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Extraction of Polysaccharides from *Saccharomyces cerevisiae* and its Immune Enhancement Activity

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Abstract: Microbial polysaccharides are located both inside the fungal cell walls and on the fungal cell surfaces and possess marked immunological properties. In this study, the extraction conditions of *Saccharomyces cerevisiae* polysaccharides (SCPS) were optimized using response surface methodology and the immune enhancement properties of SCPS were evaluated. The results indicated that the optimal extraction conditions were high pressure homogenization time of 35 min, ultrasonic power of 510 W and ultrasonic time of 26 min, respectively. Under these conditions, the maximal observed value extraction yield of *S. cerevisiae* polysaccharides (SCPS) was (29.84±0.09)%, which was agreed with predicted value 29.82%. Thin layer chromatography (TLC) exhibited SCPS may be a glucan. Pharmacological experiments indicated that SCPS could increase alkaline phosphatase (AKP), lysozyme (LZM), immunoglobulin A (IgA), immunoglobulin G (IgG) and Epidermal Growth Factor (EGF) levels in serum, increase secreted immunoglobulin A (SIgA) expression in jejunum secretion and decrease prostaglandin E_2 (PGE_2) expression in colon of immune-compromised rats at medium-dose. *Saccharomyces cerevisiae* polysaccharides has significant immune enhancement activity and could obviously protect intestine mucosa of immune-compromised rats.

Key words: Polysaccharides, extraction, immunoassay, response surface methodology, *Saccharomyces cerevisiae*

INTRODUCTION

Polysaccharides are very important class of biopolymers, which consist of long chains of repeating sugar units. Polysaccharides are widespread in many bacteria, fungi, mushrooms, algae and higher plants and have attracted attention because of bioactive and medicinal properties, such as immune-stimulating, anti-inflammatory, antimicrobial, anti-infective, antiviral, antitumor, anti-aging, antioxidant, anti-radiation, wound-healing and relatively low toxicity (Li *et al*., 2006; Caridi, 2007; Chattopadhyay *et al*., 2008; Dai *et al*., 2009; Sun *et al*., 2009; Wang *et al*., 2013). Various polysaccharides can not only activate immunocytes (Liu *et al*., 2006), but can improve secretion of cytokine. And through different approaches they activate the complement system and the reticuloendothelial system etc., thus regulate immunologic function of organism (Lai *et al*., 2010).

Yeasts are unicellular fungi and are used for baking and ethanol production for thousands of years. Nowadays, the worldwide production exceeds 2.5 million tons (Freimund *et al*., 2003). The inner parts of the cells are isolated and subsequently used as food supplements and flavor enhancers due to their high amounts of proteins and nucleotides. The outer parts of the yeast cells, the cell walls, remain as waste for which so far no commercial use has been established except as a supplement for animal feed. The cell wall of the yeast *Saccharomyces cerevisiae* is composed of a 10 nm thick layer of polysaccharides (predominantly glucans and mannoproteins) and serves as the interface between the

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cell and the neighbouring environment (Giovani *et al*., 2010). The cell walls are therefore an ideal raw material for the manufacture of polysaccharides. The polysaccharide yield is related to the ratio of broken yeast cells (Liu *et al*., 2007). In addition to safety considerations, the commercial acceptability of novel polysaccharides from yeasts will be determined by economic factors such as the yield of product. The amount of cell wall produced by the yeast and the amount of specified polysaccharides that can be extracted from the wall will govern these economics (Nguyen *et al*., 1998). But disrupting yeast cell walls is not easy due to its unique structure.

S. cerevisiae polysaccharides (SCPS) have been previously demonstrated to antitumor (Kogan and Kocher, 2007), antioxidant (Kogan *et al*., 2005), nutrition (Khalikova *et al*., 2005). Hot water technology is the main and most conventional extraction method for polysaccharides mentioned in recent studies (Yan *et al*., 2011). Extraction of polysaccharides is essential and important for its further research. Therefore, efficient extraction conditions for SCPS are needed to be optimum. However, so far there is no published information on the optimization of extraction conditions of SCPS for further application. Response Surface Methodology (RSM) is an effective mathematical and statistical technique used to optimize the conditions in pharmaceutical and food research. It can explore the interaction between the variables to obtain an optimal response (Xiong *et al*., 2009; Zhong *et al*., 2010; Gan and Latiff, 2011; Zhao *et al*., 2011).

