

## Structural Characteristics and Antioxidant Activities of Oligosaccharides from Longan Fruit Pericarp

GUOXIANG JIANG,<sup>†</sup> YUEMING JIANG,<sup>†</sup> BAO YANG,<sup>\*,†</sup> CHUNYAN YU,<sup>†</sup> RONG TSAO,<sup>‡</sup>  
 HAIYAN ZHANG,<sup>†</sup> AND FENG CHEN<sup>§</sup>

<sup>†</sup>Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China, <sup>‡</sup>Guelph Food Research Centre, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, Ontario, Canada N1G 5C9, and <sup>§</sup>Department of Food Science and Human Nutrition, Clemson University, Clemson, South Carolina 29634

Ultrasonic assisted extraction was employed to extract oligosaccharides from longan fruit pericarp (OLFP). A Box–Behnken design was applied to investigate the effects of ultrasonic temperature (30–70 °C), power (120–300 W), and time (10–50 min) on OLFP recovery. The model showed a good agreement with the experimental results on the basis of  $R^2$  of 0.9655 and  $P$ -value <0.05. From response surface plots, ultrasonic power, time, and temperature exhibited independent and interactive effects on OLFP recovery. The optimal conditions to obtain the highest OLFP recovery were determined to be 13 min, 121 W, and 65 °C. Gas chromatography analysis indicated that purified OLFP comprised Gal (71.5%), Glc (24.6%), and GalA (3.9%). The analysis of glycosidic linkages showed that the backbone consisted of  $\rightarrow$ 3)-Gal-(1 $\rightarrow$ ,  $\rightarrow$ 6)-Gal-(1 $\rightarrow$ , Glc-(1 $\rightarrow$  and  $\rightarrow$ 3)-GalA-(1 $\rightarrow$  with a molar proportion of 13:5:6:1. Furthermore, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide anion radical scavenging assays showed that OLFP exhibited strong antioxidant activities in a dose-dependent manner.

**KEYWORDS:** Longan; oligosaccharide; ultrasonic assisted extraction; response surface methodology; antioxidant activity

### INTRODUCTION

Longan (*Dimocarpus longan* Lour.), a member of the Sapindaceae family, is an important fruit in Southeast Asia. This fruit is a nonclimacteric fruit and deteriorates rapidly after harvest due to pericarp browning and decay (1). Longan fruit pericarp contains a significant amount of polysaccharides and polyphenols, which exhibit strong antibacterial, antiviral, antioxidant, anti-inflammatory, and anticarcinogenic activities (2–4). A great deal of attention has been paid to oligosaccharides for their beneficial physiological effect on the microflora of gastrointestinal tract in recent years and other biological activities (5). Oligosaccharides have become popular food ingredients because of their great potential to improve food quality, flavor, and physicochemical characteristics (6, 7). Response surface methodology is a statistical procedure frequently used for optimizing the extraction of bioactive compounds from plant materials, with a goal of finding the levels of input variables that optimize a particular response (8). The method uses quantitative data from an appropriate experimental design to determine and then to simultaneously solve multivariate problems (9). Box–Behnken design is a commonly used protocol of response surface methodology. The advantage of the Box–Behnken design is that only three levels are required to reduce experiments. Furthermore, it is more efficient to arrange and to interpret in comparison than others (10).

Ultrasonic assisted extraction is one of the important techniques for extracting bioactive compounds from plant materials (11). Recently, ultrasonic assisted extraction has been used as an alternative extraction technique at the laboratory or industry scale, which is proved to be effective and economical. It improves the extraction efficiency of target compounds by increasing the yield and shortening the extraction time, compared with that of the conventional extraction (12, 13). The enhancement in extraction obtained by ultrasound is mainly attributed to the destruction of the cellular wall, reduction of particle size, and enhancement of mass transfer through the cell walls because of the cavitation effect (14, 15).

In this work, ultrasonic assisted extraction for oligosaccharides from longan fruit pericarp (OLFP) was investigated, and then the operational parameters of the extraction were optimized using response surface methodology. Furthermore, the structural characteristics and antioxidant activity of purified OLFP were determined.

### MATERIALS AND METHODS

**Chemicals and Reagents.** 1,1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxy toluene (BHT), nitro blue tetrazolium, methionine, riboflavin, and standards of xylose (Xyl), arabinose (Ara), glucose (Glc), galactose (Gal), fructose (Fru), mannose (Man), galacturonic acid (GalA), and glucuronic acid (GlcA) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Phenol and sulphuric acid were obtained from Guangzhou Reagent Co. (Guangzhou, China). Other chemicals used were of analytical grade.

\*Corresponding author. E-mail: yangbao@scbg.ac.cn.

