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Polysaccharides in copra meal: extraction conditions, optimisation and characterisation

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Abstract Extraction conditions of polysaccharides from copra meal were optimised using an orthogonal experimental design. The characterisation of copra meal polysaccharides (CMP) was explored. Optimal extraction conditions were NaOH concentration 16% (w/v), solid-liquid ratio 1:40 (w/v), extraction temperature 70 ^{o}C and extraction time 10 h. Under these conditions, extraction yield of polysaccharides was 50.7% (w/w), closed to the predicted value of 50.3%. HPLC analysis revealed mannose as the main sugar, accounting for 99% of total sugar in CMP. FT-IR analysis demonstrated that CMP was mainly composed of polysaccharides. Results suggested alkaline extraction as an efficient method for extraction of polysaccharides from CM and CMP as raw material in the pharmaceutical, biomedical, feed and food industries.

Keywords: Copra meal, Alkaline extraction, Polysaccharides, Mannan, Orthogonal experiment design

Introduction

Copra meal (CM), an agro-industrial biomass, is the main by-product of coconut milk extraction that is generated in large qualities by coconut factories in Thailand. In 2016, Thailand produced 1,231 million tonnes of coconut generating 123 million tonnes of copra meal (Information Technology and Communication Center: Thailand Department of Agricultural Extension, 2018). Copra meal consists of 43-45% carbohydrate, 19-20% crude protein, 10-11% crude fat, 12% crude fibre (Saittagaroon *et al.*, 1983) and contains large amounts of mannose in the form of mannan. Mannan consists of beta-1,4-linked mannose and glucose in the backbone and alpha-1,6-linked galactose as side chains that generate the formation of various types of linear or branched polysaccharides. Mannan polysaccharide can be obtained from the cell walls of

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yeast and plants. Mannans are commonly prepared from plants by extraction with hot water or alkaline solution followed by precipitation with ethanol. Mannans are widely used as thickening, stabilising and gelling agents in the food industry (Yamabhai *et al.,* 2016). Mannan can be enzymatically hydrolysed using a suitable beta-mannanase to generate manno-oligosaccharide that can be used as a prebiotic. Recent studies have reported that mannan polysaccharide extracts from copra meal can resist artificial human gastric juices and stimulate the proliferation of *Lactobacillus casei* Shirota and *L. bulgaricus* (Nor *et al.,* 2017). Coconut polysaccharides extracted with sodium hydroxide (NaOH) have also been suggested as an alternative dietary replacement for the antibiotic growth promotant, avilamycin to enhance broiler performance (Sundu *et al.,* 2018).

Many methods are available for the extraction of polysaccharide from agricultural waste such as hot water extraction, solvent extraction, alcohol precipitation, ultrasonic and microwave-assisted extraction and enzymatic hydrolysis (Yanhua *et al.,* 2014). Hot water extraction is the most widely used method; however, the modest efficiency of hot water extraction gives relatively lower yields and fewer monosaccharide components. Therefore, a novel extraction method is required for polysaccharides to avoid the inadequacies of hot water extraction. Alkaline extraction improves the efficiency of polysaccharide extraction, especially for high molecular weight (MW) and acid polysaccharides. Alkaline extraction is usually carried out with an alkaline reagent such as NaOH or hydrogen peroxide (H_2O_2) (Saittagaroon *et al.,* 1983; Yao *et al.,* 2013). Numerous reports have detailed alkaline extraction methods. Saittagaroon *et al.* (1983) reported extraction yield of polysaccharides by NaOH hydrolysis from copra meal at 55%, while Chen *et al.* (2014) used the orthogonal method to optimise extraction conditions from rhizomes of *Polygonatum odoratum* with yield of polysaccharides at 17.28%. Zaidel *et al.* (2017) extracted pectin from sweet potato (*Ipomoea batabas*) peel using NaOH with polysaccharide yield at 16.78% (w/w). Extraction yield of polysaccharides is directly affected by several factors including alkaline concentration, extraction time, extraction temperature and ratio of material to water. Therefore, optimisation of extraction conditions is a very important step in the development of a solvent extraction method (RenJie, 2008).