The objectives of this study were to optimize the conditions for the extraction of SCPS using RSM design. It may facilitate a deeper understanding of the process of polysaccharide extraction from *S. cerevisiae* to provide theoretical references. Besides, the immune regulating activities of polysaccharides from *S. cerevisiae* were also investigated.

MATERIALS AND METHODS

Yeast strain and culture conditions: Yeast strain used in this study was *Saccharomyces cerevisiae*, which was provided by China General Microbiological Culture Collection Center (CGMCC) and the preservation number is CGMCC No. 3156. *S. cerevisiae* was cultured in MWU medium (8°Bx malt wort, 0.3% urea) at 30°C for 24 h in an orbital shaker (ZHWY-2102C, ZHICHENG, Shanghai, China) at 200 r min^{-1}. Then the culture medium was centrifuged at 4,000 r min^{-1} for 10 min; the precipitate was dried using Freeze Drying Equipment (K4, Edwards, UK); the *S. cerevisiae* powder was then obtained and stored in a dry and dark place.

Extraction of SCPS: *Saccharomyces cerevisiae* powder samples (10 g) were dissolved in 30 mL deionized water and then the cell walls were broken by high pressure homogeniser (NS1001L NIRO-SOAVI, Italy) and ultrasonic generator (JY92-2D Xinzhi Bio-technology Institute, Shanghai, China). The high pressure homogenization was carried out at the pressure of 60 Mpa and pressure time of 15-55 min; ultrasonic was carried out at power of 300-700 W, ultrasonic time of 15-55 min and frequency of 25 kHz. After that, the sample was extracted with boiling deionized water (200 mL) for 2 h. The extraction process was repeated three times. The extracts were combined, left to cool at room temperature and filtered. Added 150 mL of 95% ethanol (AR 500 mL, Tianjin Kaixin Chemical Industry Co., Ltd. China) for precipitation of polysaccharide at 4°C overnight, the precipitates were collected by centrifugation at 4,000 r min^{-1} for 10 min and washed with absolute ethanol (AR 500 mL, Tianjin Kaixin Chemical Industry Co., Ltd. China) then with acetone (AR 500 mL, Tianjin Kaixin Chemical Industry Co., Ltd. China), then freeze-dried. The Sevag method (York *et al*., 1986) was used to remove protein to obtain SCPS. The polysaccharide extraction yield (%) is calculated as follows:

$$\text{Extraction yield (\%)} = \frac{\text{weight of dried crude polysaccharide extraction (g)}}{\text{weight of powders (g)}} \times 100 \quad (1)$$

Response surface methodology is an empirical statistical technique employed for multiple regression analysis by using quantitative data obtained from properly designed experiments to solve multivariate equations simultaneously. Box-Behnken, a spherical and revolving design, has been applied in optimization of chemical and physical processes (Li *et al*., 2011; Maiti *et al*., 2011), because of its reasoning design and excellent outcomes. The purpose of the center points is to estimate the pure error and curvature.

On the basis of the single factor experimental results, three major influence factors and the ranges of each factors were confirmed as high pressure homogenization time of 25-45 min, ultrasonic power of 400-600 W and ultrasonic time of 15-35 min and then Box-Behnken Design (BBD) was conducted to design experimental protocol. The experiments with different high pressure homogenization time (X_1), ultrasonic power (X_2) and ultrasonic time (X_3), were employed simultaneously covering the spectrum of variables for the percentage extraction of SCPS in the BBD. In order to describe the effects of high pressure homogenization time (X_1), ultrasonic power (X_2) and ultrasonic time (X_3) on percentage of SCPS extraction, batch experiments were

conducted. Three factors chosen for this study were designated as X_1, X_2 and X_3 prescribed into three levels, coded +1, 0, -1 for high, intermediate and low value, respectively. The coded values of the extraction parameters were determined by the following equation:

$$Xi = \frac{x_t - x_o}{\Delta x},\ i = 1, 2, 3 \quad (2)$$

where, X_i is the coded value; x_i is the corresponding actual value; x_0 is the actual value of the independent variable at the center point and Δx is the step change of the variable.

