was used to examine the effects of temperature, liquid to solid (L/S) mass ratio, and extraction time on crude polysaccharides yield and total sugar. All experiments were conducted in a random order. Statistical analysis was performed using Design-Expert 13.0 (State Ease Inc., Minneapolis, MN, USA) for Windows. Furthermore, SPSS 28 statistical software was used for the analysis results in Tables 1 and 2. Oneway ANOVA and the Duncan test were used to test hypotheses, with 0.05 set as the level of significance.

3. Results and discussion

3.1 Feedstock characterization

Table 1 displays the proximate analysis of rambutan fresh seed, rambutan dried seed, and screw press cake. It was reported that moisture content in rambutan seed varies over a wide range, from 14.2 % (Hernández-Hernández et al., 2019) to 34.40 % (Md Sani et al., 2022). The drying conditions influence the moisture content of the dried seed, as well as the composition. For example, 3-day sunlight-dried seed had a moisture content of 9.0%–9.5% (Minh, 2021), whereas milled seed dried in a hot-air oven at 55°C for 5 h had a moisture content of ~5 g/100 g seeds (Sirisompong et al., 2011). After whole seeds were dried at 60°C for 8 h, the major components of the DS were carbohydrates and lipids. Despite the different sources of rambutan seed, the DS compositions agreed well with the literature.

Table 1: Proximate analysis of fresh rambutan seed, dried rambutan seed, screw-press cake, and SCCO₂ extracted screw-press cake samples (g/ 100 g sample)

Source	Moisture	Lipid	Protein	Carbohydrate	Crude fibre	Ash
FS	43.18 ± 0.56 a	18.48 ± 0.22 d	7.22 ± 0.12 ^e	26.08 ± 0.38 d	4.00 ± 0.10 ^d	1.04 ± 0.01 °
DS	6.30 ± 0.05 b	30.48 ± 0.49 a	11.90 ± 0.14 a	42.96 ± 0.15 °	6.60 ± 0.25 a	1.72 ± 0.02 b
SPC	1.85 ± 0.01 d	25.76 ± 0.40 b	9.76 ± 0.26 c	54.93 ± 0.87 b	5.81 ± 0.13 b	1.89 ± 0.01 a
SPC-SCCO ₂	1.87 ± 0.02 d	18.80 ± 0.20 °	10.04 ± 0.22 b	61.63 ± 0.74 a	5.79 ± 0.11 °	1.87 ± 0.01 a
Hydrochar *	9.56 ± 0.07 ^c	18.39 ± 0.64 d	8.95 ± 0.21 d	55.50 ± 0.68 b	6.19 ± 0.11 ^c	1.91 ± 0.01 ^a

^{*} Hydrochar is a solid residue obtained from subcritical water-ethanol extractor.

3.2 Lipid yield and extracted lipid characterization

The lipid yields that were obtained from different extraction methods are shown in Table 2. Solvent extraction is reported to be an effective method for the extraction of rambutan seed lipids. Extraction with hexane and hydrated ethanol maceration at 28°C for 8 h provided yields of 22.16% and 9.97%, respectively. Using the Soxhlet apparatus improved the yield of hexane extraction at 8 h from 22.16% to 31.76% (Yoswathana, 2013). Maceration in n-hexane with orbital shaking at 150 rpm for 1 h afforded an extraction yield of 32.6%, which was comparable with Soxhlet extraction for 6 h (33.4% yield) and 9.2 h (37.5% yield) (Lourith et al., 2016). For the SCCO₂ extraction of rambutan seed, the reported yields of fat and lipids are in the ranges of 16.0%–23.0% and 5.0%–7.6%, respectively (Eiamwat et al., 2014). The SCCO₂ extraction of ~100 g rambutan seed at 35 MPa, 45°C, and a CO₂ flow rate of 120 L/h for 44 h achieved up to 30.6% of lipid extraction yield, and the defatted rambutan seed had a fat content of 6.64 ± 0.31 g/g dry seeds (Eiamwat et al., 2016). In another study, the addition of ethanol as a co-solvent dramatically enhanced the SCCO₂ extraction yield. For example, the addition of 10 mL hydrated ethanol to 20 g of rambutan seed improved the extraction yield from ~18% to ~28.4% at 30 MPa, 50°C, with a CO₂ flow rate of 2 kg/h, and an extraction time of 2 h (Yoswathana, 2013).

