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Structural characteristics and antioxidant activities of polysaccharides from longan seed

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ABSTRACT

Ultrasound-assisted extraction was employed to extract polysaccharides from longan seed (LSP), with aids of a Box–Behnken statistical design to investigate the effects of ultrasonic power, time and liquid/solid ratio on the extraction recovery of the LSP. The structural analysis indicated that arabinose, galactose, glucose and mannose were major components of LSP, with →6)-Gal-(→1, Glc-(→1 and →6)-Glc-(1→ glycosidic linkages. In an *in vitro* antioxidant activity of the 1,1-diphenyl-2-picryldydrazyl radical-scavenging assay, LSP exhibited a dose-dependent property within the concentration range tested.

1. Introduction

Longan (*Dimocarpus longan* Lour.) is a non-climacteric subtropical fruit with high commercial value. Due to its semi-translucent to white aril, delicious taste and health benefits, longan is a preferred fruit by many consumers (Jiang, Zhang, Joyce, & Ketsa, 2002). Currently, longan arils are consumed as fresh and processed fruit. In contrast, longan seed accounting for about 17% of the whole fruit on fresh weight basis has been commonly used in traditional Chinese medicines (Xiao et al., 2004). Recent work has indicated that longan seed contains a significant amount of polyphenols which can be used as a readily accessible source of natural antioxidants (Zheng et al., 2009).

A great deal of attention has been paid to polysaccharides due to their unique bioactivities and chemical structures in recent years (Schepetkin & Quinn, 2006). Although longan polysaccharides have been reported to possess antioxidant, anti-tyrosinase, anti-glycated and immunity-modulation activities (Yang et al., 2009; Yi et al., 2012), such kind of studies and reports are scarce (Zheng, Jiang, Gao, & Zheng, 2010). One of the reasons is the lack of high efficient extraction technology of polysaccharides from longan seed. Classical extraction of longan polysaccharides uses the hot-water extraction that is considered inefficient (Li, Ding, & Ding, 2007). Therefore, it is essential and desirable to find a low cost but effective method to extract the polysaccharides from longan seed.

Ultrasonic-assisted extraction is considered as a promising techniques for extracting polysaccharides from different plant materials (Hemwimon, Pavasant, & Shotipruk, 2007; Wang, Cheng, Mao, Fan, & Wu, 2009; Zhong & Wang, 2010), which is proven to be effective and economical. The enhancement in extraction obtained by ultrasound is due to its cavitation effect, which causes destruction of cellular walls, reduction of particle size and enhancement of mass transfer through the cellular walls (Entezari, Hagh Nazary, & Haddad Khodaparast, 2004). In this study, ultrasonic-assisted extraction of the polysaccharide (LSP) was investigated using response surface methodology to optimize the operational parameters. Furthermore, the structural characteristics and antioxidant activities of LSPs were determined.

2. Materials and methods

2.1. Chemicals and reagents

Chemical standards of butylated hydroxy toluene (BHT), 1,1-diphenyl-2-picryldydrazyl (DPPH), xylose (Xyl), arabinose (Ara),

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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 in this study were of analytical grade.

2.2. Materials

Fresh fruit of longan (*Dimocarpus longan* Lour. cv. Shixia) at the commercially mature stage without disease symptoms were obtained from a commercial market of Guangzhou, China in August 2010. Fruits were selected for uniformity of shape and color. The fruit were separated manually and then the fresh seed were collected and dried for 12 h in an oven (SFG-01, Hengfeng Mechanics Co., Hubei, China) at 50 °C.