**Table 1.** Box–Behnken Design and the Responses for OLFP Recovery<sup>a</sup>

experiment	coded levels			responses
	$X_1$	$X_2$	$X_3$	
	ultrasonic time (min)	ultrasonic power (W)	ultrasonic temperature (°C)	recovery (mg GE/g DW)
1	0 (30)	-1 (120)	-1 (30)	17.80
2	0 (30)	0 (210)	0 (50)	21.98
3	1 (50)	1 (300)	0 (50)	29.41
4	1 (50)	-1 (120)	0 (50)	21.82
5	0 (30)	0 (210)	0 (50)	21.05
6	0 (30)	1 (300)	-1 (30)	27.86
7	0 (30)	0 (210)	0 (50)	21.17
8	-1 (10)	0 (210)	-1 (30)	21.79
9	0 (30)	-1 (120)	1 (70)	27.86
10	-1 (10)	0 (210)	1 (70)	25.04
11	-1 (10)	1 (300)	0 (50)	23.43
12	0 (30)	1 (300)	1 (70)	23.11
13	1 (50)	0 (210)	1 (70)	21.18
14	0 (30)	0 (210)	0 (50)	22.63
15	0 (30)	0 (210)	0 (50)	21.82
16	1 (50)	0 (210)	-1 (30)	20.70
17	-1 (10)	-1 (120)	0 (50)	29.55

<sup>a</sup>The values in parentheses mean practical levels.

**Materials.** Fresh longan fruits (*Dimocarpus longan* Lour. cv. Shixia) at the commercially mature stage were obtained from a commercial market in Guangzhou, China in August, 2008. Fruits were selected for uniformity of shape and color, and the absence of lesions. The fruit were separated manually, and the fresh pericarp tissues were collected and dried for 12 h in an oven (SFG-01, Hengfeng Mechanics Co., Hubei, China) at 50 °C.

**Ultrasonic Assisted Extraction and Quantification of OLFP.** The dried longan fruit pericarp was pulverized by a mill (DFT-50, Lingda Mechanics Co., Zhejiang, China) and screened through a 60-mesh sieve. The dried pericarp powder (4 g) was exactly weighed and mixed with 100 mL of distilled water. The extraction process was performed using an ultrasonic cleaner (SB-5200DTD, 40 kHz, Xinzhi Biotech Co., Ningbo, China.) with different ultrasonic power, temperature, and time. The extract was centrifuged at 8000g for 15 min, and the supernatant was then concentrated to 25 mL using a rotary evaporator (BC-R203, Shanghai Biochemical Equipment Co., Shanghai, China) at 65 °C under vacuum. Anhydrous ethanol (100 mL) was added to the mixture and then kept overnight at 4 °C to precipitate polysaccharides and proteins. After removing the precipitate, the mixture was partitioned with 100 mL of chloroform and ethyl acetate sequentially three times. The water phase was collected as the OLFP extract.

The content of OLFP was determined by the phenol–sulphuric acid method (16). Glucose was used to make a calibration curve. The recovery of OLFP was expressed as milligram of glucose equivalents (GE) per gram of longan fruit pericarp on a dry weight (DW) basis.

**Box–Behnken Design.** The software Design Expert (Trial Version 7.0.3, Stat-Ease Inc., Minneapolis, MN, USA) was employed for experimental design, data analysis, and model building. Box–Behnken was applied to determine the response pattern and then to establish a model. Three variables used in this study were ultrasonic power ( $X_1$ ), time ( $X_2$ ), and temperature ( $X_3$ ), respectively, with three levels for each variable, while the dependent variable was the OLFP recovery. The symbols and levels are shown in **Table 1**. The whole design consisted of 17 experimental points, which were carried out in a randomized order, to maximize the effect of unexplained variability in the observed response due to extraneous factors. The nonlinear computer-generated quadratic model is given as follows:

$$Y_0 = \beta_0 + \sum_{j=1}^K \beta_j X_j + \sum_{j=1}^K \beta_{jj} X_j^2 + \sum_{i < j} \beta_{ji} X_i X_j \quad (1)$$

where  $Y_0$  is the estimated response;  $\beta_0$ ,  $\beta_j$ ,  $\beta_{jj}$ , and  $\beta_{ji}$  are the regression coefficients for the intercept, linearity, square, and interaction; and  $X_i$  and  $X_j$  are the independent coded variables.

**OLFP Purification.** The crude OLFP obtained under the optimum conditions was purified using a column (95 × 2.5 cm) packed with XAD-7 macroporous adsorption resin (Sigma, St. Louis, MO, USA). Distilled water was employed as a mobile phase. The flow rate was 2 mL/min. Each fraction (20 mL) was collected and then analyzed with phenol–sulphuric acid reagent at 490 nm using a spectrophotometer (UV-2802, Unico Co Ltd., Shanghai, China). Fractions 10–30 which corresponded to the main peak were collected together, concentrated at 65 °C with a rotary evaporator under vacuum, and then freeze-dried for the determination of antioxidant activity.

One milliliter of 10 mg/mL OLFP was loaded onto a Sephadex G-25 column (100 × 1 cm), eluted with distilled water at a flow rate of 0.2 mL/min, and fractionated using a Redifrac fraction collector (BS-100A, Qingpu Huxi Instruments Factory, Shanghai, China). Each fraction (1 mL) was collected and analyzed with phenol–sulphuric acid reagent at 490 nm using a spectrophotometer (UV-2802, Unico Co Ltd., Shanghai, China). Fractions 34–51 corresponding to the peak were combined together and freeze-dried for structural analysis.