Table 2: Extraction yields, acid and peroxide values, and total phenolic content of rambutan seed lipid obtained from different extraction methods

Sample	Extraction Extraction yield		417	51/		DPPH	ABTS
	method	(g/100 g feed)	AV	PV	TPC	(IC ₅₀ , mg/ml)	(IC ₅₀ , mg/ml)
DS	SX-P	32.48±1.07	N/A	N/A	21.01±0.14°	78.43±0.62 ^b	310.50±0.89b
DS	SX-E	35.24±0.31	N/A	N/A	326.70±0.11a	N/A	N/A
DS	SPM	6.29±0.80	1.41±0.09 ^a	9.76±0.12 ^a	N/D	660.16±0.86a	411.36±1.01 ^a
SPC	SE-P	25.94±0.83	N/A	N/A	N/D	N/A	N/A
SPC	SX-E	28.83±1.76	N/A	N/A	43.16±0.42°	N/A	N/A
SPC-SCCO ₂	SCE	8.84±1.23	0.78±0.05 ^b	3.73±0.23 ^b	163.81±0.47 ^b	17.66±0.29°	69.34±1.22c
SCCO ₂	SCCO ₂	7.32±1.50	N/A	N/A	29.83±0.03°	N/A	N/A

N/A, not analysed, because of an insufficient quantity of sample. Different letters in the same column indicate significant differences at the 5% confidence level. N/D, not detectable

As shown in Table 2, the total phenolic content (TPC) of the extracted lipids varied between 29.831 ± 0.03 mg GAE/100 g extract (SCCO₂ without co-solvent) to 326.70 ± 0.11 mg GAE/100 g extract (Soxhlet extraction using ethanol). Furthermore, the high antioxidant activities (DPPH and ABTS) were indicated by a small half maximal inhibitory concentration (IC₅₀). It is well-known that phenolic compounds are preferably extracted by polar solvents. For example, it was reported that rambutan seed oil extracted by methanolic maceration at room temperature for 24 h had a total phenolic content of $3,410 \pm 200$ mg GAE/100 g extract (Xuan et al., 2022). In contrast, few phenolic compounds were extracted by SCCO₂ without a co-solvent, as indicated by the lowest total phenolic compound content of 29.83 ± 0.03 mg GAE/100 g extract. This work aimed to minimize phenolic compounds in SPC-SCCO₂ because they inhibit microorganism growth. Thus, the crude carbohydrates obtained in the subsequent step would not be suitable for culturing probiotics.

3.3 Effects of extraction parameters on crude polysaccharide extraction yield and total sugar content in SCW extraction

Figure 1 illustrates the effects of temperature and time on the extraction yield and total sugar in crude extracts.

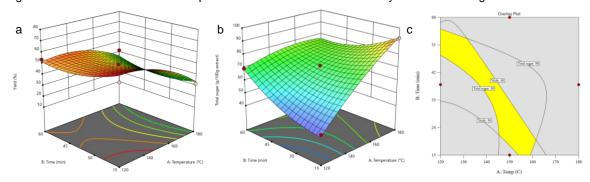


Figure 1: Response surface plot showing the effects of extraction temperature and time on (a) crude polysaccharide extraction yield and (b) total sugar content, and (c) their overlay plot at L/S ratio of 10