The complete quadratic equation is used as follows:

$$Y = \beta 0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=0}^{3} \beta_{ii} X_i^2 + \sum_{i=0}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (3)$$

where, Y is the predicted response; X_i and X_j are the coded independent variables; β_0 is the intercept coefficient; β_i is the linear coefficient; β_{ii} is the squared coefficient and β_{ij} is the interaction coefficient. Analysis of the experimental design data and calculation of predicted responses were carried out using Design Expert software (version 8.05 b, Stat-Ease, Inc., Minneapolis, USA). And a statistical program in Design Expert software 8.05 b was used for regression analysis of the data obtained and to estimate the coefficient of the regression equation. The equations were validated by the statistical tests called the ANOVA analysis. The significance of each term in the equation is to estimate the goodness of fit in each case. Response surfaces were drawn to determine the individual and interactive effects of test variable on the response.

Monosaccharide composition analysis: *Saccharomyces cerevisiae* polysaccharides (200 mg) were hydrolyzed in 30 mL of 2 mol L^{-1} sulfuric acid (AR 500 mL, Tianjin Kaixin Chemical Industry Co., Ltd. China) for 24 h at 100°C in a sealed glass tube and then added deionized water to 100 mL. The residual acid was neutralized by $BaCO_3$ (AR 500 g, Tianjin Fu Chen Chemical Reagent Factory, China) and then centrifuged at 3000 r min^{-1} for 10 min; the supernatant was hydrolysis solution. Thin Layer Chromatography (TLC) glass plates (5×10 cm glass plates with 0.2 mm thick silica gel, Qingdao Ocean Chemical Plant, China) were activated at 115°C for 1 h before use. The glucose standard (Chromatographic Pure 100 mg, Shanghai Yuanye Bio-Technology Co., Ltd. China) and the hydrolysis of SCPS (2 μL per spot) were applied on the TLC plates which were developed in TLC chambers. The solvent systems [4:5:1 (v:v:v) N-butyl alcohol-acetone-water] were run to a height of 9 cm from the origin. After drying, the plates were sprayed with 5% sulfuric acid-ethanol solution and then heated in oven at 85°C for 10 min.

Experimental animals: Male SD rats of SPF-level (6-8 weeks old, weighing around 220 g) were provided by Experimental Animal Center of Lanzhou University (Animal use permit: SCXK20009-0004). Rats were housed and maintained under specific pathogen-free conditions and experiments were carried out according to protocols approved by the Institutional Animal Care and Use Committee of Lanzhou University.

Immune enhancement activity of SCPS in rats: A total of 120 rats were randomly divided into 5 groups. Groups division: groups I, II, III were the SCPS groups. Group IV was the model control group. Group V was the normal control group. Polysaccharide contents: rats were administered with SCPS at the doses of 200, 100 and 50 mg kg^{-1} body weight marked as I, II, III, respectively. Group IV and V contained no polysaccharide in distilled water. *Saccharomyces cerevisiae* polysaccharides groups were orally administrated daily with SCPS solution according to designed Dose; Normal Saline (NS) was orally administrated at the dose of 200 mL kg^{-1} body weight in the model control group and normal control group during 30 days. Intraperitoneal injection with cyclophosphamide (100 mg kg^{-1}) (Registration No: H14023686, Shanxi Powerdone Pharmaceutics Co., Ltd. China) was given to rats to cause an immune-compromised model on the 28th and 29th days of SCPS groups and model control group, while the normal control group was injected with an equal volume of NS. Rats were sacrificed by femoral bloodletting on the 31th day.

Colorimetric examination for alkaline phosphatase (AKP) and lysozyme (LZM) in serum: On termination of the experiment, blood samples were collected; serums were separated and stored at -80°C before analysis. The colorimetric assays detected quantify AKP and LZM with kit (A059 and A050, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instruction by ultraviolet spectrophotometer (UV-2100, Shimadzu Corporation, Japan).

