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ScienceDirect
Food Chemistry 106 (2008) 685–690
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp

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Received 16 April 2007; received in revised form 15 June 2007; accepted 20 June 2007

Abstract

Ultrasonic technique was employed to extract polysaccharides from longan fruit pericarp (PLFP). The optimal conditions for ultrasonic extraction of PLFP were determined by response surface methodology. Box–Behnken design was applied to evaluate the effects of three independent variables (ultrasonic power, time and temperature) on the recovery and 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of PLFP. The correlation analysis of two mathematical-regression models indicated that quadratic polynomial model could be employed to optimize the ultrasonic extraction of PLFP. From response surface plots, ultrasonic power, time and temperature exhibited independent and interactive effects on the extraction of PLFP. The DPPH radical scavenging activity of PLFP could be improved by application of various ultrasonic power, time and temperature, which was possible due to the degradation of polysaccharides to different extent. The optimal conditions to obtain the highest recovery and the strongest DPPH radical scavenging activity of PLFP were 120 W, 22 min and 60 °C, as well as 241 W, 18 min and 51 °C, respectively. Under these optimal conditions, the experimental values agreed with the predicted ones by analysis of variance. It indicated high fitness of two models used and the success of response surface methodology for optimizing PLFP extraction.

Keywords: Longan; Polysaccharide; Ultrasonic extraction; Response surface methodology; DPPH radical scavenging activity

1. Introduction

Longan (*Dimocarpus longan* Lour.) is an important fruit in Southeast Asia (Jiang, Zhang, Joyce, & Ketsa, 2002). Longan fruit pericarp contains a significant amount of polysaccharides. A great deal of attention has been paid to polysaccharides for their unique biological, chemical and physical properties in recent years (Schepetkin & Quinn, 2006), and useful applications in developments of therapeutic drugs in modern medicine (Li, Zhou, & Han, 2006).

Ultrasonic treatment has been widely employed to extract polysaccharides from different plant materials (Hromadkova & Ebringerova, 2003), because ultrasonic treatment has mechanical effects that facilitate mass transfer between immiscible phases through a super agitation, especially at low frequency (Vinatoru et al., 1997). However, ultrasonic wave has degradation effects on polysaccharides. The changes in structure and degradation of polysaccharides depend on power and operating parameters (Mislovicova, Masarova, Bendzalova, Soltes, & Machova, 2000; Zhou & Ma, 2006).

The formation of some diseases, such as cancer, can be directly induced by free radicals, while the radical scavenging activity is one of the important functional properties for bioactive compounds (Athukorala, Kim, & Jeon,

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0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.06.031

2006). The DPPH radical scavenging activity is often used to evaluate the capacity of antioxidant compounds (Prior & Cao, 1999). Recent studies demonstrated that the antioxidant activity of polysaccharides was related to their degree of polymerization and structure (Chen & Yan, 2005). Under various ultrasonic conditions, the molecular weight and structure of PLFP would be modified, which influenced the DPPH radical scavenging activity.

The objective of this study was to investigate the effect of ultrasonic technique on polysaccharide extraction yield and bioactivity of polysaccharides during the extraction process. Response surface methodology is a statistical method that uses quantitative data from an appropriate experimental design to determine or simultaneously solve multivariate equation (Triveni, Shamala, & Rastogi, 2001). Besides, this experimental methodology can generate a mathematical model (Baş & Boyacı, 2007). In this study, ultrasonic technique was employed to extract polysaccharides from longan fruit pericarp (PLFP). Response surface methodology was used to evaluate the effects of ultrasonic power, time and temperature on the recovery and DPPH radical scavenging activity of PLFP to obtain the optimal extraction conditions.

2. Materials and methods

2.1. Materials

Fresh longan fruits (*Dimocarpus longan* Lour. cv. Shixia) at the commercially mature stage were purchased from a commercial market in Guangzhou, China. Fruits were selected for uniformity of shape and colour.

2.2. Chemicals

DPPH was purchased from Sigma chemical company (St. Louis, MO, USA). Glucose, phenol and sulphuric acid were obtained from Guangzhou Reagent Co. (Guangzhou, China). All other chemicals used were of analytical grade.