**Assay of DPPH Radical Scavenging Activity.** The DPPH radical scavenging activity was measured by the method used in previous reports (16). Aliquots of the OLFP extract were dissolved in 10 mL of distilled water to obtain different final concentrations (50, 100, 200, 300, 400, and 500 µg/mL). Two milliliters of 0.1 mM DPPH in ethanol was added to 1 mL of the OLFP solution. The absorbance was measured at 517 nm after 20 min of incubation at 25 °C. The control was carried out with water instead of extract, while ethanol was used as the blank. BHT was used as the positive control. The scavenging activity of DPPH radicals of the sample was calculated according to the following equation: DPPH radical scavenging activity (%) = (1 – absorbance of sample/absorbance of control) × 100.

**Determination of Superoxide Anion Radical Scavenging Activity.** Superoxide anion radical scavenging activity was analyzed by the method described previously (17), with some modifications. All of the solutions were prepared in 0.2 M phosphate buffer (pH 7.8). OLFP solutions at the different concentrations (50, 100, 200, 300, 400, and 500 µg/mL) were mixed with 3 mL of reaction buffer solution. The reaction mixture contained 0.5 mL of 0.2 M phosphate buffer (pH 7.8), 0.3 mL of 50 µM riboflavin, 0.3 mL of 20 mM methionine, and 0.3 mL of 0.51 mM nitro blue tetrazolium, prior to the addition of 1 mL of OLFP solution. Reaction was started by illuminating the reaction mixture using a fluorescent lamp. After 20 min of incubation, the absorbance was measured at 560 nm using a spectrophotometer (UV-2802, Unico Co Ltd., Shanghai, China). The reaction mixture without sample was used as the control, while BHT was used as the positive control. The scavenging activity of the superoxide anion radical was calculated using the following equation: superoxide anion radical scavenging activity (%) = (1 – absorbance of sample/absorbance of control) × 100.

**Analysis of Monosaccharide Composition.** OLFP (10 mg) was hydrolyzed by 10 mL of 2 M trifluoroacetic acid at 100 °C for 4 h (18). Derivatization of the released monosaccharides was then carried out by the trimethylsilylation reagent according to the method described previously (19). The hydrolyzed OLFP mixture was dried at low pressure by a rotary evaporator (RE52AA, Yarong Instrument Co, Shanghai, China). Pyridine (1 mL), hexamethyldisilazane (0.4 mL), and trimethylchlorosilane (0.2 mL) were added and kept at room temperature (25 °C) for 5 min. The trimethylsilylated derivatives were centrifuged at 8000g for 15 min, and the supernatant was loaded onto a GC-2010 gas chromatography system (Shimadzu, Shanghai, China) equipped with a RTX-5 capillary column and a flame ionization detector. The following program was adopted for gas chromatography analysis: injection temperature, 230 °C; detector temperature, 230 °C; column temperature programmed from 130 to 180 at 2 °C/min, holding for 3 min at 180 °C, then increasing to 220 at 10 °C/min, and finally holding for 3 min at 220 °C. Nitrogen was used as the carrier gas and was maintained at 40.0 mL/min. The speed of air and hydrogen gas was 400 and 40 mL/min, respectively. The split ratio was set as 10:1. Inositol was used as the internal standard to quantify the monosaccharide content.

**Methylation Analysis.** Methylation of OLFP was carried out according to the method described previously (20), with minor modifications. Five milligrams of dry OLFP were weighed precisely and dissolved in

5.0 mL of DMSO before 200 mg of NaOH was added. The mixture was then treated by ultrasonic wave with an ultrasonic cleaner (KQ-300DE, Kunshan Ultrasonic Equipment Co, Kunshan, China, 40 kHz) for 10 min. After incubation for 1 h at room temperature (25 °C), methyl iodide (1.5 mL) was added for OLFP methylation. The sample was kept in the dark for 1 h before 4.0 mL of distilled water was used to decompose the remaining methyl iodide. The methylated oligosaccharides were extracted by 3 × 2 mL of chloroform and dried at low pressure by a rotary evaporator (RE52AA, Yarong Instrument Co, Shanghai, China). After hydrolysis by 10 mL of 2 M trifluoroacetic acid, the OLFP hydrolysates were dissolved into 4 mL of 1% (w/w) NaOH. Twenty milligrams of NaBH<sub>4</sub> was added to reduce the hemiacetal bond. After incubation at 40 °C for 30 min, 100 μL of glacial acetic acid was used to terminate the reduction. The sample was dried under low pressure and then acetylated by 2 mL of acetic anhydride and 2 mL of pyridine. The reaction was kept at 100 °C for 1 h. Two milliliters of distilled water was used to decompose the remained acetic anhydride. The acetylated derivatives were extracted by 4 mL of methylene chloride. A gas chromatography/mass spectrometer (GCMS-QP 2010, Shimadzu, Kyoto, Japan) was used to analyze glycosidic linkage. The acetylated derivatives were loaded into an HP-1 capillary column. The temperature program was set as follows: the initial temperature of the column was 15 °C, increased to 180 at 10 °C/min, then to 260 at 15 °C/min, and held for 5 min at 260 °C; the injection temperature was 220 °C. The ion source of the mass spectrometer was set at 200 °C. One microliter of sample was injected, and the split ratio was 50:1.

**Statistical Analysis.** All of the experiments were carried out in triplicate, and the average of the OLFP recovery was taken as a responsive value. Analyses of variance were used to determine the significant difference between results.

