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EXTRACTION AND ANTIOXIDATION OF POLYSACCHARIDE FROM *PORPHYRA HAITANENSIS* USING RESPONSE SURFACE METHOD

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Abstract

This paper deals with the preparation and antioxidation of polysaccharide from *Porphyra haitanensis*. The ratio of water to raw material, extraction temperature and extraction time were taken in sequence as independent variables in single factor test, and polysaccharide yield as response value. Using Box-Benhnken central combination experimental design principles and response surface methodology, interactions of variables and their influence on polysaccharide yield of *P. haitanensis* were studied and the prediction model of quadratic polynomial regression equation was inferred by simulation, in which the optimum parameters for preparing polysaccharide from *P. haitanensis* were 88.4°C of extraction temperature, 1.97 h of extraction time and 40:1 (ml/g) of ratio of water to raw material, and polysaccharide of 15.19 % in yield from *P. haitanensis* was verified after two parallel test. Furthermore, the polysaccharide of *P. haitanensis* showed good antioxidant capacity which could be used as potential natural antioxidant products in food additives industries.

Key words: *Porphyra haitanensis*; Polysaccharide extraction; Antioxidant activity.

Introduction

Porphyra haitanensis, commonly known as Wucai, is a kind of important economic seaweed mainly distributed in Zhejiang and Fujian Provinces in China. As the main content of *P. haitanensis*, polysaccharide has anticoagulation (Zhang *et al.*, 2010a), antianaphylaxis (Shi *et al.*, 2015), anti-oxidation(Ying *et al.*, 2015; Zhang *et al.*, 2009a) and prolonging life span(Zhao *et al.*, 2008) activities. Moreover, the antioxidant capacity of *P. haitanensis* polysaccharide would be enhanced after chemical modification (Zhang *et al.*, 2004; Zhang *et al.*, 2009b; Zhang *et al.*, 2010b; Zhao *et al.*, 2006) to protect human body against harmful reactive oxygen species (Wang *et al.*, 2016a). Therefore, this polysaccharide has potential in food additives as natural antioxidant.

Different extraction methods could affect the yield of polysaccharide from *P. haitanensis*. Traditional procedures include water extraction and alcohol precipitation, ultrafiltration and ultrasonic-assisted extraction (Zhang *et al*., 2004; Zhang *et al*., 2009a; Zhao *et al*., 2006). These processes proved to be suitable due to their simplicity and safety. In this work, we improved the hot-water extraction process of *P. haitanensis* using Box-Behnken design, a type of response surface methodology, which has been used in polysaccharides preparation (Chen *et al.*, 2015; Pu *et al.*, 2015; Sharma *et al.*, 2016). The purified polysaccharides were then conducted for antioxidant assays.

Material and Methods

P. haitanensis was collected from Fuding sea area, Fujian Province. The total antioxidant capacity assay kit with FRAP method was from Beyotime Institute of Biotechnology, and hydroxyl radical kit from Nanjing Jiancheng Bioengineering Institute.

Polysaccharide extraction: 10 g of dried *P. haitanensis* powder were soaked in 75% alcohol for 12 h to remove pigment. After filtration and drying, distilled water was added to the dried powder followed by water bath at constant temperature. Extraction temperature, extraction time, and the ratio of water to raw material were selected according to experimental design. After centrifugation in 10,000 g for 20 min (Supra 22k High-speed Freezing Centrifuge, Korea, Hanil Science), the supernatant was precipitated in final ethanol concentration of 75% at 4°C for 24h and then centrifugated to collect sediment. The sediment was freeze-dried by vertical freeze-drying system (LabConco Co., US). Carbohydrate content in polysaccharide was detected by phenol-sulfuric acid method.

Polysaccharide extraction using response surface methodology: Based on the results of single factor experiment, orthogonal experiments of three factors and three levels were designed according to Box-Behnken central combination experimental design principles. And the extraction conditions of polysaccharides from *P. haitanensis* were optimized through response surface method. The zero level and fluctuating zone of three factors were selected based on the results of single factor experiment (Table 1).

Table 1. Design and results of response surface analysis.