2.3. Extraction and quantification of PLFP

Pericarp tissues (4 g) of longan fruit were immersed into 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 filtered through a Whatman No. 1 filter paper and the filtrate was then concentrated to 25 ml with a rotary evaporator at 65 °C under vacuum. The proteins in the extract were removed using the Sevag reagent (Navarini et al., 1999). After removal of the Sevag reagent, 100 ml of anhydrate ethanol was added, then the mixture was kept in a beaker overnight at 4 °C to precipitate polysaccharides. PLFP was obtained by centrifugation at 3860*g* for 15 min. The content of polysaccharides was determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Glucose was used to construct a standard curve. The recovery of PLFP was expressed as mg of glucose equivalents (GE) per gram of longan fruit pericarp on dry weight (DW) basis.

2.4. Assay of DPPH radical scavenging activity

The DPPH radical scavenging activity was measured by the method of Yang et al. (2006). PLFP extract was dissolved in 10 ml of distilled water to a final concentration of 100 μg/ml. Two millilitre of 0.2 mM DPPH in ethanol was added to 1 ml of the PLFP solution. The absorbance was measured at 517 nm after 20 min of incubation at 25 °C. Distilled water was used as the control. The scavenging activity of DPPH radicals by the sample was calculated according to the following equation: DPPH radical scavenging activity (%) = (1 − absorbance of sample/absorbance of control) × 100.

2.5. 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. A Box–Behnken design with three variables (Box & Behnken, 1960) was used 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), with three levels of each variable, while the dependent variables were the recovery and DPPH radical scavenging activity of PLFP, respectively. The symbols and levels are shown in Table 1. Five replicates at the centre of the design were used to allow for estimation of a pure error sum of squares. Experiments were randomised to maximise the effects of unexplained variability in the observed responses due to extraneous factors. A full quadratic equation or the diminished form of this equation, shown as follows, was used for this model

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j, \quad (1)$$

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

3. Results and discussion

3.1. Effects of ultrasonic power, time and temperature on the recovery of PLFP

The mechanism of ultrasonic extraction involves two processes of physical activity: the dissolution of the extractive substances near the particle surface (rinsing) and the diffusion from the solid particles to the bulk of the liquid extract (slow extraction) (Vinatoru, 2001). The effects of

Table 1
Box–Behnken design and the responses for the recovery and DPPH radical scavenging activity of PLFP

Experiments	Coded levels			Responses	
	*X*1	*X*2	*X*3	Recovery (mg GE/g DW)	DPPH radical scavenging activity (%)
	Ultrasonic power (W)	Ultrasonic time (min)	Ultrasonic temperature (°C)		
1	−1 (120)	0 (20)	−1 (30)	5.02	23.3
2	−1 (120)	−1 (10)	0 (45)	4.66	23.9
3	−1 (120)	0 (20)	+1 (60)	5.43	29.6
4	−1 (120)	+1 (30)	0 (45)	4.95	28.0
5	0 (210)	−1 (10)	−1 (30)	4.19	23.5
6	0 (210)	−1 (10)	+1 (60)	4.49	33.4
7	0 (210)	0 (20)	0 (45)	4.25	36.2
8	0 (210)	0 (20)	0 (45)	4.16	38.6
9	0 (210)	0 (20)	0 (45)	4.35	37.8
10	0 (210)	+1 (30)	+1 (60)	4.06	34.9
11	0 (210)	+1 (30)	−1 (30)	3.64	33.2
12	0 (210)	0 (20)	0 (45)	4.14	36.4
13	0 (210)	0 (20)	0 (45)	4.26	39.9
14	+1 (300)	0 (20)	+1 (60)	3.82	32.9
15	+1 (300)	−1 (10)	0 (45)	3.89	38.6
16	+1 (300)	+1 (30)	0 (45)	3.36	30.4
17	+1 (300)	0 (20)	−1 (30)	3.60	28.0

ultrasonic power, time and temperature on the recovery of PLFP as well as their interactions are shown in Fig. 1. The extending ultrasonic time could result in a higher extraction recovery. However, the recovery of PLFP decreased with the extension of ultrasonic time when a high ultrasonic power was used, which was possibly due to the degradation of polysaccharides by ultrasonic wave. According to the reports of Li, Guo, and Li (2005) as well as Sivakumar and Pandit (2001), application of high ultrasonic power results in degradation effect. At high ultrasonic temperature, the liquid viscosity and density decreases and has fast mass transfer (Hemwimol, Pavasant, & Shotipruk, 2006). Furthermore, high ultrasonic temperature leads to the increase of cavitation bubble number and surface contact area (Palma & Barroso, 2002). Thus, an appropriate high ultrasonic temperature can enhance the extraction efficiency.