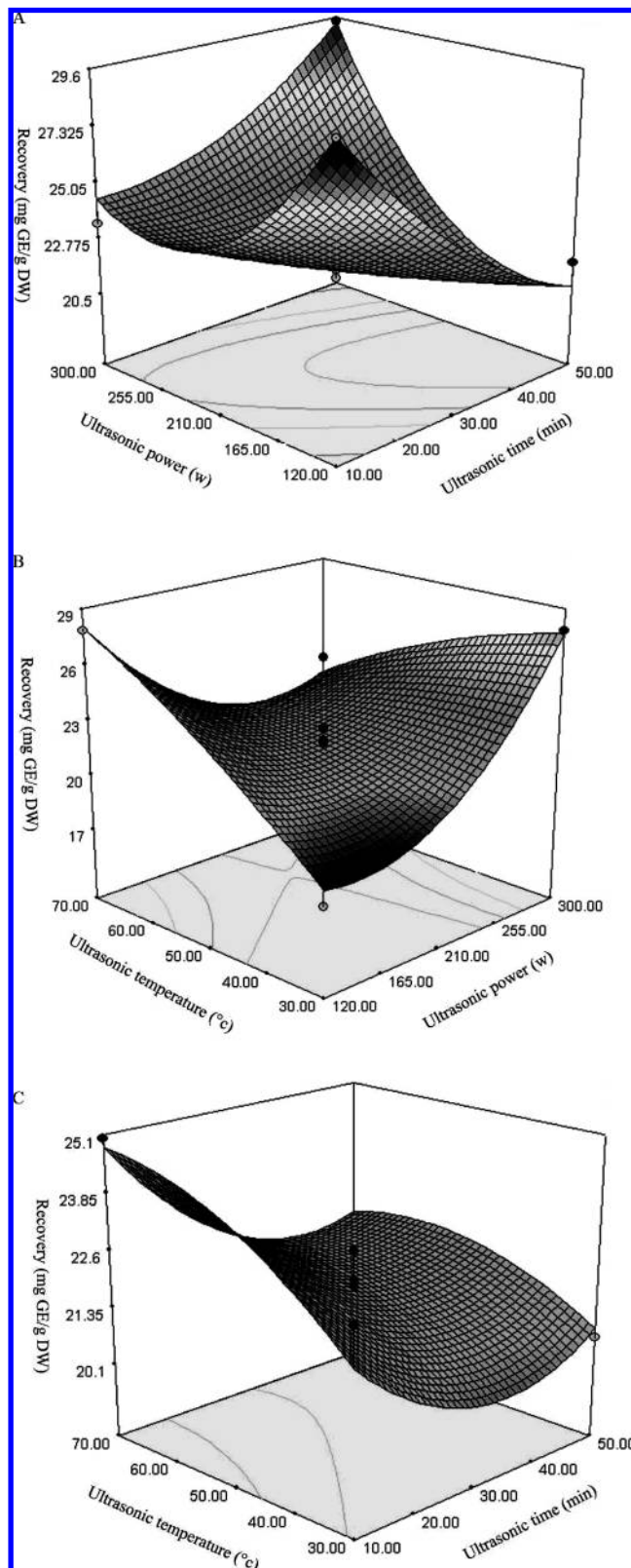
## RESULTS AND DISCUSSION

**Effects of Ultrasonic Power, Time, and Temperature on OLFP Recovery.** The effects of ultrasonic power and time on the recovery of OLFP as well as their interactions are shown in **Figure 1A**. The OLFP recovery decreased with increasing ultrasonic power when a short ultrasonic time was chosen. However, a reverse interaction between OLFP recovery and ultrasonic power was observed when the ultrasonic time was close to 50 min. A positive relationship was found between ultrasonic temperature and OLFP recovery (**Figure 1B** and **C**). The OLFP recovery increased with the increase of ultrasonic temperature, especially when the temperature fell within the range of 40–70 °C. However, OLFP recovery decreased with increasing ultrasonic temperature when a high ultrasonic power was used. It is generally accepted that increasing ultrasonic temperature reduces liquid viscosity and density, and consequently increases mass transfer (13). Furthermore, high ultrasonic temperature leads to the increase of cavitation bubble number and surface contact area (21). Therefore, an appropriately high ultrasonic temperature can enhance extraction efficiency.

**Model Fitting.** The values of response (OLFP recovery) at different experimental combinations are given in **Table 1**, which showed that there was a considerable variation in OLFP recovery within the range of the ultrasonic assisted extraction conditions. The statistical model, representing OLFP recovery as a function of the independent variables under investigation, can be expressed by the following quadratic equation:

$$Y = 22.51570 - 0.5798X_1 - 0.099415X_2 + 0.67671XX_1X_2 - 1.10625X_1X_3 - 2.05833X_2X_3 + 3.12469X_1^2 + 3.68256X_2^2 - 1.60969X_3^2 \quad (2)$$

where  $Y$  is OLFP recovery (mg GE/g DW),  $X_1$ ,  $X_2$ , and  $X_3$  are the coded variables for ultrasonic power, time, and temperature, respectively.