2.3. Ultrasonic-assisted extraction and quantification of LSP

The dried longan seed was pulverized by a mill (DFT-50, Lingda Mechanics Co., Zhejiang, China) and screened through a 60-mesh sieve. The dried seed powder was refluxed three times with 80% ethanol at 80 °C in a water bath for 2 h and then filtered through Whatman No. 1 filter paper to remove monosaccharides and other ethanol-soluble impurities (Wen et al., 2011). The obtained residue was dried at 45 °C for 12 h and the dried residue powder (4 g) was exactly weighed and mixed with a specified amount 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 liquid/solid ratios. The extract was centrifuged at 8000 × *g* 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. The resulting solution was mixed with four volumes of dehydrated ethanol at the ethanol final ethanol concentration of 80%, kept overnight at 4 °C, and then centrifuged for 30 min at 8000 × *g* to obtain crude LSP.

The content of LSP was determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Glucose was used to make a calibration curve. The extraction yield of LSP was expressed as mg of glucose equivalents (GE) per gram of longan seed on the dry weight (DW) basis.

2.4. 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 design was employed for optimization. Three variables used in this study were ultrasonic time (X_1), ultrasonic power (X_2) and liquid/solid ratio (X_3), with three levels for each variable, while the dependent variable was the extraction yield of LSP. The symbols and levels are shown in Table 1. The whole design consisted of 17 experimental points carried out in a randomized order to maximize the effect of unexplained variability in the observed response due to extraneous factors. The non-linear computer-generated quadratic model is given as

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

where Y_0 is the estimated response, and β_0, β_j, β_{jj} and β_{ji} are the regression coefficients for intercept, linearity, square and interaction while X_i and X_j are the independent coded variables, respectively.

2.5. Preparation of crude polysaccharides

The crude LSP was obtained under the optimum conditions when the proteins in the crude LSP were removed by the Sevag reagent (Navarini et al., 1999). Then, anhydrous ethanol was added into the extract to obtain a final concentration of 40% and maintained overnight at 4 °C to precipitate polysaccharides with large molecular weight (LSP 1) which was then obtained after centrifugation at 4000 × *g* for 15 min. Ethanol was then added into the supernatant to obtain a final concentration of 80%. The above program was repeated to obtain polysaccharides with small molecular weight (LSP 2).

2.6. Separation and purification of the polysaccharides

The above-mentioned LSP 2 was redissolved in ultra-pure water, and then applied to a DEAE-Sepharose Fast Flow column (2.4 × 60 cm) for further separation. After being loaded with sample, the column was eluted with distilled water for 300 ml at 0.5 ml/min, followed by 300 ml of 0.5 M NaCl aqueous solution at 0.5 ml/min. Each fraction (2 ml) was collected and then analyzed with the phenol–sulphuric acid reagent at 490 nm using a spectrophotometer (UV-2802, Unico Co. Ltd., Shanghai, China).The major polysaccharide fraction eluted with water was collected using a fraction collector (BS-100A, Qingpu Huxi Instruments Factory, Shanghai, China) for further purified. One milliliter of the above-mentioned major polysaccharide fraction at 10 mg LSP/ml was loaded onto a Sephadex G-100 gel column (100 × 1 cm), and then eluted with distilled water at a flow rate of 0.2 ml/min and fractionated using a fraction collector. Each fraction (1 ml) was collected and analyzed at 490 nm using a UV spectrophotometer (UV-2802, Unico Co. Ltd., Shanghai, China). Fractions 34–51 corresponding to the peak were combined together and freeze dried to obtain the purified polysaccharide (LSP 3).

2.7. Analysis of monosaccharide composition

LSP (10 mg) was hydrolysed by 10 ml of 2 M trifluoroacetic acid at 120 °C for 2 h (Erbing, Jansson, Widmalm, & Nimmich, 1995). Derivatization of the released monosaccharides was then carried out by the trimethylsilylation reagent according to the method described by Guentas et al. (2001). The hydrolysed LSP mixture was dried at low pressure by a rotary evaporator (RE52AA, Yarong Instrument Co., Shanghai, China). Then 1 ml pyridine, 0.4 ml hexamethyldisilazane and 0.2 ml trimethylchlorosilane were added and kept at 25 °C for 5 min. The trimethylsilylated derivatives were centrifuged at 8000 × *g* for 15 min and then the supernatant was collected and 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 °C at 2 °C/min, holding for 3 min at 180 °C, then increasing to 220 °C at 10 °C/min and finally holding for 3 min at 220 °C. Nitrogen was used as the carrier gas and 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.