3.2. Effects of ultrasonic power, time and temperature on the DPPH radical scavenging activity of PLFP

The effects of ultrasonic power, time and temperature on the DPPH radical scavenging activity of PLFP as well as their interactions are shown in Fig. 2. As shown in Fig. 2a, the DPPH radical scavenging activity of PLFP increased with the extension of ultrasonic time at low ultrasonic power, but decreased at high ultrasonic power. When the ultrasonic time and temperature were kept constant within the range under investigation, the DPPH radical scavenging activity of PLFP increased to a value with elevating ultrasonic power, thereafter decreased (Fig. 2a and b). The similar phenomenon was also found for ultrasonic temperature used in this study (Fig. 2b and c).

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods (Yuan et al., 2005). This method is based on the reduction of ethanolic $DPPH^-$ solution in the presence of a hydrogen donating antioxidant, leading to the formation of non-radical form DPPH-H. The polysaccharide extract is able to reduce the stable radical $DPPH^-$ to yellow-coloured diphenylpicrylhydrazine. The positive correlation between polysaccharide concentration and its antioxidant activity is well documented (Li, Li, & Zhou, 2007). The antioxidant activity of polysaccharides is high related to their chemical structure (Rao & Muralikrishna, 2006). In this study, ultrasonic power, time and temperature showed apparent influences on the DPPH radical scavenging activity of PLFP. The possible mechanism should be the degradation of PLFP and further changes in chemical structure induced by ultrasonic treatment.

3.3. Model fitting

The mathematical models representing the recovery and DPPH radical scavenging activity of PLFP as a function of the independent variables within the region under investigation were expressed by the following equation:

$$Y_1 = 4.23 - 0.67X_1 - 0.15X_2 + 0.17X_3 - 0.21X_1X_2 - 0.048X_1X_3 + 0.03X_2X_3 + 0.18X_1^2 - 0.19X_2^2 + 0.058X_3^2 \quad (2)$$

$$Y_2 = 37.78 + 3.14X_1 + 0.89X_2 + 2.85X_3 - 3.08X_1X_2 - 0.35X_1X_3 - 2.05X_2X_3 - 5.18X_1^2 - 2.38X_2^2 - 4.15X_3^2, \quad (3)$$