**Figure 1.** (A) Response surface plot showing the effect of ultrasonic power and time on OLFP recovery. The ultrasonic temperature was constant at 50 °C. (B) Response surface plot showing the effect of ultrasonic temperature and power on OLFP recovery. The ultrasonic time was constant at 30 min. (C) Response surface plot showing the effect of ultrasonic temperature and time on OLFP recovery. The ultrasonic power was constant at 210 W.

In general, exploration and optimization of a fitted response surface may produce poor or misleading results unless the model



exhibits good fitness, which essentially means checking of the model adequacy (22). The *P*-value of the model was significant ( $P < 0.05$ ), while the lack of the fitted value of the model was 0.0686 ( $P > 0.05$ , not significant). Both of the values indicated that the model exhibited a good fitness to the true behavior.

$R^2$  is defined as the ratio of the explained variation to the total variation, which reflects the degree of fitness (23). The small value of  $R^2$  indicates the poor relevance of the dependent variables in the model. In this experiment, the value of  $R^2$  (0.9655) indicated a good agreement between the experimental and predicted values of OLFP recovery. The value of adj- $R$  (0.9211) suggests that the total variation of 92% for OLFP recovery was attributed to the independent variables, and only about 8% of the total variation could not be explained by the model.

The coefficient estimates of the model equation, along with the corresponding *P*-values and *F*-values, are present in **Table 2**. The *P*-value is used as a tool to check the significance of each coefficient, which also indicates the interaction strength between each independent variable. A small *P*-value indicates a more significant coefficient (24). It can be seen from **Table 3** that  $X_1$ ,  $X_2$ ,  $X_3$ ,  $X_1X_2$ ,  $X_2X_3$ ,  $X_1^2$ , and  $X_2^2$  were significant model terms.

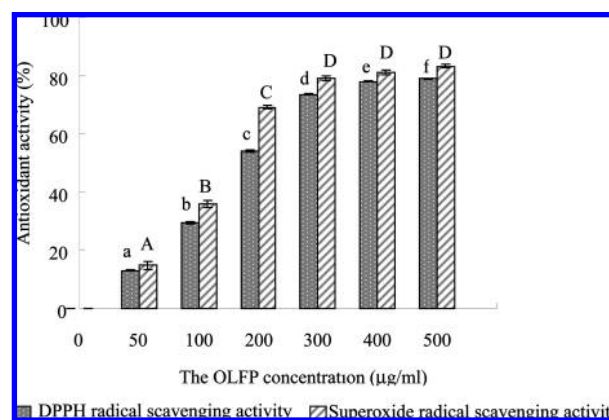
**Validation of the Model.** In order to validate the adequacy of the model equation (eq 2), a verification experiment was carried out under the optimal conditions (13 min, 121 W, and temperature 65 °C) within the experimental range. Under the optimal conditions, the model predicted a maximum response of 32.1 mg GE/g DW. To ensure that the predicted result was not biased toward the practical value, experimental validation was performed using these deduced optimal conditions;  $32.9 \pm 1.4$  mg GE/g DW of OLFP recovery was obtained from practical experiments, not significantly different from the predicted value within the 95% confidence interval. The good correlation between the predicted and practical values confirmed that the response model was adequate for reflecting the expected optimization. The results also indicated that the experimental value was in good agreement with the predicted value and suggested that the model of eq 2 was satisfactory and accurate.

**DPPH Radical Scavenging Activity of OLFP.** For investigation of the radical scavenging activity of OLFP, the DPPH radical scavenging assay was used. This assay is a widely accepted tool for

rapidly estimating the free radical scavenging activities of antioxidants (25). In this experiment, the DPPH radical scavenging activities of OLFP solutions at different concentrations were measured. As shown in **Figure 2**, the DPPH radical scavenging activity increased with increasing OLFP concentration up to 500  $\mu\text{g}/\text{mL}$ . This result was similar to the early report that feruloyl oligosaccharides showed a dose-dependent behavior on DPPH radical scavenging activity (26). The highest DPPH radical scavenging activity of OLFP was 78.85% at a concentration of 500  $\mu\text{g}/\text{mL}$ . By the calculation of  $\text{IC}_{50}$  (50% scavenging activity concentration), OLFP ( $177.09 \pm 2.1$   $\mu\text{g}/\text{mL}$ ) had a higher value than BHT ( $8.46 \pm 1.5$   $\mu\text{g}/\text{mL}$ ).

DPPH is a stable nitrogen-centered free radical with typical deep purple color, and its color changes from violet to yellow when reduced by the process of hydrogen donation. The principle of the DPPH• method is based on the reduction of DPPH• solution to DPPH-H in the presence of a hydrogen-donating antioxidant, leading to the formation of yellow-colored diphenylpicrylhydrazine (27). It is well accepted that the antioxidants can scavenge the DPPH radical through hydrogen-donating behavior (28, 29). In this study, the OLFP exhibited a dose-dependent behavior on DPPH radical scavenging activity within the test range of 50–500  $\mu\text{g}/\text{mL}$ , which might be due to their hydrogen-donating ability.