ELISA examination for immunoglobulin A (IgA), immunoglobulin G (IgG), epidermal growth factor (EGF) in serum, secreted immunoglobulin A (SIgA) in jejunum secretion and prostaglandin E_2 (PGE_2) in colon: Serums were separated and stored with the same methods like AKP and LZM. Jejunum contents were rinsed three times with PBS for 10 min, collected washer liquid; centrifugation for 10 min at 3500 r min^{-1}, supernatant was stored at -80°C before assay. Colon was rinsed by NS at 4°C, blotted with filter paper, then weighed 200 mg for

tissue homogenate. The tissue homogenate was incubated for 15 min at 37°C and then centrifugation for 15 min at 3500 r min^{-1}, supernatant was stored at -80°C before assay. Two-site sandwich enzyme-linked immunosorbent assays (ELISA) were performed for quantify IgA and IgG, EGF in serum, SIgA in jejunum secretion and PGE_2 in colon with kit (Sigma, USA) according to the manufacturer's instruction. The absorbance in each well was measured by microliter enzyme-linked immunosorbent assay reader (SpectreMax M_2 Molecular Devices, USA) at a wave length of 450 nm.

Statistical analyses: Statistical analysis was performed using Design-Expert (Version 8.05b, Stat-Ease, Inc., Minneapolis, USA) and SPSS (Version 17.0) statistical software. Significance of difference between two groups was evaluated using Student's t-test. For multiple comparisons, one-way analysis of variance (ANOVA) was used. Mean values were considered significantly different when p-value was at or below 0.05.

RESULTS AND DISCUSSION

Statistical analysis and the model building: The design matrix and the corresponding results of RSM experiments to determine the effects of the three independent variables including high pressure homogenization time (X_1), ultrasonic power (X_2) and ultrasonic time (X_3) are shown in Table 1.

By applying multiple regression analysis on the experimental data, the dependent variable and independent variable are related by the following second-order polynomial equation:

$$Y = 29.80 + 0.0025X_1+0.2X_2+0.08X_3-0.025X_1X_2+ 0.00001X_1X_3-0.12X_2X_3-0.14X_1^2-0.71X_2^2-0.3X_3^2$$

The above model can be used to predict the extraction yield within the limits of the experimental factors. The significance of each coefficient was determined using the F-test and P-value in Table 1. The ANOVA of the quadratic regression model demonstrated that the model F-value of 88.49 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob>F" less than 0.05 indicate model terms are significant. The corresponding variables would be more significant if the absolute F-value becomes greater and the p-value becomes smaller (Qiu *et al*., 2010). The data in Table 1 indicated that the variables with the largest effect were the linear terms of ultrasonic power (X_2) and the quadratic term of ultrasonic power (X_2^2) and ultrasonic time (X_3^2) ($p<0.001$). Besides, the linear terms of ultrasonic time (X_3) and the interaction effects of ultrasonic power and ultrasonic time ($X_2 X_3$) and the quadratic term of high pressure homogenization time (X_1^2) were also found significant ($p<0.01$). The lack of fit test measures the failure of the model to represent data in experimental domain at points which are not included in the regression (Zhao *et al*., 2011). The "lack of fit F-value" of 5.86 implied the lack of fit was not significant relative to the pure error, which indicated that the model equation was adequate for predicting the yield of SCPS under any combination of values of the variables.

Table 1: Analysis on variance (ANOVA) of response surface methodology (RSM) for *S. cerevisiae* polysaccharides extraction

Variables	Sum of squares	DF	Mean square	F-value	p-value prob>F
X_1	0.00005	1	0.00005	0.013	0.9140
X_2	0.30	1	0.30	76.24	<0.0001**
X_3	0.051	1	0.051	12.83	0.0089*
X_1X_2	0.0025	1	0.0025	0.63	0.4546
X_1X_3	0.000	1	0.000	0.000	1.0000
X_2X_3	0.058	1	0.058	14.44	0.0067*
X_1^2	0.084	1	0.084	20.98	0.0025*
X_2^2	2.13	1	2.13	533.46	<0.0001**
X_3^2	0.37	1	0.37	92.46	<0.0001**
Model	3.18	9	0.35	88.49	<0.0001**
Residual	0.028	7	0.00399		
Lack of fit	0.026	3	0.00855	5.86	0.0521
Pure error	0.00228	4	0.00057		
Cor total	3.21	16			