No.	Level			Polysaccharide yield/%
	Extraction temperature/ °C (A)	Extraction time/h (B)	Ratio of water to raw materials /(g/ml) (C)	
1.	75	1	40	1.0072
2.	95	1	40	0.7791
3.	75	3	40	0.9557
4.	95	3	40	0.8138
5.	75	2	30	0.8814
6.	95	2	30	0.3712
7.	75	2	50	0.986
8.	95	2	50	1.5767
9.	85	1	30	1.0125
10.	85	3	30	1.1533
11.	85	1	50	1.2062
12.	85	3	50	0.9772
13.	85	2	40	1.1346
14.	85	2	40	1.2584
15.	85	2	40	1.4538
16.	85	2	40	1.6854
17.	85	2	40	1.7175

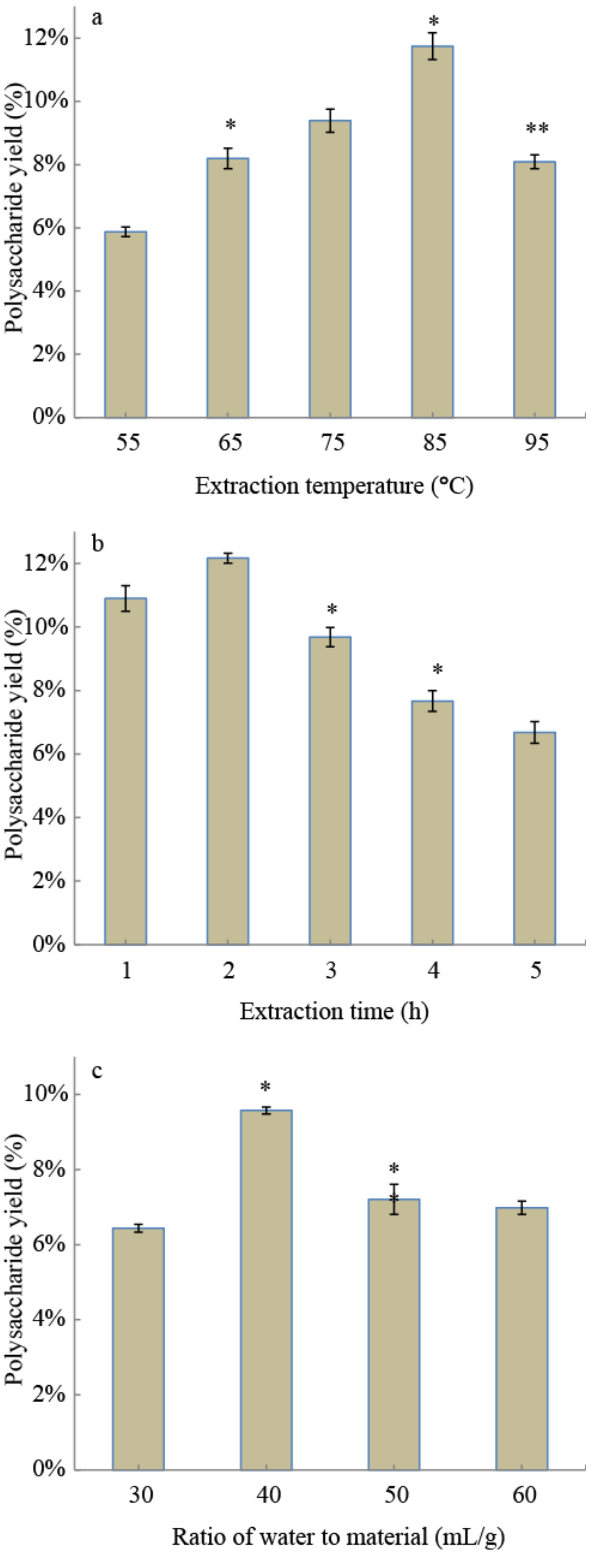

Fig. 1. Effects of single factor on yield of polysaccharides.
a. Influence of extraction temperature on yield under extraction timeof 3h and ratio of water to raw material (ml/g) of 40, **b.** Influence of extraction time on yield under extraction temperatureof 75°C and ratio of water to raw material (ml/g) of 40, **c.** Influence of ratio of water to raw material on yield under extraction time of 3 h and temperature of 75°C. Compare with the previous group, * means p<0.05, ** means p<0.01, n=3

Antioxidant capacity: Reducing power of polysaccharides was determined according to Wang *et al*'s method (Wang *et al.*, 2013), with some modifications. In brief, 1 mL of 0.5-3 mg/mL sample was well-mixed with 1 mL potassium ferricyanide (1%, w/v). After thermostatic waterbath at 50°C for 20 min, 2 mL trichloroacetic acid was added to terminate reaction. Finally, 1.2 mL ferric chloride was added and the absorbance at 700 nm was measured. Total antioxidant capacity of polysaccharide was detected according to the instructions of total antioxidant capacity assay kit (Muhammad *et al.*, 2017) while hydroxyl radical scavenging activity was detected using hydroxyl radical kit based on fenton reaction principles (Halliwell & Gutteridge, 1992).