2.8. Methylation analysis

Methylation of LSP was carried out according to the method of Needs and Sevendran (1993) with some modifications. The dried LSP (5 mg) was weighted precisely and then dissolved in 5.0 ml

Table 1
Box–Behnken design and the responses for the LSP recovery. The value in the bracket means the practical level of each parameter.

Experiment	Coded levels			Responses
	X_1 Ultrasonic time (min)	X_2 Ultrasonic power (W)	X_3 Liquid/solid ratio (g/ml)	Recovery (mg GE/g DW)
1	1 (45)	0 (210)	−1 (5)	3.96
2	1 (45)	0 (210)	1 (15)	5.82
3	0 (25)	−1 (120)	1 (15)	3.19
4	0 (25)	1 (300)	−1 (5)	2.59
5	−1 (5)	−1 (120)	0 (10)	2.69
6	−1 (5)	0 (210)	1 (15)	2.93
7	1 (45)	−1 (120)	0 (10)	3.78
8	0 (25)	0 (210)	0 (10)	3.93
9	0 (25)	−1 (120)	−1 (5)	2.64
10	0 (25)	0 (210)	0 (10)	4.70
11	0 (25)	1 (300)	1 (15)	4.43
12	0 (25)	0 (210)	0 (10)	4.69
13	−1 (5)	0 (210)	−1 (5)	2.66
14	−1 (5)	1 (300)	0 (10)	2.63
15	0 (25)	0 (210)	0 (10)	4.74
16	1 (45)	1 (300)	0 (10)	5.48
17	0 (25)	0 (210)	0 (10)	4.62

of DMSO before 200 mg NaOH was added. The mixture was then treated for 10 min by ultrasonic wave using an ultrasonic cleaner (KQ-300DE, Kunshan Ultrasonic Equipment Co., Kunshan, China, 40 kHz). After incubation for 1 h at 25 °C, methyl iodide (1.5 ml) was added to conduct LSP methylation. The sample was kept in dark for 1 h before 4.0 ml of distilled water was used to decompose the remaining methyl iodide. The methylated polysaccharides were extracted by 3 × 2 ml of chloroform and then 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 LSP hydrolysates were dissolved into 4 ml of 1% (w/w) NaOH. Twenty milligrams of $NaBH_4$ was added to reduce 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 with 2 ml acetic anhydride and 2 ml pyridine. The reaction was kept at 100 °C for 1 h. Two milliliters of distilled water was used to decompose the remaining acetic anhydride. The acetylated derivatives were extracted with 4 ml methylene chloride. A gas chromatography/mass spectrometer (GCMS-QP 2010, Shimadzu, Kyoto, Japan) was used to analyze the glycosidic linkage. The acetylated derivatives were loaded into a HP-1 capillary column. The temperature program was set as follows. The initial temperature of column was 15 °C, and then was increased from 15 to 180 °C at 10 °C/min, from 180 to 260 °C at 15 °C/min and held for 5 min at 260 °C. Injection temperature was 220 °C. The ion source of mass spectrometer was set at 200 °C. One microliter of sample was injected. The split ratio was 50:1.

2.9. Assay of DPPH radical scavenging activity

The DPPH radical scavenging activity was measured by the method of previous reports (Yang, Zhao, Shi, Jiang, & Yang, 2008). Aliquots of the LSP extract were dissolved in 10 ml of distilled water to obtain different final concentrations. Two milliliters of 0.1 mM DPPH in ethanol was added to 1 ml of the LSP solution. The absorbance was measured at 517 nm after 20 min of incubation at 25 °C. The control contained all reagents except the sample while distilled water was used as a 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.