R^2 = 0.9913, R^2_{Adj} = 0.9801, R^2_{Pred} = 0.8709, C.V.% = 0.22, Adeq Precision = 28.939, **Means significance ($p<0.001$), *Means significance ($p<0.01$)

The total determination coefficient (R^2) was 0.9913, indicating a reasonable fit of the model to the experimental data. In addition the adjusted coefficient of determination (R^2_{Adj} = 0.9801) and the coefficient of variation (C.V.% = 0.22) are shown in Table 1. These values indicated a high degree of precision and a good reliability of the experimental data. And the R^2_{Pred} of 0.8709 was in reasonable agreement with the R^2_{Adj} of 0.9801. Adeq precision measured the signal to noise ratio. A ratio greater than 4 was desirable. The ratio of 28.939 indicated an adequate signal. This model could be used to navigate the design space.

Optimization of the process: In this study, the aim of optimization was to find the conditions which give the maximum extraction yield of polysaccharides. The 3-D response surface and 2-D contour plots were the graphical representations of regression function. The optimal values of the selected variables were obtained by solving the regression equation using the Design-Expert software. The 3-D response surface and 2-D contour plots showed the type of interactions between two tested variables and the relationship between responses and experiment levels of each variable. Two variables within

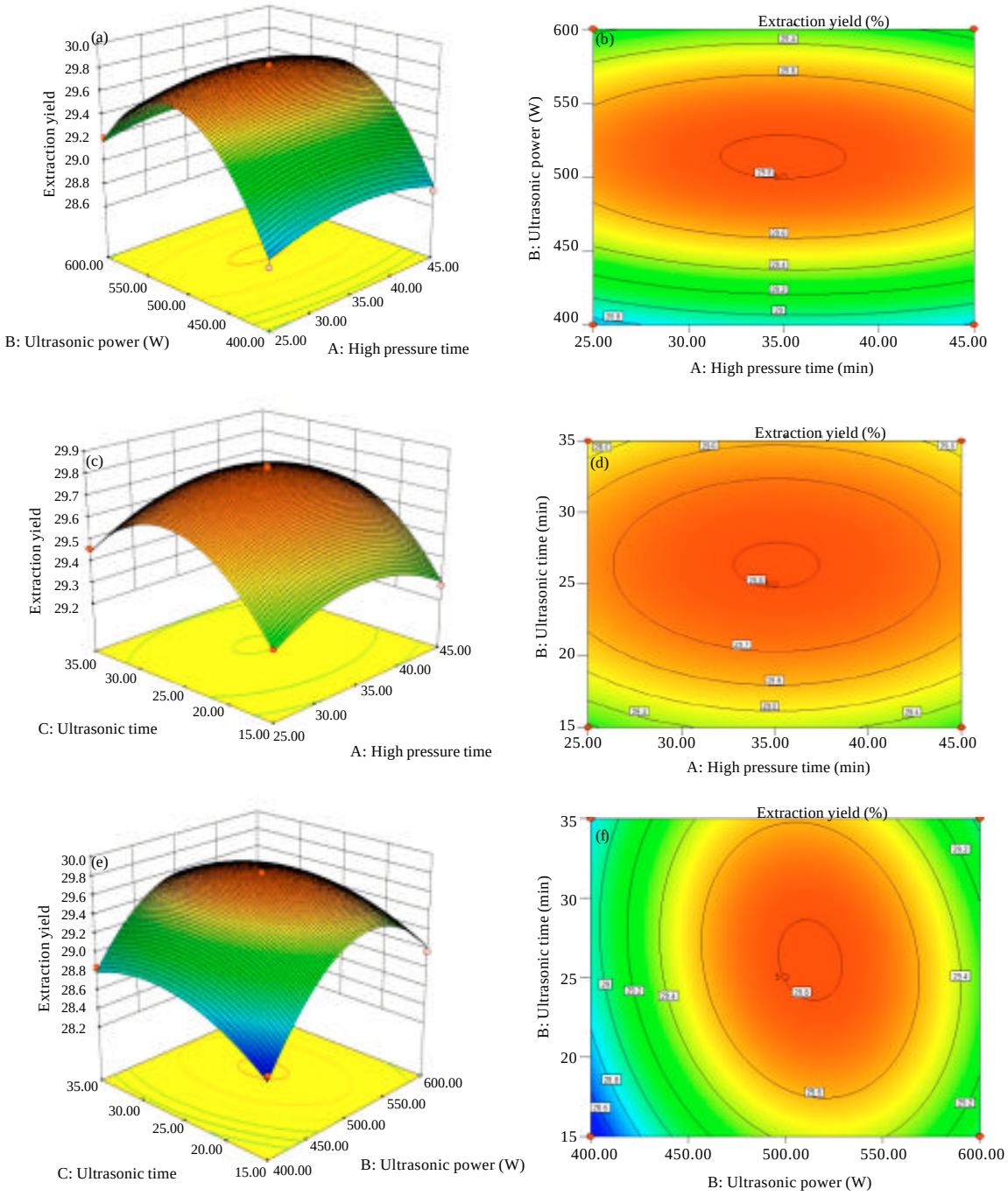

Fig. 1(a-f): Response surface (a, c, e) and contour plots (b, d, f) for the effect of high pressure homogenization time (X_1), ultrasonic power (X_2) and ultrasonic time (X_3) on the polysaccharides yield. Where (a, b) is X_1 and X_2; (c, d) is X_1 and X_3; (e, f) is X_2 and X_3

the experimental range are depicted in 3-D surface plots when the third variable is kept constant at zero level and different shapes of the contour plots indicated different interactions between the variables. Figure 1a and b showed the 3-D surface plot and the contour plot of the effect of high pressure homogenization time (X_1) and