Orthogonal test design is a popular method used to analyse multi-factors and multi-conditions of scientific test data (Ni *et al.,* 2018). There are many advantages of orthogonal test design including reducing the number of testing treatments, thereby saving experimental time and cost to analyse results. Tian *et al.* (2011) used orthogonal test design to optimise the extraction of polysaccharides from *Paeonia sinjiangensis* K.Y. Pan, while, Ni *et al.* (2018)

applied orthogonal experimental design to optimise the parameters of laser-induced breakdown spectroscopy of aluminium alloy.

The extraction parameters were optimised using an orthogonal experimental design, and investigated the chemical characterisation of polysaccharides.

Materials and methods

Preparation of copra meal

Copra meal (CM) was obtained from a coconut milk factory in Nakhon Pathom, Thailand, sieved to obtain 40-60 mesh fractions and Soxhlet-extracted with hexane for 4 h to remove lipids. After treatment, defatted copra meal was extracted with distilled water at 80 ^{o}C for 2 h. The hot water-insoluble fraction was separated by Whatman filter paper No.1, washed with distilled water and dried in an oven at 60 ^{o}C for 2-3 days. The insoluble powder was then delignified with acidified sodium chlorite ($NaClO_2$) solution. Holocellulose copra meal was treated with a commercial protease in glycine buffer solution, pH 9.5, at 40 ^{o}C for 24 h to remove protein. The deproteinised copra meal (DeCM) was filtered, washed with distilled water and acetone, and dried at 60 ^{o}C for 6 h before storing in plastic bags at room temperature until required for use. Total protein content of DeCM was determined by following the Kjeldahl method at 0.55% (w/w).

Polysaccharide extraction procedure

Dried DeCM was extracted with NaOH solution at different concentrations. The mixture was incubated in a thermostat-controlled water bath shaker at the required temperature and time. After complete extraction, the supernatant was separated by filtration using Whatman filter paper No.1 and adjusted to pH 5.0-6.0 with acetic acid. The supernatant was precipitated using three volumes of 95% (v/v) ethanol and kept at 4 ^{o}C overnight. Copra meal polysaccharide (CMP) was obtained by centrifugation at 4 ^{o}C and 13,000 g for 15 min and freeze-dried. Extraction yield of polysaccharide was calculated using the following formula:

$$\text{Extraction yield of polysaccharide (\%)} = \frac{\text{Dry weight of polysaccharide (g)}}{\text{Dry weight of DeCM (g)}} \text{ x } 100$$

Single-factor experiments

Single-factor experiments were performed to obtain the preliminary range of extraction factors as NaOH concentrations (4, 8, 12, 16 and 20% (w/v)), solid-liquid ratios (1:20, 1:30, 1:40, 1:50 and 1:60 (w/v)), extraction time (4, 6, 8, 10, and 12 h) and extraction temperature (4, 30, 50, 70 and 90 ^{o}C). Only one factor was changed in each experiment while other factors remained the same. After each single-factor experiment was completed, an orthogonal experiment was conducted to determine interaction between the single factors. An orthogonal experiment (L_{16} (4^4)) with four factors and four levels was chosen to examine optimal extraction conditions for maximal yield of CMP. Factors and levels of orthogonal design are shown in Table 1.

Table 1. Orthogonal design factors and extraction levels

Factor	**Level**			
	1	2	3	4
A, NaOH concentration (%)	8	12	16	20
B, Solid-liquid ratio (g ml-1)	1:30	1:40	1:50	1:60
C, Extraction time (h)	6	8	10	12
D, Extraction temperature (oC)	30	50	70	90

FT-IR analysis

Fourier transform infrared (FT-IR) spectroscopy (Bruker, USA) was investigated the functional polysaccharide groups in CMP. The polysaccharide was ground with potassium bromide (KBr) power and then pressed into a pellet for FT-IR analysis. Spectrum range was 374-4000 cm^{-1} with resolution 4 cm^{-1} and 16 scans were collected.