2.10. Statistical analysis

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

3. Results and discussion

3.1. Effects of ultrasonic time, power and liquid/solid ratio on the yield of LSP

As shown in Fig. 1, three-dimensional response surface plots and two-dimensional contour plots indicated the interaction effects between response factors. There was an increase in the extraction yield of LSP with extending ultrasonic time (Fig. 1A). The extraction yield of LSP was found to increase rapidly with increasing liquid/solid ratio from 10 to 15. As shown in Fig. 1B, the yield of LSP increased with increasing ultrasonic power at a low level and reached the maximum value at ultrasonic power from 165 to 255 W (Fig. 1B). Obviously, the LSP yield increased with extending ultrasonic time at the same ultrasonic power. As displayed in Fig. 1C, the yield of LSP increased with increasing liquid/solid ratio at a low ultrasonic power, but it decreased at high ultrasonic power. In this investigation, as the ultrasonic time and liquid/solid ratio were kept constant, the yield of LSP increased to a value with elevating ultrasonic power, and decreased thereafter (Fig. 1B and C). The similar phenomenon was also found for liquid/solid ratio used in this study (Fig. 1A and C).

It is generally accepted that the enhanced extraction yield obtained by ultrasound is mainly attributed to the cavitation effect. Ultrasonic cavitation can cause super high temperature and great pressure in micro-environment (Kanthale, Gogate, Pandit, & Wilhelm, 2003), which promotes the mass transfer and facilitates the diffusion of extracts by improving the osmotic pressure difference between inside and outside of the cells (Sun, Liu, Chen, Ch Ye, & Yu, 2011). The similar result by higher extraction yield at suitable liquid/solid ratio was also observed by Vongsangnak, Gua, Chauvatcharin, and Zhong (2004) and Wen et al. (2011).

3.1.1. Model fitting

The experimental conditions and the results of the extraction yield of LSP according to the factorial design are shown in Table 1, which showed that there was a considerable variation in the LSP

