Extraction, Molecular Weight Distribution, and Antioxidant Activity of Oligosaccharides from Longan (*Dimocarpus Longan* Lour.) Pulp

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Abstract Ultrasonic-microwave synergistic extraction (UMSE) was optimized for the extraction of oligosaccharides from longan pulp (OLP). Box-Behnken design was used to evaluate the effects of temperature (35–55°C), ultrasonic time (5–25 min), and water to material ratio (10–30 mL/g) on the extraction efficiency of crude OLP. A regression model was developed and its validity was statistically demonstrated. Significant interaction between temperature and water to material ratio was observed. The following optimal conditions for the extraction yield of crude OLP were determined: extraction temperature 55°C, ultrasonic time 18.52 min, and water to material ratio 10 mL/g. The extracted OLP were purified for the determination of molecular weight distribution and antioxidant activity. Results of matrix-assisted laser desorption ionization time-of-flight mass spectrometry revealed that the molecular weight distribution of the purified OLP ranged from m/z 495.138 to 795.511. The purified OLP exhibited a dose-dependent behavior in 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity.

Keywords: longan pulp, oligosaccharide, ultrasonic-microwave synergistic extraction, molecular weight distribution, antioxidant activity

Introduction

Longan (*Dimocarpus longan* Lour.) belongs to the Sapindaceae family and grows commercially in many subtropical countries. It is not only preferred for its desirable flavor but also considered as a traditional nutritional food and Chinese medicinal resource (1). In recent years, studies on bioactive compounds of longan fruit have attracted increasing interest to researchers. Current reports show that the longan fruit is rich in polyphenolic compounds, which contribute to antioxidant, antiglycated, antityrosinase, and anticancer activities in the longan pulp, pericarp, or seed extract (2). Another important bioactive compound in longan fruit is polysaccharide. Evidence shows that while polysaccharides extracted from the fruit pericarp exhibit remarkable effects on the radical scavenging, antioxidant, antiglycated, and antityrosinase activities, polysaccharides extracted from the pulp have excellent scavenging activities, potent immune-modulatory, and great antitumor effects (3). There are also reports about oligosaccharides from longan fruit pericarp (4). However, there is no study focusing on oligosaccharides from longan pulp (OLP). Oligosaccharides are regarded as bioactive components in food. Increasing interest has been given to oligosaccharides for their

varied benefits including antioxidant, immune-modulatory, anti-inflammatory, hypotensive, antiallergic, hyperlipemic, and neuroprotective to anticancer activities (5–8).

Ultrasonic extraction has been widely employed because of its capillary effects and enhancing influence on mass transfer and cell disruption (6). Microwave can penetrate into the plant matrix and generate heat within the cells, which also results in cell rupture and mass transfer intensification (9). By combining the two methods, ultrasonic-microwave synergistic extraction (UMSE) can realize fast and efficient extraction under mild temperature (10).

The main objective of this work was to evaluate the effects of UMSE conditions on the extraction yields of crude OLP and optimize the operating parameters using response surface methodology. In addition, the extracted OLP were purified and their molecular weight distribution and antioxidant activity were determined to preliminarily understand the properties of OLP.

Materials and Methods

Chemicals and reagents 2,2-Diphenyl-picrylhydrazyl (DPPH) was

obtained from Sigma Chemical Company (St. Louis, MO, USA). Ethanol, phenol, and sulfuric acid were purchased from Guangzhou Reagent Co. (Guangzhou, China). Other chemicals used were of analytical grade.

Dried longan (*Dimocarpus longan* Lour. cv. Shixia) fruits were procured from a local market in Guangzhou, China and were further dried using a hot air circulation dryer (GHRH-20; Guangdong Agri-machinery Research Institute, Guangzhou, China) at 50°C. Then the longan pulps were manually separated from the fruits, collected, grinded, and stored in a desiccator at ambient temperature before use (no more than one month).

UMSE of crude OLP The dried grinded longan pulps (6 g, moisture content 16.67%) were carefully weighed and extracted with distilled water. A CW-2000 ultrasonic-microwave synergistic extractor (Shanghai New Billiton Microwave Dissolving Sample Testing Technology Co., Ltd., Shanghai, China) was used for extraction, operated with different ultrasonic powers, temperatures, and times.