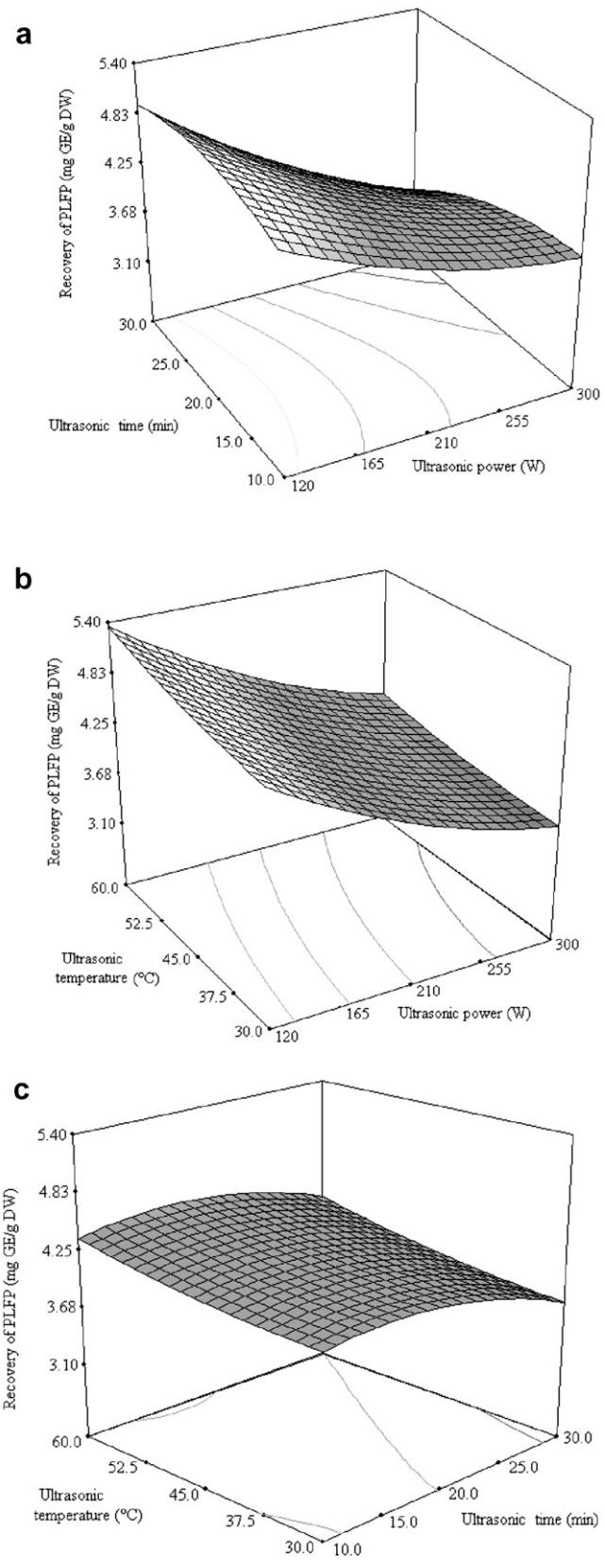

Fig. 1. Response surface plots showing effects of ultrasonic power, time and temperature on the recovery of PLFP and their interaction. (a) The ultrasonic temperature was constant at 45 °C. (b) The ultrasonic time was constant for 20 min. (c) The ultrasonic power was constant at 210 W.