Monosaccharide composition analysis

Copra meal (CM) and copra meal polysaccharides (CMP) were hydrolysed according to the method of Sritrakul *et al.* (2017) with some modifications. Concentrations of monosaccharide were determined by a high performance liquid chromatography (HPLC) system equipped with an Asahipak NH_2P-50 4E column (4.6 mm x 250 mm, Shodex, Japan) and a refractive index detector (Shimadzu, Japan). The mobile phase was composed of water and acetonitrile (25:75 v/v) at a flow rate of 1 ml/min and column temperature of 25 ^{o}C. Concentration of each sugar was calculated using peak

area compared with a mixture of each standard sugar such as glucose, xylose, galactose, mannose and arabinose.

Statistical analysis

Optimisation of alkaline extraction conditions for CMP was performed using MINITAB software (version 16). Statistical significance was analysed by ANOVA.

Results

Chemical composition of copra meal

Total monosaccharide sugar in CM accounted for 64% w/w dry weight, consisting of mannose (54.3%), glucose (7.1%), xylose (1.5%) and arabinose (1.1%) while no galactose was detected. Fat content of CM was 13.3% (w/w) with protein content 7.9% (w/w). Results suggested that CM showed potential as a mannan production source in the feed and food industries.

Effect of NaOH concentration on extracted yield of polysaccharide

Different NaOH concentrations at 4, 8, 12, 16 and 20% (w/v) were investigated for extracted yield from DeCM. Other extraction parameters were extraction temperature 30 ^{o}C, solid-liquid 1:40 (w/v) and extraction time 12 h. As shown in Figure 1A, the extracted yield of polysaccharide increased from 13% to 41% as NaOH concentration increased from 4% to 16% (w/v). With increasing NaOH concentration to 20% (w/v), extracted yield of polysaccharide decreased. Our results suggested optimal NaOH concentration at 16% (w/v).

Effect of solid-liquid ratio on extraction yield of polysaccharide

Solid-liquid ratio is an important parameter that influences extraction yield of polysaccharide. The solid-liquid extraction yield of polysaccharide from DeCM was conducted at ratios of 1:20, 1:30, 1:40, 1:50 and 1:60% (v/w). Other parameters were set as NaOH concentration of 12% (w/v), extraction temperature was 30 ^{o}C and extraction time was 12 h. As shown in Figure 1B, the extracted yield of polysaccharide increased with increasing solid-liquid ratio and reached maximal value (51.7%) when solid-liquid ratio was 1:50. Extracted yield of polysaccharide decreased at solid-liquid ratios above 1:50. Therefore, optimal solid-liquid ration was set at 1:50 (w/v).

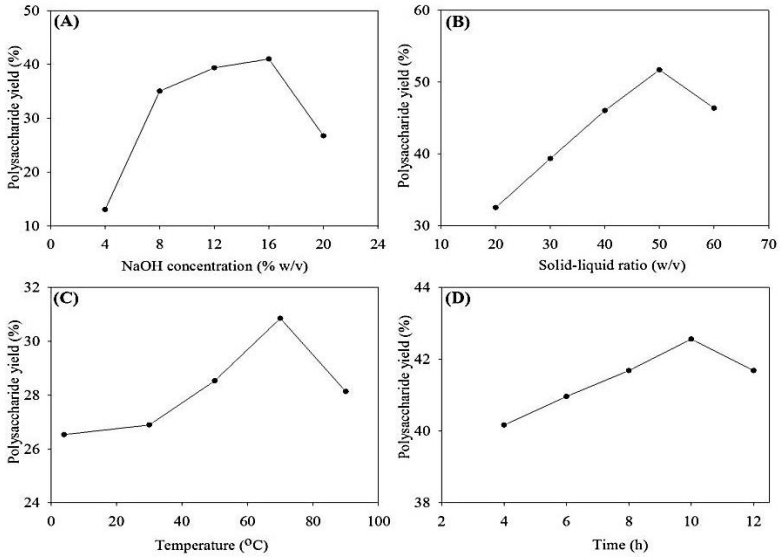

Figure 1. Effect of different factors on extraction yield of polysaccharide from copra meal: NaOH concentration (A), solid-liquid ratio (B), extraction temperature (C) and extraction time (D)