After UMSE, the extracted slurry was centrifuged at 1,613xg force for 20 min. Then the supernatant was collected and concentrated to 20 mL using a rotary evaporator (EYELA N-1000; Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 50°C under vacuum. The solution was added to dehydrated ethanol to the final concentration of 85% and kept overnight to precipitate proteins and polysaccharides at 4°C. After removing the precipitates, the supernatant was sequentially partitioned with ethyl acetate and chloroform three times. The water phase was collected, concentrated at 45°C with the rotary evaporator under vacuum, and freeze dried to obtain the crude oligosaccharides. The percentage of crude OLP yield (%) is calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of dried crude OLP (g)}}{\text{Weight of longan pulp powder (g)}} \times 100 \quad (1)$$

Box-Behnken design The optimization of UMSE for crude OLP was performed through the Box-Behnken design. The three extraction variables were temperature (X_1), ultrasonic time (X_2), and water to material ratio (X_3). The levels of each variable are displayed in Table 1. Experimental data were fitted to a quadratic polynomial model to obtain the regression coefficients. The applied quadratic polynomial model was expressed as follows:

$$Y = \beta_0 + \sum_{j=1}^k \beta_j X_j + \sum_{j=1}^k \beta_{jj} X_j^2 + \sum_{\substack{i=1 \\ j>i}}^{k-1} \sum_{i=2}^k \beta_{ji} X_i X_j \quad (2)$$

where Y is the response variable; X_i and X_j are the independent coded variables; and β_0 , β_j , β_{jj} , and β_{ji} are the regression coefficients for intercept, linearity, square, and interaction, respectively.

Purification of crude OLP The crude OLP were purified through an anion-exchange column of DEAE-52 cellulose (2.5×60 cm). Samples were loaded on the column, and elution was carried out with 500 mL of distilled water at 2.0 mL/min. Each fraction (15 mL) was mixed

with the phenol-sulphuric acid reagent and measured at 490 nm by a spectrophotometer (UV1800; Shimadzu Corp., Kyoto, Japan). The first independent elution peak was collected, concentrated at 45°C with the rotary evaporator under vacuum, and freeze dried to obtain purified OLP.

Analysis of molecular weight distribution Purified OLP were deposited onto a stainless steel target plate and dehydrated at room temperature. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was carried out on a Bruker Ultra FleXtreme mass spectrometer (Bruker Daltonics, Bremen, Germany) operated in reflectron positive-ion mode.

Determination of DPPH radical scavenging activity The DPPH radical scavenging activity of the purified OLP was evaluated according to Brand-Williams (11) with some modification. Aliquots of the purified OLP extract were mixed with different volumes of distilled water to prepare different purified OLP concentrations (10, 20, 30, 40, and 50 mg/mL). The sample of a 0.2-mL solution with different concentrations of the purified OLP solution was properly mixed with 2.8 mL DPPH solution (0.1 mmol/L) and kept in the dark at ambient temperature for 30 min. Then the absorbance of the mixture was measured at 517 nm. The control was performed by using distilled water to replace the sample. The blank was carried out using ethanol. The DPPH radical scavenging activity was determined through the following formula:

$$\begin{aligned} \text{DPPH radical scavenging activity (\%)} \\ = \left(1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}} \right) \times 100 \end{aligned} \quad (3)$$

Statistical analysis The Box-Behnken design, model fitting, and the corresponding analysis of variance were performed using the Design Expert software. All the experiments were carried out in triplicate. The results were expressed as average±standard deviation.

Results and Discussion

Optimization of UMSE for crude OLP

Model fitting The yields of crude OLP at different design points are presented in Table 1. Results revealed considerable variations in the yields of crude OLP under different UMSE conditions. The obtained model was expressed by the quadratic polynomial equation as follows:

$$\begin{aligned} Y = 6.07 + 0.50X_1 + 0.39X_2 + 0.14X_3 + 0.052X_1X_2 - 0.48X_1X_3 + 0.12X_2X_3 \\ - 0.064X_1^2 - 0.45X_2^2 + 0.70X_3^2 \end{aligned} \quad (4)$$

where Y is the crude OLP yield and X_1 , X_2 , and X_3 are the coded variables for temperature, ultrasonic time, and water to material ratio, respectively.