ultrasonic power (X_2) on extraction yield. The ultrasonic power (X_2) demonstrated an exponential increase at a range of 400-600 W on extraction yield was observed. But the effect of high pressure homogenization time (X_1) did not display an increase on the response at a range of 25-45 min. Figure 1c and d depicted the effect of high pressure homogenization time (X_1) and ultrasonic time (X_3) on the extraction yield. And the figures showed extraction yield increased at a range of 15-35 min in ultrasonic time (X_3). Figure 1e and F showed the effect of ultrasonic power (X_2) and ultrasonic time (X_3) on the extraction yield and extraction yield increased with the increase in ultrasonic power (X_2) was observed. Also, extraction yield increased with the increase in ultrasonic time (X_3). By analyzing the plots, the predicted maximum value (29.82%) of the tested variables for extraction yield, lied in the following condition: high pressure homogenization time (X_1) 34.98 min, ultrasonic power (X_2) 512.74 W and ultrasonic time (X_3) 26.10 min. In order to better control conditions, abovementioned conditions were optimized: high pressure homogenization time (X_1) 35 min, ultrasonic power (X_2) 510 W and ultrasonic time (X_3) 26 min. In the optimal conditions, the experimental yield was (29.84±0.09)%, which agreed with the predicted value. Therefore, the research confirmed that these conditions were optimal for extraction yield.

Monosaccharide composition analysis: Polysaccharides contain between hundreds or thousands of monosaccharides that are linked by glycosidic bonds. The bonds of polysaccharides can be broken up (hydrolyzed) by acid hydrolysis. Breaking of the bonds can turn a polysaccharide into monosaccharide. Thin layer chromatography is a common laboratory procedure used to qualitatively measure compounds. Every compound has a different Rf value and for this reason, Rf values can be used to determine unknown compounds.

Thin layer chromatography was used to qualitatively analyze the hydrolysis reactions of SCPS in this experiment. The results showed the hydrolysis of SCPS had only one spot in plate and the Rf value was the same to glucose's (Rf = 0.69) (Fig. 2). So, SCPS may be a glucan in this study.