Statistical analysis: For single factor experiment and antioxidation research, all data were shown as mean±standard deviation. Statistical differences between the experimental groups were determined by one way ANOVA, and differences were considered to be statistically significant or very significant if p<0.05, or p<0.01, respectively.

For response surface study, polysaccharide extraction test were designed using multiple linear regression analysis program of Design Expert 8.06 software, with second-order polynomial fitted. Then, using significance comparison (F test) of regression equation coefficients, the ratio of water to raw material, extraction temperature and extraction time on polysaccharide yield was studied. Finally, the yield was optimized based on response surface method.

Results and Discussion

Single factor analysis: The variation of polysaccharide yield along with extraction temperature was obtained under extraction timeof 3h and ratio of water to raw material (ml/g) of 40 (Fig. 1a). As temperature increased, more solvent was allowed to penetrate into raw material to rise the polysaccharides yield (Wang *et al.*, 2016b), which reached the maximum at 85°C followed by polysaccharides degradation (Thirugnanasambandham *et al.*, 2015). Therefore, 85°C was selected as zero level of extraction temperature (A) of independent variable.

Extraction time is another important factor affecting the extraction efficiency. Under extraction temperatureof 75°C and ratio of water to raw material (ml/g) of 40, the polysaccharide yield was increased, followed by a decline after 2 h (Fig. 1b) due to degradation of polysaccharides (Liu *et al.*, 2015), which was selected as zero level of extraction time (B).

Ratio of water to raw material would affect the yield of polysaccharides (Wang *et al*., 2016b). Under extraction time of 3 h and temperature of 75°C, the polysaccharide yield increased when the ratio of water to raw material rose (Fig. 1c). At ratio of 40,polysaccharide yield reached the maximum due to the increase of driving force for the mass transfer of the polysaccharides, which was selected as zero level (C) of ratio of water to raw material.

Table 2. Variance analysis of regression model.

Variation source	Quadratic sum	Variance	Mean square	F-Value	P- Value
Model	1.31	1	0.22	3.49	0.0397
A	0.01	1	0.01	0.17	0.6914
B	1.38E-03	1	1.38E-03	0.022	8.85E-01
C	0.22	1	0.22	3.51	0.0904
AC	0.3	1	0.3	4.83	0.0527
A^2	0.53	1	0.53	8.48	0.0155
B^2	0.21	1	0.21	3.3	0.0991
Residual	0.63	10	0.063		
Lack of fit	0.36	6	0.061	0.92	
Pure error	0.26	4	0.066		
Sum	1.94	16			

R^2=0.7435

Table 3. Test of significance of regression equation coefficient.

Coefficient terms	Regression coefficient	Variance	Standard error	Confidence lower limit	Confidence upper limit
Intercept	1.39	1	0.1	1.17	1.61
A	-0.036	1	0.089	-0.23	0.16
B	-0.013	1	0.089	-0.21	0.18
C	0.17	1	0.089	-0.031	0.36
AC	0.28	1	0.13	-3.82E-03	0.55
A^2	-0.36	1	0.12	-0.63	-0.083
B^2	-0.22	1	0.12	-0.49	0.05

Analysis from response surface: Based on the principle of Box-Behnken central combination design, three levels of each factor were taken in the experiment, which composed a total of 17 experimental points (Table 1). The regression model of response surface had achieved significant level (p<0.05) and the sequence of primary and secondary factors affecting the polysaccharide yield of *P. haitanensis* was C>A>B. In this model, A^2 had significant influence on yield of polysaccharide. As R^2=0.7435, this model had good fitting degree with experiment result (Table 2). The influence of each factor on response value was not simple linear relationship. After regression analysis, the influence of test factor on response value could be expressed as regression equation (Table 3):

$$Y=1.39-0.036A-0.013B+0.17C+0.28AC-0.36A^2-0.22B^2$$

The response surface with gentle slope in Fig. 2a shows that the yield is not sensitive to the variations of extraction temperature and time. The slope angle of response surface is not so steep, indicating that there is a wide suitable range of extraction temperature and time. Fig. 2b shows that slope angle of response surface is relatively steep, indicating that yield is sensitive to the variations of extraction temperature and ratio of water to raw material. From response surface, the ratio of water to raw material has larger influence on the polysaccharide yield of *P. haitanensis*. In Fig. 2c, the slope of response surface is a little gentle, showing that yield is some sensitive to the interaction of ratio of water to raw material and extraction time. Based on the contour, response value is significantly affected by the variation of ratio of water to raw material.