**Superoxide Anion Radical Scavenging Activity.** In the present work, the scavenging activity of OLFP against the superoxide anion radical was investigated. As illustrated in **Figure 2**, OLFP exhibited a dose-dependent behavior for the superoxide anion radical scavenging activity within the tested concentration range of 50–500  $\mu\text{g}/\text{mL}$ . The result corroborates the earlier report that



**Figure 2.** DPPH and superoxide anion radical scavenging activities of OLFP. Each value represents the mean  $\pm$  standard deviation of three replicates. For each activity, the values having the same letter are not significantly different ( $P > 0.05$ ).

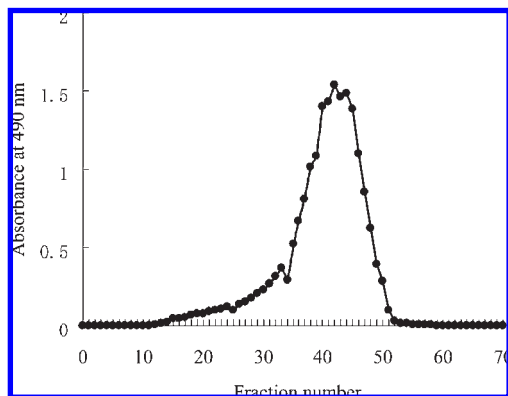
**Table 2.** Analysis of Variance for the Response Surface Quadratic Model for OLFP Recovery

source	degrees of freedom	sum of squares	mean square	<i>F</i> -value	<i>P</i> -value
model	9	170.40	18.93	21.76	0.0003
residual	7	6.09	0.87		
lack of fit	3	4.88	1.63	5.39	0.0686
pure error	4	1.21	0.30		
total	16	176.49			

**Table 3.** Regression Coefficient Estimates and Their Significance Test for the Quadratic Model for OLFP Recovery

model term	coefficient estimate	standard error	sum of squares	mean square	<i>F</i> -value	<i>P</i> -value	significance <sup>a</sup>
intercept	21.8	0.42					
$X_1$	-0.96	0.33	7.42	7.42	8.53	0.0223	**
$X_2$	0.85	0.33	5.74	5.74	6.60	0.0370	**
$X_3$	1.01	0.33	8.10	8.10	9.30	0.0186	**
$X_1X_2$	3.42	0.47	46.92	46.92	53.92	0.0002	**
$X_1X_3$	-0.44	0.47	0.78	0.78	0.90	0.3743	
$X_2X_3$	-3.71	0.47	54.91	54.91	63.10	<0.0001	***
$X_1^2$	1.25	0.45	6.58	6.58	7.56	0.0285	**
$X_2^2$	2.98	0.45	37.46	37.46	43.06	0.0003	**
$X_3^2$	-0.64	0.45	1.75	1.75	2.01	0.1996	

<sup>a</sup>\*\*Significance at 0.01 levels. \*\*\*Significance at 0.001 levels.



**Figure 3.** Sephadex G-25 gel filtration chromatogram of OLFP.

**Table 4.** Relative Molar Percentages of the Glycosidic Linkages and Monosaccharide Content of OLFP

monosaccharide content	relative molar percentage	
Gal	71.5%	
Glc	24.6%	
GalA	3.9%	

mass spectrum interpretation	glycosidic linkage	molar percentages
1,5-di- <i>O</i> -acetyl-2,3,4,6-tetra- <i>O</i> -methyl- <i>D</i> -glucitol	Glc-(1→	24.1%
1,5,6-tri- <i>O</i> -acetyl-2,3,4-tri- <i>O</i> -methyl- <i>D</i> -galactitol	→6)-Gal-(1→	20.2%
1,3,5-tri- <i>O</i> -acetyl-2,4,6-tri- <i>O</i> -methyl- <i>D</i> -galactitol	→3)-Gal-(1→	55.7%

either the polysaccharide or the mixture of oligosaccharides exhibited a similar scavenging activity against the DPPH and the superoxide anion radical in vitro (30). The highest superoxide anion radical scavenging activity of OLFP was 83.25% at 500  $\mu\text{g}/\text{mL}$ . The  $\text{IC}_{50}$  value of OLFP ( $145.09 \pm 1.5 \mu\text{g}/\text{mL}$ ) was much lower than that of BHT ( $471.58 \pm 1.1 \mu\text{g}/\text{mL}$ ). This result revealed that the OLFP had strong superoxide anion radical scavenging activity.

The superoxide anion is a reduced form of molecular oxygen created by receiving one electron. It is also an initial free radical formed from mitochondrial electron transport systems (31). The superoxide anion is a weak oxidant, but it gives rise to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (32). The results clearly suggested that the antioxidant activity of OLFP was related to its ability to scavenge the superoxide anion. The superoxide anion can react with the active hydrogen moiety in antioxidants to form a stable radical (33, 34). This might be a mechanism for OLFP to scavenge the superoxide radical.