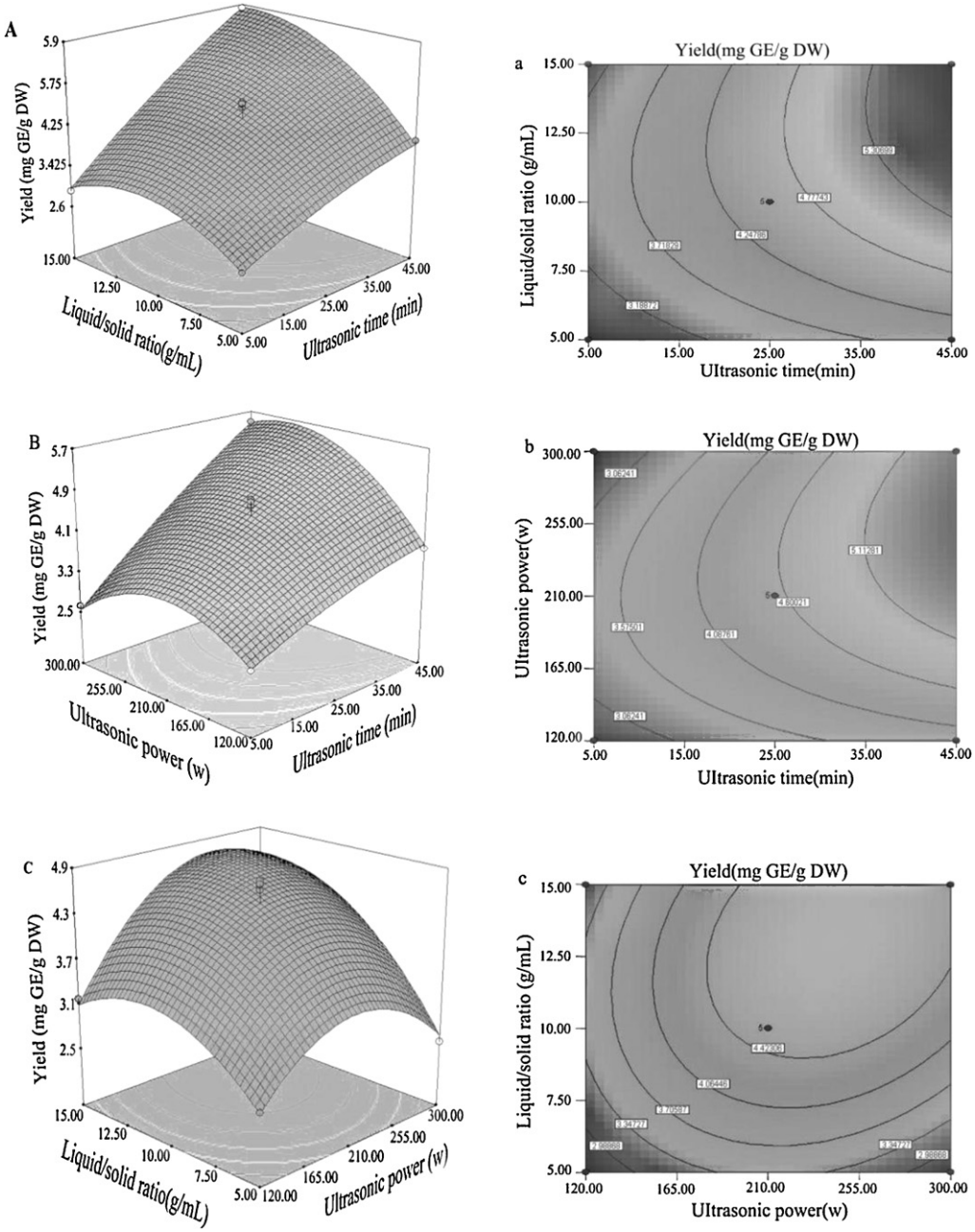

Fig. 1. Response surface plots and countour plots showing effects of ultrasonic time, power and liquid/solid ratio on the yields of LSPs and their interaction. (a) The ultrasonic power was constant at 210 W; (b) the liquid/solid ratio was constant at 10 mg/ml; and (c) the ultrasonic time was constant for 25 min.

yield under the ultrasonic-assisted extraction conditions. The multiple regression analysis was applied to the experiment data, and the response variable and the test variables can be expressed by the following second-order polynomial equation:

$$Y = 4.54 + 1.01X_1 + 0.36X_2 + 0.57X_3 + 0.44X_1X_2 + 0.40X_1X_3 + 0.33X_2X_3 - 0.14X_1^2 - 0.76X_2^2 - 0.56X_3^2 \quad (2)$$

where Y is the LSP yield (mg GE/g DW) and X_1, X_2 and X_3 are the coded variables for ultrasonic time, power and liquid/solid ratio, respectively.

In general, poor fitness model would produce poor or misleading results and examination of the model adequacy is essential (Liyana-Pathirana & Shahidi, 2005). In the experiment, the P-value of the model was significant ($P < 0.05$) while the lack of the fitted value of the model was 0.9489 ($P > 0.05$). Both the values indicated that the model exhibited a good fitness to the true behaviour. Furthermore, in this experiment, the value of R^2 (0.9727) indicated a good agreement between the experimental and predicted values of the LSP yield as R^2 reflects the degree of fitness while a large value of R^2 indicates the more relevance of the dependent variables in the model. The value of adj-R (0.9376) suggested that the total variation of 93% for the LSP recovery was attributed to the independent variables and only about 7% of the total variation could not be explained by the model. The coefficient estimates of model equation along with the corresponding P-values and F-values are presented in Table 3. 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 (Muralidhar, Chirumamila, Marchant, & Nigam, 2001). A small P-value less

Table 2
Analysis of variance for the response surface quadratic model for the yield of LSP.