Effect of SCPS on AKP and LZM content in serum: Table 2 showed the effect of SCPS on serum AKP and LZM levels of rats. AKP and LZM play an important role in phagotrophy and sterilization ability of macrophages. AKP activity can reflect the growth performance of animals; improving AKP activity helping to improve Average Daily Gain (ADG) (Zhou *et al*., 2010) and AKP can enhance the nonspecific immunity function. LZM is an effector organ of specific immune and mainly secretes

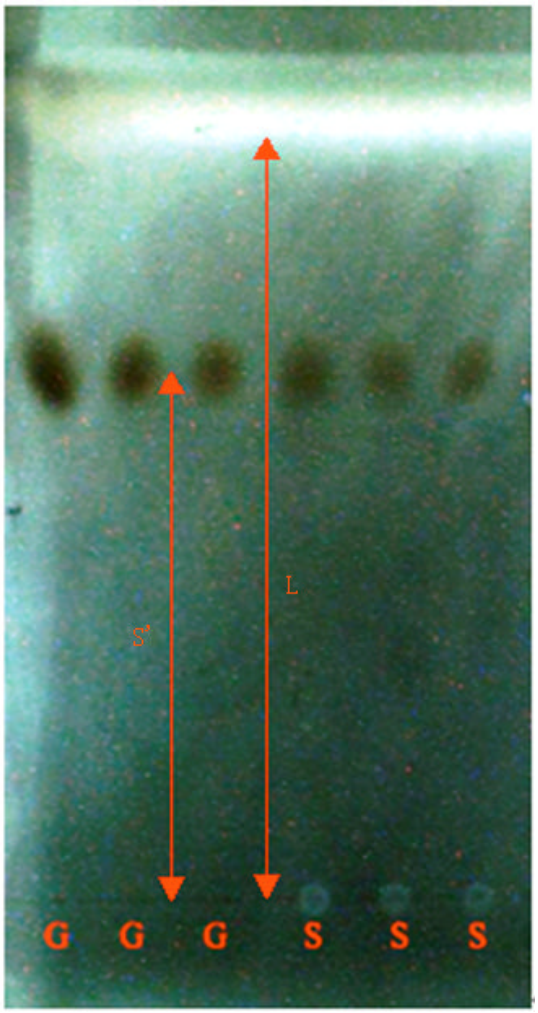

Fig. 2: Monosaccharide composition analysis of SCPS by TLC. Rf = Distance from origin to spot/Distance from origin to solvent front = S'/L = 0.69. Capital letter G and S correspond to glucose standard and hydrolysis of SCPS, respectively

by macrophages. The concentration of serum LZM is important index of nonspecific immune. As shown in Table 2, the AKP and LZM levels of model control group (IV) were significantly lower than that of the normal control group (V) ($p<0.05$), which showed that the immune-compromised model was successfully built. Compared with the model control group, the AKP and LZM levels of SCPS medium-dose group significantly increased ($p<0.05$), suggesting that SCPS was conducive to the increase immune function of immune-compromised rats. *Saccharomyces cerevisiae* polysaccharides enhances the function of nonspecific immunity maybe through increasing the activities of serum AKP and LZM.

Table 2: Comparisons of alkaline phosphatase (AKP) and lysozyme (LZM) content in serum between-group design after treatment with *S. cerevisiae* polysaccharides (multiple comparisons)

Groups	Dose (mg kg^{-1} day^{-1})	AKP (mg 100 mL^{-1})	LZM (U mL^{-1})
High-dose group (I)	200	46.71±12.64^{bc}	147.62±23.28^{c}
Medium-dose group (II)	100	51.57±10.47^{b}	181.71±21.83^{b}
Low-dose group (III)	50	38.64±6.11^{cd}	162.56±34.94^{bc}
Model control group (IV)	-	38.39±3.58^{cd}	165.67±28.78^{bc}
Normal control group (V)	-	82.36±15.87^{a}	219.86±22.02^{a}

Results are represented as the Means±SD, Values in the same column followed by different letters are significantly different ($p<0.05$), Normal control group and model control group were treated with equal volume of distilled water