The predicted maximum yield of polysaccharide was 15.94%, and corresponding values of factors were 88.4°C, 1.97 h and 40 ml/g, respectively. Under such optimal conditions, two parallel verification experiments were conducted, and the average polysaccharide yield was 15.19%, which was similar to the predictive value.

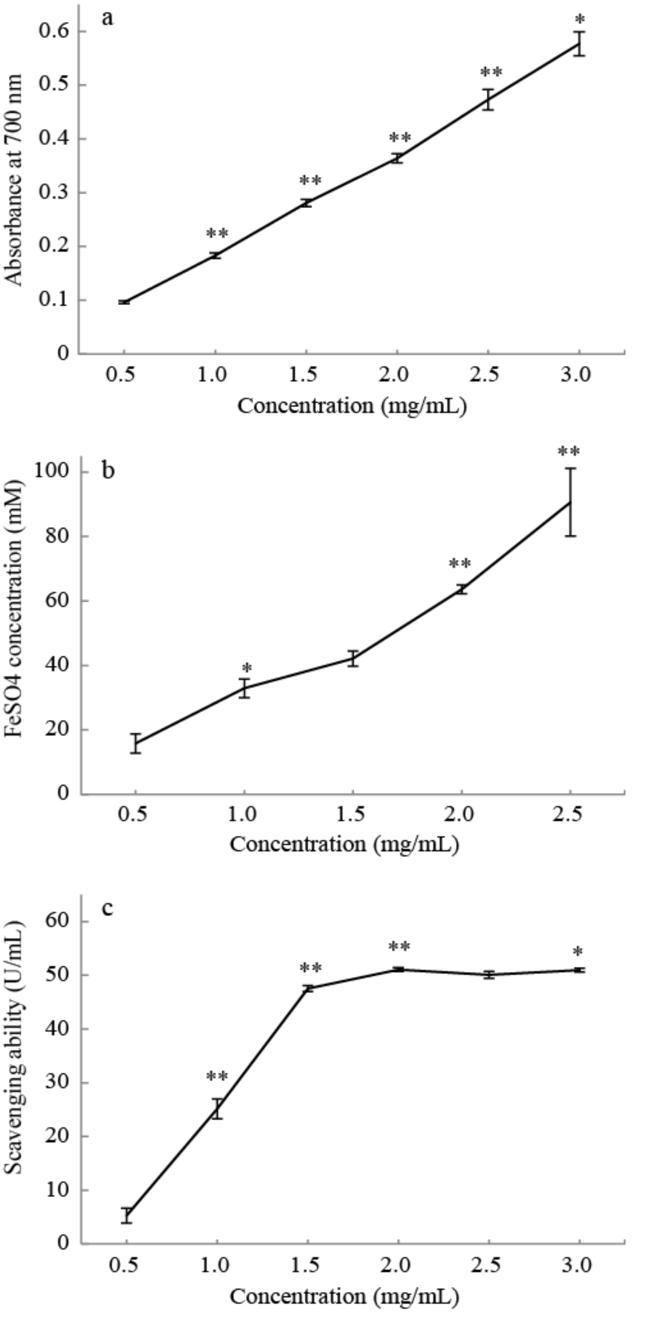

Fig. 3 Antioxidant capacity of polysaccharides using different methods. a. reducing power, b. antioxidant capacity, c. scavenging of hydroxyl radical. compare with blank control, * means p<0.05, ** means p<0.01. n=3

Detection of antioxidant capacity: With the rise of concentration, reducing power, which is in line with electron transfer capability (Khaskheli *et al.*, 2015), and antioxidant capacity of polysaccharide (Jiang *et al.*, 2015) increased linearly (Fig. 3a). The antioxidant capacity of polysaccharide reached the maximum value (Fig. 3b) at the concentration of 2.5mg/mL polysaccharide. As a kind of main reactive oxygen, hydroxyl radicals can destroy all the adjacent biomolecules in living cell (Chen *et al.*, 2016), while scavengers will protect deoxyribose from damage(Khaskheli *et al.*, 2015). It was indicated that polysaccharide of *P. haitanensis* has strong scavenging activity to hydroxyl radical. Within the range of 0.5-1.5mg/mL, the polysaccharide has good dose-dependent effect (Fig. 3c).