Source	Degrees of freedom	Sum of squares	Mean square	*F*-value	*P*-value Prob > *F*
Model	9	17.82	1.98	27.70	0.0001
Residua	7	0.50	0.071		
Lack of fit	3	0.039	0.013	0.11	0.9489
Pure error	4	0.46	0.12		
Total	16	18.32			
X_1	1	8.24	8.24	115.31	<0.0001
X_2	1	1.03	1.03	14.48	0.0067
X_3	1	2.59	2.59	36.28	0.0005
X_1X_2	1	0.77	0.77	10.79	0.0134
X_1X_3	1	0.64	0.64	8.95	0.0202
X_2X_3	1	0.45	0.45	6.25	0.0410
X_1^2	1	0.079	0.079	1.11	0.3278
X_2^2	1	2.40	2.40	33.64	0.0007
X_3^2	1	1.30	1.30	18.24	0.0037

than 0.05 indicate a significant coefficient. As shown in Table 2, X_1, X_2, X_3, X_1X_2, X_1X_3, X_2X_3, X_2^2 and X_3^2 were significant model terms.

3.1.2. Validation of the model

In order to validate the adequacy of the model equation, a verification experiment was carried out under the optimal conditions (35 min, 268.7 W and liquid/solid ratio 14.3 ml/g). The model predicted a maximum response of 5.51 mg GE/g DW. To ensure the predicted result that was not based on the practical value, the experimental validation was performed under the optimal conditions. The recovery of LSP (5.57 ± 0.18 mg GE/g DW) was obtained from practical experiments, which was not significantly different to the predicted value within 95% confidence interval. The good correlation between the predicted value and the practical value confirmed that the response model was adequate for reflecting the expected optimization (Jiang et al., 2009; Wen et al., 2011). The results also indicated that the experimental value was in a good agreement with the predicted value and suggested that the model was satisfactory and accurate.

3.2. Structural characteristic of LSP

3.2.1. Monosaccharide composition of LSP

The monosaccharide composition of LSP 1, 2 or 3 was determined, as shown in Table 3. GC analysis of the trimethylsilyl derivatives showed the differences in these three LSPs, with the presence of Fuc, Fru, Xyl, Man, Ara, Gal, and Glc in the molar ratio of 1:1.19:1.61:3.51:6.16:9.95:41.99 for LSP 1, Rha, GalA, Man, Ara, Fru, Fuc, Glc and Gal in the molar ratio of 1.00:1.07:6.53:8.30:8.99:9.62:29.25:34.86 for LSP 2 and Man, Ara, GalA, Glc, and Gal in the molar ratio of 1:1.40:1.90:6.65:7.70 for LSP 3. These data revealed further great differences in the polysaccharide distribution of xylose, mannose, glucose, galactose, and fructose, strongly indicating that the polysaccharides were heterogeneous. Furthermore, galactose and glucose constructed the backbone of LSP. Galactose was the dominant monosaccharide with relative molar percentage of 35.56% and 40.58% in LSP 2 or 3, but the percentage was only 14.93% in LSP 1. In addition, LSP 1 was precipitated by 40% ethanol, which had predominant monosaccharides of glucose, galactose, arabinose and mannose. This result was similar to the early report of a water-soluble polysaccharides isolate from *Dendrobium nobile* Lindl stem (Luo et al., 2009). For LSP 2 or 3, glucose was the major monosaccharide, but their proportions were lower than galactose. LSP 2 and 3 had similar monosaccharide composition; the major difference between them was LSP 3 did not have Rha, Fuc and Fru, but higher GalA. This difference might be relate to the purification process removed some heterogeneous polysaccharides.

Table 3
The relative molar percentage of monosaccharide in LSP 1, 2 and 3.

