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

Xian Lin, Jinling Chen, Gengsheng Xiao*, Yujuan Xu, Daobang Tang, Jijun Wu, Jing Wen, and Weidong Chen

Sericultural & Agri-Food Research Institute, Guangdong Academy of Agricultural Sciences/Key Laboratory of Functional Foods, Ministry of Agriculture/Guangdong Key Laboratory of Agricultural Products Processing, Guangzhou 510610, China

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).

Ultrasound 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 Agrimachinery 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 1,613 x g 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 (X₁), ultrasonic time (X₂), and water to material ratio (X₃). 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:

\[
Y = \beta_0 + \sum_{j=1}^{3} \beta_j X_j + \sum_{i=1}^{3} \sum_{j>i}^{3} \beta_{ij} X_j X_i \quad (2)
\]

where Y is the response variable; X₁ and X₂ are the independent coded variables; and \(\beta_0\), \(\beta_j\), and \(\beta_{ij}\) 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-S2 cellulose (2.5 x 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 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:

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

**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:

\[
Y = 6.07 + 0.50 X_1 + 0.39 X_1^2 + 0.14 X_2 + 0.052 X_2^2 + 0.48 X_3 + 0.12 X_1 X_2 - 0.064 X_1^3 - 0.45 X_2^3 + 0.70 X_3^2 \quad (4)
\]

where Y is the crude OLP yield and X₁, X₂, and X₃ are the coded variables for temperature, ultrasonic time, and water to material ratio, respectively.