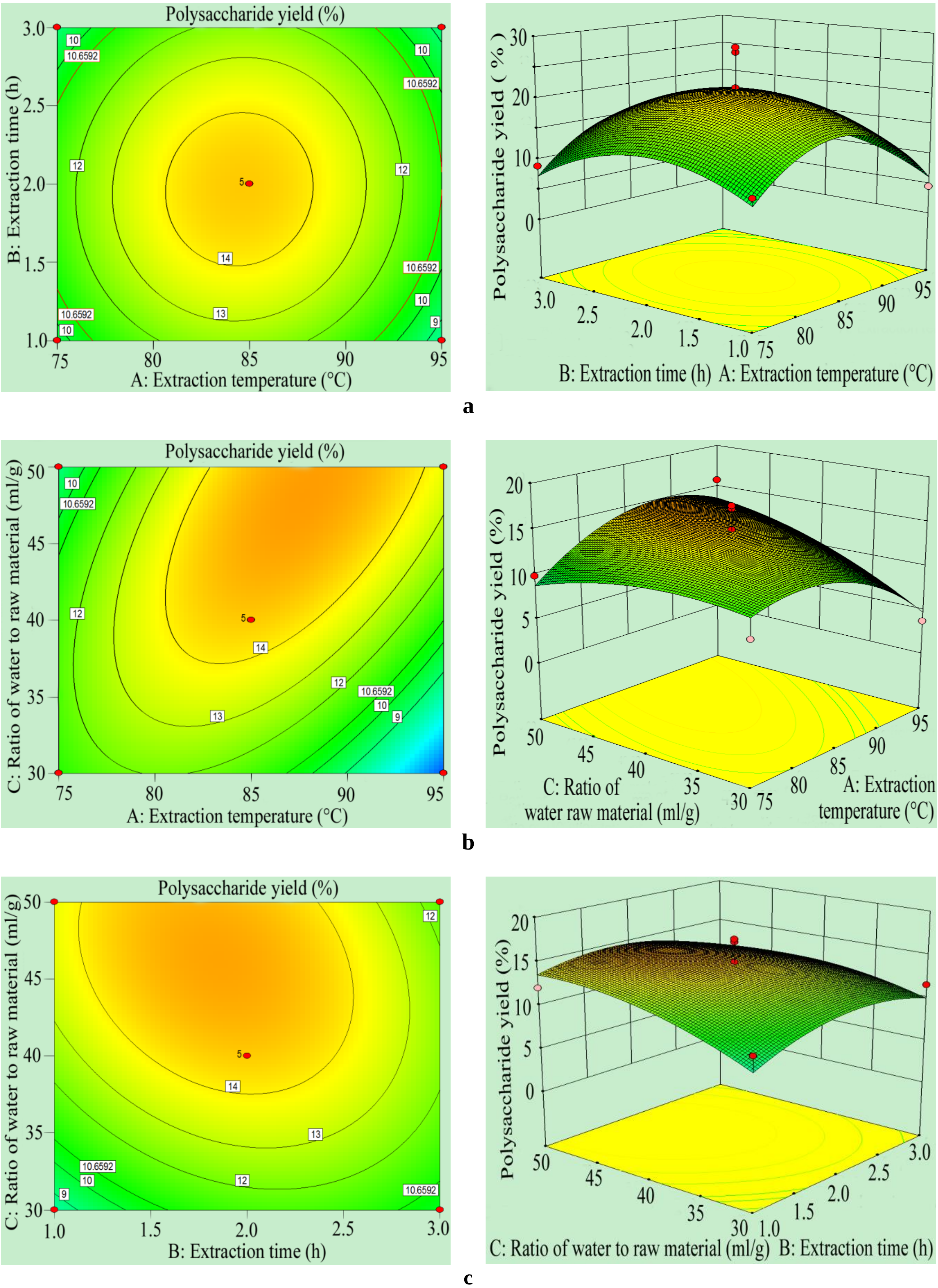

Fig. 2. The graph of response surface methodology including 3D and corresponding contour line.
a. Extraction temperature and time, C: Ratio of water to raw material (ml/g) =40; **b.** Extraction temperature and ratio of water to material, B: Extraction time (h)=2; c. extraction time and ratio of water to material, A: Extraction temperature (°C)=85.

Conclusions

The extraction process of polysaccharide from *P. haitanensis* was optimized using response surface method and the optimum parameters for preparing polysaccharide were 88.4°C of extraction temperature, 1.97 h of extraction time and 40:1 (ml/g) of ratio of water to raw material, and polysaccharide of 15.19% in yield from *P. haitanensis* was verified after two parallel test. Furthermore, the polysaccharide of *P. haitanensis* showed good antioxidant capacity which could be used as potential natural antioxidant products in food additives industries.

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