Monosaccharide	LSP 1	LSP 2	LSP 3
Ara	9.25 ± 0.02	8.47 ± 0.01	7.38 ± 0.02
Rha		1.02 ± 0.07	
Fuc	1.50 ± 0.17	9.81 ± 1.26	
Xyl	2.42 ± 0.24		
Man	5.65 ± 0.26	6.66 ± 0.39	5.27 ± 0.08
Fru	1.79 ± 0.33	9.17 ± 0.15	
Gal	14.93 ± 0.2	35.56 ± 0.14	40.58 ± 0.6
Glc	62.98 ± 0.02	29.84 ± 0.31	35.06 ± 0.03
GalA		1.10 ± 0.18	9.99 ± 0.03

3.2.2. Glycosidic linkage of LSP

The glycosidic linkage of LSP 1, 2 or 3 was determined by methylation method and analyzed by GC/MS. As shown in Table 4, the glycosidic linkages of LSP 1 were in coincidence with those of LSP 2. In LSP 1, 2 or 3, →4)-Ara-(→1, →6)-Gal-(→1, Glc-(→1 and →6)-Glc-(1→ was detected. However, →4)-Xyl-(→1 terminal was only found in LSP 1. Glucose exhibited two linking forms, which were Glc-(→1 and 6)-Glc-(1→, respectively. The glycosidic linkage of mannose in LSP 1, 2 or 3 was significantly different. LSP 3 showed →3)-Man-(1→ and →6-Man-(1→, while LSP 2 only had →3)-Man-(1→ and LSP 1 only exhibited →6-Man-(1→. Fuc and Fru were found to be linked as →3)-Fuc-(→1 and →3)-Fru-(1→. Only two types of glycosidic linkage were found for Gal, which were →6)-Gal-(1→ and →3)-Gal-(1→. As GalA residue could not be linked as→6)-Gal-(1→, due to the uronic acid group, the glycosidic linkage of GalA was determined to be →3)-GalA-(1→ (Jiang et al., 2009; Yang et al., 2009). Further analysis of glycosidic linkage indicated that LSP 1, 2 or 3 had similar linkage types, but they were significant differences in the relative mole percentages. →4)-Ara-(→1, Glc-(→1 and 6)-Glc-(1→ were the major linking types for LSP 1 and →6)-Gal-(→1, Glc-(→1 and 6)-Glc-(1→ were the major linking types for LSP 2, while →3)-Gal-(→1, Glc-(→1 and 6)-Glc-(1→

Table 4
The relative molar percentage of glycosidic linkages in LSPs 1, 2 and 3.

Glycosidic linkages	LSP 1	LSP 2	LSP 3
→4)-Ara-(→1	9.25	8.47	7.38
→3)-Fuc-(→1	1.50	9.81	
→4)-Xyl-(→1	2.42		
→3)-Man-(→1	5.65	1.79	3.71
→6)-Man-(→1		4.87	1.56
→5)-Rha-(→1		1.02	
→3)-Fru-(→1	1.79	9.17	
→3)-Gal-(→1	6.41	1.10	13.18
→6)-Gal-(→1	8.52	35.56	37.39
Glc-(→1	9.82	13.02	12.23
→6)-Glc-(→1	53.16	16.82	22.83

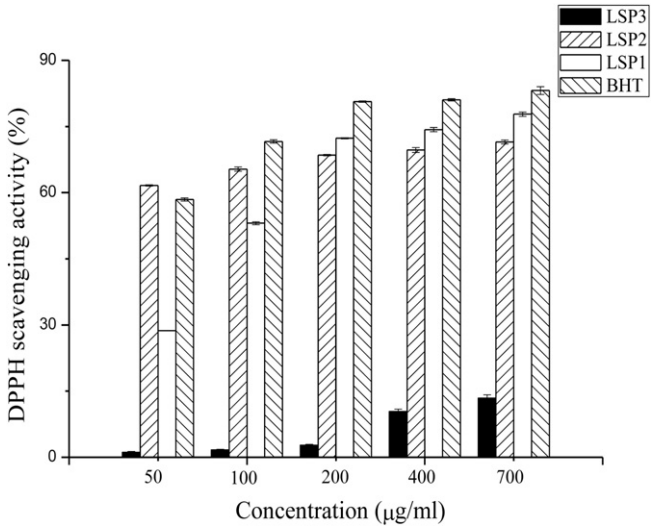

Fig. 2. DPPH radical scavenging activities of LSPs. Each value represents the mean ± standard deviation ($n = 3$).