Effect of temperature on extraction yield of polysaccharide

Temperature was set at 4, 30, 50, 70 and 90 ^{o}C while other parameters were set as NaOH concentration 12% (w/v), solid-liquid ratio 1:40 (v/w) and extraction time 12 h. The extracted yield of polysaccharide gradually increased from 26.5% to 30.9% as temperature increased from 4 to 70 ^{o}C (Figure 1C). When temperature exceeded 70 ^{o}C, extracted yield of polysaccharide decreased. Thus, extraction temperature was selected at 70 ^{o}C.

Effect of extraction time on extraction yield of polysaccharide

Extraction time plotted against extracted yield of polysaccharide is displayed in Figure 1D. Extractions were performed at 4, 6, 8, 10 and 12 h while other parameters were set as NaOH concentration 12% (w/v), solid-liquid ratio 1:40 (v/w) and extraction temperature 30 ^{o}C. Extracted yield of polysaccharide increased with increasing extraction time. Increase in extraction time from 6 to 10 h resulted in an increase of extracted yield of polysaccharide from 41.1% to 42.6% When extraction time exceeded 10 h, extracted yield of polysaccharide decreased. Therefore, an appropriate extraction time for the experiment was set at 10 h.

Table 2. Orthogonal experimental results for alkaline extraction of copra meal polysaccharide

No.	Factor				
	A NaOH concentration (%w/v)	**B Solid-liquid ratio (v/w)**	**C Extraction time (h)**	**D Extraction temperature (^{o}C)**	**Yield of CMP (%)**
1	8	1:30	6	30	27.17
2	8	1:40	8	50	38.73
3	8	1:50	10	70	37.13
4	8	1:60	12	90	35.39
5	12	1:30	8	70	36.54
6	12	1:40	6	90	48.45
7	12	1:50	12	30	40.84
8	12	1:60	10	50	46.34
9	16	1:30	10	90	42.94
10	16	1:40	12	70	49.84
11	16	1:50	6	50	43.66
12	16	1:60	8	30	39.90
13	20	1:30	12	50	33.81
14	20	1:40	10	30	34.40
15	20	1:50	8	90	35.36
16	20	1:60	6	70	41.64
K1	30.58	30.69	31.77	30.84	
K2	32.61	32.45	31.44	32.04	
K3	32.76	31.80	31.97	32.14	
K4	31.09	32.11	31.87	32.03	
R	2.18	1.75	0.53	1.30	

Table 3. Variance analysis of alkaline extraction of polysaccharide

Factor	DF	SS	MS	F-value	p-value
A (NaOH concentration)	3	14.24	4.74	12.32	0.03
B (Solid-liquid ratio)	3	6.93	2.31	6.03	0.09
C (Extraction time)	3	0.63	0.21	0.55	0.68
D (Extraction temperature)	3	4.55	1.52	3.96	0.14
Error	3	1.14	0.38		
Total	15	27.51			

Note: Degree of freedom (DF), Sum of squares (SS) and Mean square (MS)