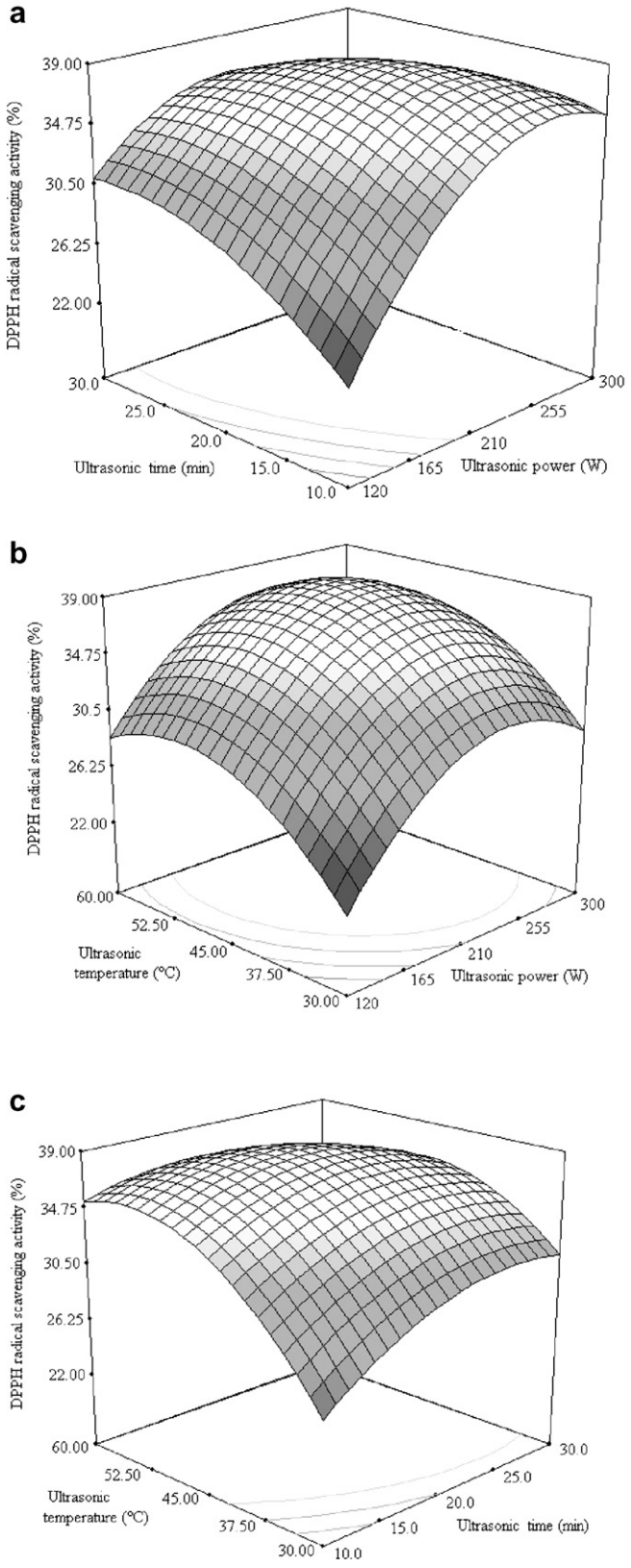

Fig. 2. Response surface plots showing effects of ultrasonic power, time and temperature on the DPPH radical scavenging activity of PLFP. (a) The ultrasonic temperature was constant at 45 °C. (b) The ultrasonic time was constant for 20 min. (c) The ultrasonic power was constant at 210 W.

Table 2
Analysis of variance for the response surface quadratic model for the recovery and DPPH radical scavenging activity of PLFP

Source	Degrees of freedom	Sum of squares	Mean square	*F*-value	*P*-value
The recovery					
Model	9	4.52	0.50	22.78	0.0002
Residual	7	0.15	0.022		
Lack of fit	3	0.13	0.042	5.84	0.0606
Pure error	4	0.029	0.007		
Total	16	4.67			
The DPPH radical scavenging activity					
Model	9	436.21	48.47	6.90	0.0093
Residual	7	49.20	7.03		
Lack of fit	3	39.63	13.21	5.52	0.0661
Pure error	4	9.57	2.39		
Total	16	485.42			

where Y_1 and Y_2 are the recovery and DPPH radical scavenging activity of PLFP, respectively, whereas X_1, X_2 and X_3 are the coded variables for ultrasonic power, time and temperature, respectively.

In general, exploration and optimization of a fitted response surface may produce poor or misleading results unless the model exhibits a good fit, which checks the model adequacy essential (Liyana-Pathirana & Shahidi, 2005). The *P*-values of two models for the recovery and DPPH radical scavenging activity of PLFP were 0.0002 and 0.0093 (Table 2), respectively, which indicated that the fitness of both models were significant. However, the fit values of two models exhibited 0.0606 and 0.0661, respectively, without significant difference.

Coefficient (R^2) of determination is defined as the ratio of the explained variation to the total variation and is a measurement of the degree of fitness (Nath & Chattopadhyay, 2007). The small value of R^2 indicates the poor relevance of the dependent variables in the model. The model can fit well with the actual data when R^2 approaches unity (Sin, Yusof, Hamid, & Rahman, 2006). Analysis of variance, the R^2 values of the two models for the recovery and DPPH radical scavenging activity of PLFP were determined to be 0.967 and 0.899, respectively, which showed that the regression models defined well the true behavior of the system.

By prediction of computing program, the optimal conditions to obtain the highest recovery and DPPH radical scavenging activity of PLFP were determined as follows: 120 W, 22 min and 60 °C, and 241 W, 18 min and 51 °C, respectively. After extraction of PLFP under these optimal conditions, the recovery and DPPH radical scavenging activity of PLFP were 5.47 ± 0.16 mg GE/g DW and $38.72 \pm 0.19\%$, but they were not significantly different to predicted values 5.37 mg GE/g DW and 38.78% within 95% confidence interval.

4. Conclusions

The high correlation of two mathematical models indicated that quadratic polynomial model could be employed to optimize ultrasonic extraction process and DPPH radical scavenging activity of PLFP. From response surface plots, three factors (ultrasonic power, time and temperature) significantly influenced the extraction efficiency of PLFP, independently and interactively. The optimal conditions to obtain the highest recovery and strongest DPPH radical scavenging activity of PLFP were determined to be 120 W, 22 min and 60 °C, as well as 241 W, 18 min and 51 °C, respectively. Under the optimal conditions, the experimental values agreed with the predicted values by analysis of variance. Thus, this methodology could provide a basis for the model to search for non-linear nature between independent variables and response in a short-term experiment.

Acknowledgements

The financial support provided by National Natural Science Foundation of China (Grant No. 30425040), Eleventh-five-year National Key Technology R&D Program (No. 2006BAD27B03), and Guangzhou Scientific Research Foundation (No. 2004Z1-E0061 and 2005Z2-E0151) was appreciated.

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