According to Figure 1(a), temperature posed negative effects on the extraction yields, while extraction time slightly influenced the crude polysaccharide extraction yields. Regardless of the extraction temperature, it could be assumed that the maximum crude polysaccharide extraction yields were obtained at 15 min. The temperature above 120°C reduced the crude polysaccharide extraction yield because of the exceed thermal hydrolysis (Sakdasri et al., 2022). As shown in Figure 1(b), the total sugar contents in the extracts enhanced with both increasing temperature and extraction time, excepted at temperature of 180°C. In other words, the effects of temperature at extraction time of 15 min were stronger than that at other extraction times. Because the effects of temperature and extraction time on both responses were distinguished, the response surfaces of crude polysaccharide extraction yield and total sugar were correlated and superimposed as shown in Fig.1(c). The optimal regions were in the yellowed area where the objective functions are the crude polysaccharide extraction yield in range of 40–50 g/100 g DS and the total sugar higher in range of 80–90 g/100 g extract were obtained. The saddle regions were allocated with changing L/S ratios; thus, the minimum L/S ratio was selected to reduce water usage in the process. At L/S ratio of 10, the optimal temperatures were in range of 145–149°C and the extraction times were in the range of 15–24 min.

Pissanumhon Food Products Company Limited provided information that the fresh rambutan seed was sold at 0.06 USD/kg. However, the leftover rambutan seed accounted for approximately 300 tons/year. The extracted lipids were shown to form a biocomposite film with konjac glucomannan, gum Arabic, agar, and glycerol in a previous work (Nilmat et al., 2022). The developed biocomposite film showed antimicrobial and moisture-resistance properties. Rambutan fat was also developing as specialty fat for cosmetics (Lourith et al., 2016), non-dairy liquid creamer (Wanthong & Klinkesorn, 2020) and cocoa butter improver (Azzatul et al., 2020). The 37.74 kg of solid residue from the SCW extractor contained high amounts of water-insoluble carbohydrates as confirmed by FT-IR (data not shown). Owing to the high protein and carbohydrate contents, it could be used as feedstock for animal feed and fertilizer. As the mass balance has been calculated, economic analysis could be attempted in further study. Recycling 11.5 kg of CO₂, 118.3 kg of ethanol (co-solvent), and 424.73 kg of ethanol in 748.26 kg of filtrate could be conducted to improve this extraction process.

4. Conclusions

A screw press machine and a SCCO₂-ethanol extractor could extract crude lipid from dried rambutan seed with extraction yields of 6.29 and 8.84 g/100 g feed, respectively. Pre-extraction with a screw press improved the extraction yield of the SCE extraction to 11.69 g/100 g feed. However, the high lipid content of the SPC-SCCO₂ extraction, of 18.80 g/100 g sample, can be improved further. Lipids obtained from different extraction methods had various properties, for example, crude lipid extracted by SCCO₂-ethanol had a high TPC of 163.81 mg GAE/100 g extract, whereas crude lipid extracted by a screw press machine had a high acid value. For crude polysaccharides obtained by SCW extraction, temperature significantly impacted on all responses. The overall mass balance showed that rambutan seed could be fractionated to yield 9% of crude lipids and 16% of crude carbohydrates. For industrial applications, rambutan seed lipids could be used as cosmetic and food ingredients, and the extracted crude polysaccharide could be used as alternative carbohydrate. Further studies on the utilization of the extracted lipids, such as to produce biocomposite films, and the extracted carbohydrates, such as their probiotic properties, should be conducted in the future.

Nomenclature

FS – fresh rambutan seed
DS – dried rambutan seed
SPC – screw-press cake
SPC-SCCO₂ – screw-press cake extracted by
supercritical carbon dioxide
AV – acid value (mg KOH/g lipid)
PV – peroxide value (meg/kg)

TPC – total phenolic content (mg GAE/100 g extract) SX-P – Soxhlet extraction (petroleum ether)

SX-E - Soxhlet extraction (ethanol)

SPM – Screw press machine

SCE - supercritical carbon dioxide extraction with

ethanol as co-solvent

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