Optimisation of extraction conditions of polysaccharides from copra meal

Various parameters affectted the extraction process and optimisation of extraction conditions that is an important step in the development of an alkaline extraction method. Optimisation of all the selected conditions in polysaccharide extraction was conducted using an orthogonal L_{16} (4^4) experimental design. Each experiment was performed in triplicate under the same conditions. Results of the orthogonal experiment are presented in Table 2. The R value was influenced by NaOH concentration, solid-liquid ratio, extraction time and extraction temperature as 2.18, 1.75, 0.53 and 1.30 respectively. According to the R value, results revealed that NaOH concentration (factor A) had the most significantly affected on the extracted yield of polysaccharides followed by solid-liquid ratio (factor B), extraction temperature (factor D) and extraction time (factor C). Variance analysis of the results of polysaccharide alkaline extraction (Table 3) indicated that NaOH concentration was significant ($p<0.05$) while solid-liquid ratio, extraction time and extraction temperature were not significant ($p>0.05$). Predicted optimal conditions of NaOH extraction from orthogonal design were set as NaOH concentration 16% (w/v), solid-liquid ratio 1:40 (w/v), extraction temperature 70 ^{o}C and extraction time 10 h. To validate the predicted results, three experiments were conducted under optimal extraction conditions. Results revealed that the extracted yield of polysaccharides was 50.7% which was closed to the predicted value of 50.3%. Findings showed good agreement between experimental and predicted values. Therefore, optimal conditions were used for extraction of polysaccharides from CM.

Copra meal polysaccharide characterisations

CMP was obtained from DeCM as a water-insoluble white power by alkaline extraction (Figure 2) with chemical composition presented in Table 4. Total sugar content was determined by the phenol-sulphuric acid method, total sugar content was 91 g/100 g dry CMP. HPLC results revealed that CMP contained mannose and glucose while xylose, arabinose and galactose were not detected. Monosaccharide composition of CMP revealed that mannose was the main type accounting for 99% of the monosaccharide sugar in CMP.

Table 4. Chemical composition of copra meal polysaccharide

Component	**% (w/w) dry weight**
Total sugar*[a]	91
Monosaccharide sugar*[b]	
Mannose	80.4
Glucose	0.84
Xylose	-
Arabinose	-
Galactose	-

a: Analysed by the phenol-sulphuric acid method
b: Analysed by HPLC

Characterisation of functional groups in copra meal polysaccharides

Available function groups and bonding configurations in CMP were investigated by FT-IR spectroscopy with functional groups shown in Figure 3. Strong absorption at 3383 cm^{-1} was attributed to stretching of the hydroxyl groups, while the absorption band at 2925 was due to C-H stretching of methyl groups, considered as characteristic absorption of polysaccharide. The band in the region of 1745 cm^{-1} represented the ester bond C=O, while the peak at 1414 cm^{-1} was due to C-H deformation of crystalline cellulose and the absorption peak at 1248 was attributed to C-O stretching in the carboxyl group. The band at 1091 cm^{-1} was due to C-O vibration. All data demonstrated that CMP was mainly composed of polysaccharides.