Table 1. Variables and responses of Box-Behnken design for UMSE of crude OLP

No.	A: Temperature (°C)		B: Ultrasonic time (min)		C: Water to material ratio (mL/g)		Yield (%)
	X_1	Code X_1	X_2	Code X_2	X_3	Code X_3	
1	45.00	0	15.00	0	20.00	0	6.13
2	45.00	0	15.00	0	20.00	0	6.06
3	45.00	0	25.00	1	30.00	1	6.97
4	45.00	0	15.00	0	20.00	0	5.98
5	35.00	1	25.00	1	20.00	0	5.30
6	35.00	1	5.00	1	20.00	0	4.64
7	35.00	1	15.00	0	10.00	1	5.68
8	45.00	0	5.00	1	10.00	1	5.93
9	45.00	0	5.00	1	30.00	1	5.92
10	45.00	0	25.00	1	10.00	1	6.48
11	45.00	0	15.00	0	20.00	0	6.11
12	55.00	1	5.00	1	20.00	0	5.71
13	45.00	0	15.00	0	20.00	0	6.08
14	35.00	1	15.00	0	30.00	1	6.94
15	55.00	1	15.00	0	30.00	1	6.79
16	55.00	1	25.00	1	20.00	0	6.58
17	55.00	1	15.00	0	10.00	1	7.44

Table 2. Analysis of variance of regression coefficients of the fitted quadratic model for UMSE of crude OLP

Variables	Sum of squares	df	Mean square	F-value	p-value Prob. >F
Model	7.14	9	0.79	65.60	<0.0001
A-Temperature	1.96	1	1.96	162.09	<0.0001
B-Ultrasonic time	1.22	1	1.22	101.26	<0.0001
C-Water to material ratio	0.15	1	0.15	12.28	0.0099
AB	0.011	1	0.011	0.91	0.3715
AC	0.91	1	0.91	75.41	<0.0001
BC	0.062	1	0.062	5.17	0.0572
A ²	0.017	1	0.017	1.40	0.2747
B ²	0.86	1	0.86	70.82	<0.0001
C ²	2.09	1	2.09	172.55	<0.0001
Residual	0.085	7	0.012		
Lack of fit	0.071	3	0.024	7.04	0.0549
Pure error	0.013	4	3.370E-003		
Correlation total	7.22	16			
				$R^2=0.9883$	$R^2_{adj}=0.9732$

Results of the analysis of variance are listed in Table 2 for the fitted model for extraction yields of crude OLP. The *p*-value was applied to estimate the significance of each variable. Results suggested that the model had a very low *p*-value ($p<0.0001$). Meanwhile, the *p*-value of the lack of fit was 0.0549 ($p>0.05$), confirming that the model equation was adequate for the prediction of crude OLP extraction yield under different combinations of the variables (12).

In addition, Table 2 indicated that the determination coefficient (R^2) was 0.9883, implying that only 1.17% of the total variations were not explained by the obtained model. The adjusted determination coefficient was 0.9732, further verifying the validity of the model (13).

As shown in Table 2, all the independent variables (A, B, and C) and the two quadratic terms (B² and C²) significantly affected the yields of crude OLP. It was also observed that interaction between

temperature (A) and water to material ratio (C) was significant, while interaction between temperature (A) and ultrasonic time (B), as well as interaction between ultrasonic time (B) and water to material ratio (C) were not significant ($p>0.05$).

Effects of temperature, ultrasonic time, and water to material ratio on the yield of crude OLP

The effects of temperature, ultrasonic time, and water to material ratio as well as their interactive relationships on the yields of crude OLP are shown in Fig. 1. Response surface plots and their corresponding contour plots showed a visual description of the interactions between every two variables and the point with maximum response. For the shape of the contour plot, the circular contour plot indicates negligible interactions between variables, while the elliptical contour plot suggests significant interactions (14). As shown in Fig. 1A and 1B,

Fig. 1. Response surface plots (A, C, and E) and contour plots (B, D, and F) of the interactions between every two factors of temperature, ultrasonic time, and water to material ratio for UMSE of crude OLP

when the water to material ratio was fixed, the extraction yields of crude OLP increased significantly with the increase in temperature. Higher temperature enhanced the mass transfer between the solvent and OLP, thus increasing extraction yields. However, temperature beyond a certain degree was not suitable for heat-sensitive bioactive compounds such as oligosaccharides. High temperature may cause possible decomposition and structure damage, resulting in the loss of their bioactivity (15). It was reported that hydrolysis of fructo-oligosaccharides occurred when the incubation temperature was 60°C (16). So for the extraction of OLP, the highest temperature was fixed at 55°C. The extraction yield of crude OLP was observed to increase rapidly as ultrasonic time increased and reached the maximum level at around 20 min. When extraction time was prolonged, crude OLP yields decreased slightly, which might be attributed to the degradation of OLP (17). As shown in Fig. 1C and 1D, when extracting for a fixed time, water to material ratio and temperature exhibited obvious interactions. As the water to material ratio decreased and temperature increased, the yield of crude OLP reached a higher level. Similar results of higher extraction yield at lower water to material ratio were also observed by You and Vongsangnak (10,18). The