**Monosaccharide Compositions and Glycosidic Linkages of OLFP.** The OLFP which were obtained by purification of the Sephadex column (Figure 3) were hydrolyzed by trifluoroacetic acid into individual monosaccharides. They were further trimethylsilylated for gas chromatography analysis. The results are shown in Table 4. Three monosaccharides, including Glc, Gal, and GalA, were identified after comparison of the retention time with that of the monosaccharide standards. Their molar percentages were 71.5%, 24.6%, and 3.9%, respectively. The results suggested that Gal constructed the backbone in combination with Glc for OLFP. Table 4 shows the glycosidic linkages of OLFP determined by GC/MS. Only two types of glycosidic linkage were found for Gal, which were →6)-Gal-(1→ and →3)-Gal-(1→. The GalA residue could not be linked as →6)-Gal-(1→ because of the uronic acid group; therefore, the glycosidic linkage of GalA was →3)-GalA-(1→ (35). By calculation of the molar

percentage, the proportion of →3)-Gal-(1→, →6)-Gal-(1→, Glc-(1→ and →3)-GalA-(1→ was approximately 13:5:6:1. Moreover, Glc had only one linkage, Glc-(1→, which indicated that glucose was the first monosaccharide of the backbone.

In general, the response surface methodology was proved to be useful for the optimization of oligosaccharide extraction. The statistical analysis showed that the optimal conditions for ultrasonic assisted extraction were 121 W, 13 min, and 65 °C. Under the optimal conditions, the experimental value agreed well with the predicted value by analysis of variance. OLFP showed good antioxidant activities in both DPPH and NBT systems. Gas chromatography analysis suggested that OLFP comprised Gal, Glc, and GalA with molar percentages of 71.5%, 24.6%, and 3.9%, respectively. The assay of glycosidic linkage showed that the backbone of OLFP consisted of →3)-Gal-(1→, →6)-Gal-(1→, Glc-(1→ and →3)-GalA-(1→ with a molar proportion of 13:5:6:1. Further bioactivity investigation of OLFP is worth doing in future work to better exploit this substance.

#### ABBREVIATION USED

DPPH, 1,1-diphenyl-2-picrylhydrazyl; BHT, butylated hydroxy toluene; OLFP, oligosaccharides from longan fruit pericarp; GE, glucose equivalents; DW, dry weight; Xyl, xylose; Ara, arabinose; Glc, glucose; Gal, galactose; Fru, fructose; Man, mannose; GalA, galacturonic acid; GlcA, glucuronic acid.

#### LITERATURE CITED

- Jiang, Y. M.; Zhang, Z. Q.; Joyce, D. C.; Ketsa, S. Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.). *Postharvest Biol. Technol.* **2002**, *26*, 241–252.
- Rangkadilok, N.; Worasuttayangkurn, L.; Bennett, R. N.; Satayavivad, J. Identification and quantification of polyphenolic compounds in longan (*Euphoria longana* Lam.) fruit. *J. Agric. Food Chem.* **2005**, *53*, 1387–1392.
- Sun, J.; Shi, J.; Jiang, Y. M.; Xue, S. J.; Wei, X. Y. Identification of two polyphenolic compounds with antioxidant activities in longan pericarp tissues. *J. Agric. Food Chem.* **2007**, *55*, 5864–5868.
- Prasad, N. K.; Hao, J.; Shi, J.; Liu, T.; Li, J.; Wei, X. Y.; Qiu, S. X.; Jiang, Y. M. Antioxidant and anticancer activities of high pressure-assisted extract of longan (*Dimocarpus longan* Lour.) fruit pericarp. *Innov. Food Sci. Emerg. Technol.* **2009**, Article in press.
- Roberfroid, M.; Slavin, J. Nondigestible oligosaccharides. *Crit. Rev. Food Sci. Nutr.* **2000**, *40*, 461–480.
- Crittenden, R. G.; Playne, M. J. Production, properties and applications of food-grade oligosaccharides. *Trends Food Sci. Technol.* **1996**, *7*, 353–361.
- Rivero-Urgell, M.; Santamaria-Orleans, A. Oligosaccharides: Application in infant food. *Early Hum. Dev.* **2001**, *65*, S43–S52.
- Dhungana, P.; Eskridge, K. M.; Weiss, A.; Baenziger, P. S. Designing crop technology for a future climate: An example using response surface methodology and the CERES–wheat model. *Agric. Syst.* **2006**, *87*, 63–79.
- Erbay, Z.; Icier, F. Optimization of hot air drying of olive leaves using response surface methodology. *J. Food Eng.* **2009**, *91*, 533–541.
- Ferreira, S. L. C.; Bruns, R. E.; Ferreira, H. S.; Matos, G. D.; David, J. M.; Brandão, G. C. Box–Behnken design: An alternative for the optimization of analytical methods. *Anal. Chim. Acta* **2007**, *597*, 179–186.
- Vilkhu, K.; Mawson, R.; Simons, L.; Bates, D. Applications and opportunities for ultrasound assisted extraction in the food industry: A review. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 161–169.
- Toma, M.; Vinatoru, M.; Paniwnyk, L.; Mason, T. J. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrason. Sonochem.* **2001**, *8*, 137–142.
- Hemwimol, S.; Pavasant, P.; Shotpruk, A. Ultrasound-assisted extraction of anthraquinones from roots of *Morinda citrifolia*. *Ultrason. Sonochem.* **2006**, *13*, 543–548.