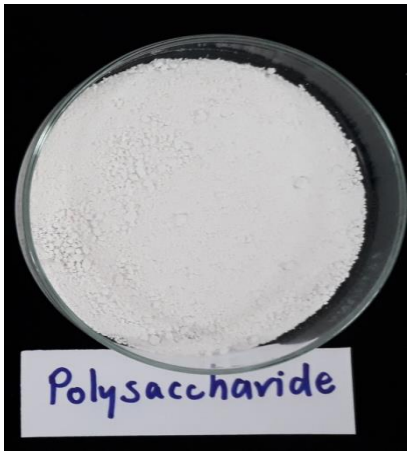

Figure 2. Photograph of copra meal polysaccharide powder

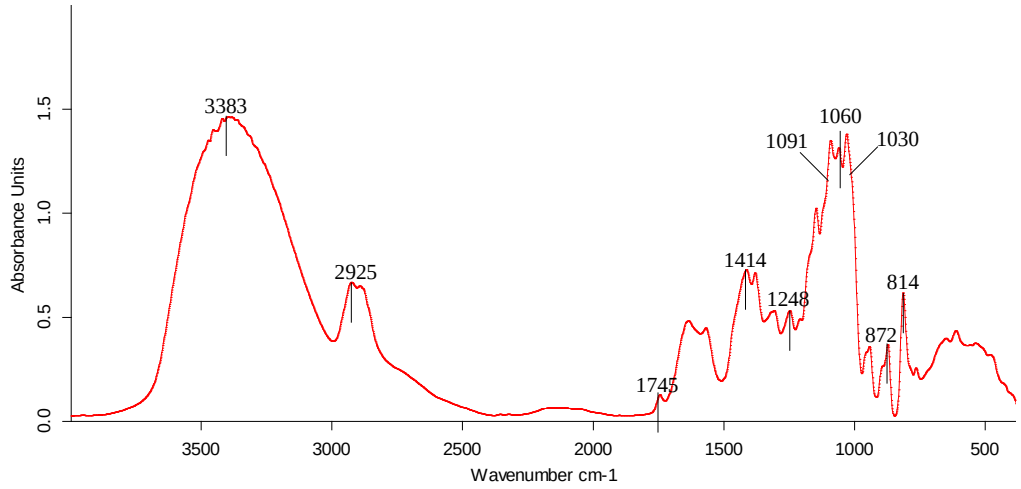

Figure 3. FT-IR spectrum of polysaccharide from copra meal

Discussion

Results of the orthogonal experimental design and variance analysis revealed that NaOH concentration significantly affected extracted yield of polysaccharides ($p<0.05$) while solid-liquid ratio, extraction time and extraction temperature were not significant ($p>0.05$). Optimal extraction conditions for alkaline extraction of CMP were NaOH concentration 16% (w/v), solid-liquid ratio 1:40 (w/v), extraction temperature 70 ^{o}C and extraction time 10 h. Under these conditions, extraction yield of polysaccharides was 50.7% which was close to the predicted yield (50.3%). Our results concurred with other alkaline polysaccharide extraction studies from various types of raw material. Chen *et al.* (2014) found that ratio of NaOH to solid was the most important factor for extracted yield of polysaccharides from rhizomes of *Polygonatum odoratum* among three other factors as extraction temperature, NaOH concentration and extraction time, while Balasubramaniam (1976) reported the extracted yield of 61% from copra kernel extracted twice with 4% NaOH and once with 17% NaOH. Saittagaroon *et al.* (1983) revealed yield of alkaline extraction from CM at 55% when extracted three times with 16% NaOH at room temperature under nitrogen atmosphere for 24 h, while Cai and Pang (2019) determined the extracted yield of polysaccharide from millet bran at 11.46% by alkaline extraction under NaOH concentration 0.7 mol/l, extraction temperature 80 ^{o}C, ratio of NaOH to solid 1:15 and extraction time 5 h.

Monosaccharide contents of CMP were analysed by HPLC. Results revealed mannose as the main type, accounting for 99% of total monosaccharide sugar. Similar results were reported by Saittagaroon *et al.* (1983) who found that mannose was the main sugar accounting for 98% of total monosaccharides in polysaccharides. For other biomass, Bello *et al.* (2018) stated that extracts of palm kernel with 2% NaOH contained mannose (38.8%), galactose (15.5%), glucose (12.9%), rhamnose (1.7%), xylose (0.9%), and arabinose (0.5%). Their results indicated that polysaccharides obtained by this procedure can be assigned as pure mannan. Several authors have reported galactomannan and galactoglucomannan as the main polysaccharides in coconut kernel and copra meal respectively. Results obtained by FT-IR analysis indicated that CMP was mainly composed of polysaccharides. Glucomannan presentation, in addition to the 1030 cm^{-1} glucan and band at 1060 cm^{-1}, occurred due to mannose units. In the anomeric region (950-700 cm^{-1}), adsorption peaks at 872 and 814 cm^{-1} exhibited the existence of mannose (Peng *et al.*, 2003), with the former indicating the presence of beta-glycosidic bonding (Ghosh *et al*., 2015).

Our results suggested alkaline extraction as an efficient method for extraction of polysaccharides from CM and CMP for use as raw material in the pharmaceutical, biomedical, feed and food industries. Our findings presented a basic information for alkaline extraction process prediction, process scale-up and equipment design.

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