reason might be that water dispersed and absorbed the microwave energy at an extraction condition with higher water to material ratio, thus leading to a decline in the extraction yield. Fig. 1E and 1F revealed that the yield of crude OLP was improved as ultrasonic time increased. A higher extraction yield of crude OLP was observed at higher or lower water to material ratio. In conclusion, the optimal conditions for UMSE of crude OLP were determined to be obtained at extraction temperature 55°C, ultrasonic time 18.52 min, and water to material ratio 10 mL/g.

Validation of the model An experiment under the optimal conditions (extraction temperature 55°C, ultrasonic time 18.52 min, and water to material ratio 10 mL/g) was accomplished to check the accuracy of the model. Results showed that the practical value was $7.57 \pm 0.91\%$, which aligned with the predicted value ($7.61 \pm 0.62\%$, $p > 0.05$).

Purification of crude OLP An anion-exchange column of DEAE-52 cellulose was used for the purification of crude OLP. The first independent elution peak (Fig. 2) was collected as the target fraction.

Fig. 2. Purification of crude OLP by a column of DEAE-52 cellulose

Fig. 3. MALDI-TOF-MS spectrum of purified OLP

The target fraction was concentrated at 45°C with the rotary evaporator under vacuum, and freeze dried. Then purified OLP were obtained with a yield of 42.48%. Jovanovic-Malinovska, Kuzmanova, and Winkelhausen conducted a survey on oligosaccharide levels in 32 fruits (19). Considering the extraction yield of crude OLP and the moisture content of dried longan pulps, the oligosaccharide level of the longan pulp was higher than the majority of the selected fruits but similar to those of blueberry, pear, and raspberry. However, this survey only took five specific compounds of fructooligosaccharides and raffinose family oligosaccharides into account, which may have just partially represented the oligosaccharide levels of the fruits.

Molecular weight distribution of purified OLP MALDI-TOF-MS was used for the determination of the molecular weight (Mw) of purified OLP. MALDI-TOF-MS has been introduced as a rapid and sensitive method to analyze carbohydrate mixtures. It enables the process of soft ionization to cause little or no fragmentation of analytes, allowing the molecular ions of analytes to be identified (20,21). The mass profiling of purified OLP, as shown in Fig. 3, represented a series of mass ranging from m/z 70.830-795.511, where mass peaks ranging from m/z 495.138-795.511 were attributed to oligosaccharides and their derivatives. Sugar moieties with Mw of 795.511, 766.548, 576.571, 551.791, 523.438, and 495.138 were oligosaccharides or their derivatives identified in the purified OLP. Thus, it can be inferred

Fig. 4. DPPH radical scavenging activity of purified OLP

that the degree of polymerization of OLP was 3-5. The molecular distribution of purified OLP was in a range similar to that of pitaya (22). However, the mixed oligosaccharides of pitaya were made up of only four components. Oligosaccharides in Cabernet Sauvignon, Syrah, Monastrell, Carignan, and Merlot red wines contained components with higher molecular weights (23,24).

DPPH radical scavenging activity of purified OLP In biological systems, oxidative damage caused by reactive oxygen species is believed to be the main reason for some degenerative diseases. Antioxidants have earned the distinction as protecting cells from damage caused by reactive oxygen species. For the evaluation of the antioxidant activity of pure compounds and food extracts, the DPPH assay is a frequently used method. This method provides a rapid way to determine antioxidant activity by monitoring the decrease in its absorbance at a characteristic wavelength during reaction with certain antioxidant (11,25,26).

The DPPH radical scavenging activities of the purified OLP solution with different concentrations were determined. As shown in Fig. 4, a significant dose-dependent behavior in DPPH radical scavenging activity was observed within the tested concentration range. When the purified OLP concentration was 10 mg/mL, 16.76% of the scavenging percentage was obtained. This scavenging effect was improved significantly when concentration increased and reached 74.65% at 50 mg/mL ($p < 0.05$). The IC_{50} (50% scavenging activity concentration) of purified OLP was calculated to be 31.97 ± 2.08 mg/mL.

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Disclosure The authors declare no conflict of interest.

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