were the main linkages for LSP 3. The major difference between LSP 2 and LSP 3 was LSP 3 did not have →3)-Fuc-(→1, →5)-Rha-(→1 and →3)-Fru-(→1, but higher →3)-Man-(→1, →3)-Gal-(→1 and →6)-Glc-(→1. This maybe relate to LSP 2 was a crude polysaccharide. →6)-Gal-(→1, was also detected as an important component of longan pericarp polysaccharides (Yang et al., 2009). Moreover, Glc had one linkage Glc-(1→, which indicated that glucose was the first monosaccharide of the backbone.

3.3. DPPH radical scavenging activity of LSP

For investigation of the radical scavenging activity of LSP, the DPPH radical scavenging assay was used. This assay is a widely accepted tool to evaluate the free radical scavenging activity of antioxidant (Jiang et al., 2009; Yuan et al., 2005). In this experiment, the DPPH radical scavenging activities of LSP solutions at different concentrations were measured. As shown in Fig. 2, the DPPH radical scavenging activity increased with increasing LSP concentration up to 700 (g/ml, while LSP 3 showed a lower antioxidant activity than LSP 1 and LSP 2. This result was similar to the early report that the crude polysaccharide had better antioxidant effects than the purified components of the polysaccharide (Chen, Xie, Nie, Li, & Wang, 2008). Lo et al. (2011) reported that the monosaccharide compositions such as Ara, Man, and Glu in the polysaccharide, →6)-Glc-(→1 and →4)-Ara-(→1 linkages and the side chain structure in the monosaccharide (mainly Rha) played an important role in the scavenging DPPH radicals. The hydroxyl groups from the specific monosaccharide compositions and their side chain linkages of polysaccharides can act as electron donors to bind radicals and radical ions, and, consequently, terminate radical chain reactions (Chen et al., 2008; Li, Li, & Zhou, 2007). According to the experimental results and previous literature, it could be inferred that a lower DPPH radicals scavenging activity of LSP 3 than LSP 2 might be related to the removed some heterogeneous polysaccharides in the purification process, which had higher Ara, Man, and Rha content and →4)-Ara-(→1 linkages.

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 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 (Sentandreu, Navarro, & Sendra, 2008). It is well accepted that the antioxidant activity of polysaccharides is highly related to their structural charccteristic (Rao & Muralikrishna, 2006; Yang, Zhao, Prasad, Jiang, & Jiang, 2010), such as molecular weight and composition of monosaccharide (Tsiapali et al., 2001). However, it is still unclear about the relationship between the polysaccharides structural characteristics and the antioxidant activity. The antioxidant activity of polysaccharides might be a combination of several factors. In this study, the LSP exhibited a dose-dependent behavior on DPPH radical scavenging activity within the tested concentration range, which might be attributable to its hydrogen-donating ability.

4. Conclusions

In general, the response surface methodology was proven to be useful for optimizing polysaccharide extraction from longan seeds, for which a mathematical model with high fitness was constructed. The monosaccharide compositions and glycosidic linkages of LSP 1, 2 and 3 were determined. Ara, Man, Gal and Glc were detected in LSP 1, 2 and 3. →6)-Gal-(→1, Glc-(→1 and →6)-Glc-(1→ was the major glycosidic linkages of LSP 1, 2 and 3. The LSP exhibited a dose-dependent behavior on DPPH radical scavenging activity within the tested concentration range. Furthermore, LSP 2 had a better DPPH radical scavenging activity than LSP 1 at a low concentration. Further work will be conducted to elucidate the structure/bioactivity relationship for LSP in the future.

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