Table 3: Comparisons of immunoglobulin A (IgA), immunoglobulin G (IgG) and epidermal growth factor (EGF) levels in serum, secreted immunoglobulin A (SIgA) expression in jejunum secretion and prostaglandin E_2 (PGE_2) in colon between-group design after treatment with *S. cerevisiae* polysaccharides (multiple comparisons)

Groups	Dose (mg kg^{-1} day^{-1})	IgA (μg mL^{-1})	IgG (μg mL^{-1})	EGF (pg mL^{-1})	SIgA (μg mL^{-1})	PGE_2 (ng L^{-1})
High-dose group (I)	200	7.60±0.48^{cd}	3.14±0.25^{c}	113.77±3.06^{b}	10.79±1.38^{b}	61.03±7.66^{ab}
Medium-dose group (II)	100	8.86±1.05^{a}	3.18±0.11^{b}	118.61±6.49^{a}	13.73±2.01^{a}	46.62±9.04^{cd}
Low-dose group (III)	50	8.04±0.49^{bc}	3.19±0.06^{b}	113.10±4.09^{b}	9.56±1.39^{b}	53.63±15.45^{bc}
Model control group (IV)	-	7.70±0.52^{cd}	3.12±0.05^{c}	113.21±1.98^{b}	10.53±2.38^{b}	75.73±7.36^{a}
Normal control group (V)	-	8.25±0.59^{ab}	3.36±0.20^{a}	114.28±2.84^{b}	9.68±0.81^{b}	55.18±13.36^{bc}

Results are represented as the Means±SD, Values in the same column followed by different letters are significantly different ($p<0.05$), Normal control group and model control group were treated with equal volume of distilled water

Effect of SCPS on IgA, IgG, EGF in serum, SIgA in jejunum secretion and PGE_2 in colon: Effect of SCPS on IgA, IgG and EGF in serum, SIgA in jejunum secretion and PGE_2 in colon was determined by ELISA. As shown in Table 3, the IgA, IgG and PGE_2 levels of model control group (IV) were statistically significant difference ($p<0.05$) compared with that of normal control group (V), which showed that the immune-compromised model was successfully built. Host defense depends on the presence of capsule-specific antibodies. The level of serum immunoglobulin improved in a range can maintain health. IgA is overwhelmingly the most important immunoglobulin isotype at mucosal sites and can interfere with bacterial attachment to mucosal epithelia (Childers *et al*., 1989; Macpherson *et al*., 1996). IgA has been shown to neutralise viruses in culture and to form part of the protective response *in vivo* (Macpherson *et al*., 2001). Therefore, the level of IgA is one of the standards used to estimate mucosal immunity. IgG antibody is the predominant isotype-specific response following immunization (Butler *et al*., 1993). In the study, compared with the model control group (IV), the serum IgA and SIgA in jejunum secretion were significantly increased in SCPS medium-dose group (II) ($p<0.05$). A similar observation was indicated in the serum IgG. *Saccharomyces cerevisiae* polysaccharides markedly augmented serum IgA and IgG and SIgA in jejunum secretion of rats and were predicted to be protective for immune-compromised rats. The results were in agreement with the level of serum immunoglobulin could be affected by yeast glucan (Krakowski *et al*., 2002; Wang *et al*., 2008). EGF is a single-chain polypeptide activating factor secreted by maternal organs, which is cytoprotective for intestinal epithelial cells development (Pillai *et al*., 1999). EGF has positive effects on meiotic maturation (Coticchio *et al*., 2004). PGE_2 are known produced by many tumors and has been associated with immune suppressive (Gabrilovich and Nagaraj, 2009). As shown in Table 3, the EGF and PGE_2 levels of SCPS medium-dose group (II) were statistically significant difference compared with model control group (IV) and normal control group (V) ($p<0.05$), which indicated that the SCPS had noticeable effects on immune enhancement of rats.

CONCLUSION

In conclusion, the present study observed that the maximal extraction yield of *Saccharomyces cerevisiae* polysaccharides (SCPS) was 29.84±0.09% after the extraction conditions were optimized using response surface methodology. Monosaccharide composition analysis exhibited SCPS may be a glucan. Pharmacological experiments indicated that SCPS can enhance the immune function and can obviously protect intestine mucosa of immune-compromised rats.

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