- (14) Entezari, M. H.; Hagh Nazary, S.; Haddad Khodaparast, M. H. The direct effect of ultrasound on the extraction of date syrup and its micro-organisms. *Ultrason. Sonochem.* **2004**, *11*, 379–384.
- (15) Paniwnyk, L.; Beaufoy, E.; Lorimer, J. P.; Mason, T. J. The extraction of rutin from flower buds of *Sophora japonica*. *Ultrason. Sonochem.* **2001**, *8*, 299–301.
- (16) Yang, B.; Zhao, M. M.; Shi, J.; Jiang, Y. M.; Yang, N. Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp. *Food Chem.* **2008**, *106*, 685–690.
- (17) Duan, X. W.; Wu, G. F.; Jiang, Y. M. Evaluation of antioxidant properties of phenolics from litchi fruit in relation to pericarp browning prevention. *Molecules* **2007**, *12*, 759–771.
- (18) Erbing, B.; Jansson, P. E.; Widmalm, G.; Nimmich, W. Structure of the capsular polysaccharide from the *Klebsiella* K8 reference strain 1015. *Carbohydr. Res.* **1995**, *273*, 197–205.
- (19) Guentas, L.; Pheulpin, P.; Michaud, P.; Heyraud, A.; Gey, C.; Courtois, B.; Courtois, J. Structure of a polysaccharide from a *Rhizobium* species containing 2-deoxy- $\beta$ -D-arabino-hexuronic acid. *Carbohydr. Res.* **2001**, *332*, 167–173.
- (20) Needs, P. W.; Sevendran, R. R. Avoiding oxidative degradation during sodium hydroxide methyl iodide-mediated carbohydrate methylation in dimethyl sulfoxide. *Carbohydr. Res.* **1993**, *245*, 1–10.
- (21) Palma, M.; Barroso, C. G. Ultrasound-assisted extraction and determination of tartaric and malic acids from grapes and wine-making by-products. *Anal. Chim. Acta* **2002**, *458*, 119–130.
- (22) Liyana-Pathirana, C.; Shahidi, F. Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chem.* **2005**, *93*, 47–56.
- (23) Nath, A.; Chattopadhyay, P. K. Optimization of oven toasting for improving crispness and other quality attributes of ready to eat potato-soy snack using response surface methodology. *J. Food Eng.* **2007**, *80*, 1282–1292.
- (24) Muralidhar, R. V.; Chirumamila, R. R.; Marchant, R.; Nigam, P. A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. *Biochem. Eng. J.* **2001**, *9*, 17–23.
- (25) Hu, F. L.; Lu, R. L.; Huang, B.; Ming, L. Free radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. *Fitoterapia* **2004**, *75*, 14–23.
- (26) Yuan, X. P.; Wang, J.; Yao, H. Y.; Chen, F. Free radical-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber. *LWT Food Sci. Technol.* **2005**, *38*, 877–883.
- (27) Sentandreu, E.; Navarro, J. L.; Sendra, J. M. Reduction kinetics of the antiradical probe 2,2-diphenyl-1-picrylhydrazyl in methanol and acetonitrile by the antiradical activity of protocatechuic acid and protocatechuic acid methyl ester. *J. Agric. Food Chem.* **2008**, *56*, 4928–4936.
- (28) Chen, C. W.; Ho, C. T. Antioxidant properties of polyphenols extracted from green and black tea. *J. Food Lipids.* **1995**, *2*, 35–46.
- (29) Kikuzaki, H.; Hisamoto, M.; Hirose, K.; Akiyama, K.; Taniguchi, H. Antioxidant properties of ferulic acid and its related compounds. *J. Agric. Food Chem.* **2002**, *50*, 2161–2168.
- (30) Yuan, H. M.; Zhang, W. W.; Li, X. G.; Lu, X. X.; Li, N.; Gao, X. L.; Song, J. M. Preparation and *in vitro* antioxidant activity of k-carrageenan oligosaccharides and their oversulfated, acetylated, and phosphorylated derivatives. *Carbohydr. Res.* **2005**, *340*, 685–692.
- (31) Siddhuraju, P.; Becker, K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chem.* **2007**, *101*, 10–19.
- (32) Meyer, A. S.; Isaksen, A. Application of enzymes as food antioxidants. *Trends Food Sci. Technol.* **1995**, *6*, 300–304.
- (33) Sun, T.; Xie, W. M.; Xu, P. X. Superoxide anion scavenging activity of graft chitosan derivatives. *Carbohydr. Polym.* **2004**, *58*, 379–382.
- (34) Xing, R. E.; Liu, S.; Guo, Z. Y.; Yu, H. H.; Zhong, Z. M.; Ji, X.; Li, P. C. Relevance of molecular weight of chitosan-N-2-hydroxypropyl trimethyl ammonium chloride and their antioxidant activities. *Eur. J. Med. Chem.* **2008**, *43*, 336–340.
- (35) Yang, B.; Jiang, Y. M.; Zhao, M. M.; Chen, F.; Wang, R.; Chen, Y. L.; Zhang, D. D. Structural characterization of polysaccharides purified from longan (*Dimocarpus longan* Lour.) fruit pericarp. *Food Chem.* **2009**, *115*, 609–614.

---

Received July 22, 2009. Revised manuscript received August 24, 2009. Accepted August 25, 2009. The financial support from National Natural Science Foundation of China (Nos. 30425040, 30910103040, and 30700557), International Foundation of Science (No. F/4451-1), and the CAS/SAFEA International Partnership Program for Creative Research Teams and Scientific Research Foundation of South China Botanical Garden (No. 200807) is highly appreciated.