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ORIGINAL RESEARCH PAPER

EXTRACTION AND ANTIOXIDANT ACTIVITIES ASSAY OF POLYSACCHARIDES FROM WHITE HYACINTH BEAN AND PROMOTING GROWTH TO PROBIOTICS

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The extraction parameters of water-soluble polysaccharides (WPs) from white hyacinth bean were optimized using single-factor and orthogonal experiment. The antioxidant activities of WPs were presented by assaying three different radicals, 2,2-diphenyl-1-picrylhydrazy radical (DPPH radical), hydroxyl radical and superoxide radical scavenging ability. In addition, the effects of WPs obtained on the growth of three probiotic strains (*Lactobacillus acidophilus LA5*, *Bifidobacterium bifidum BB01* and *Lactobacillus bulgaricus LB6*) were also determined by measuring the OD and pH value of culture medium. According to the results, the optimum extraction parameters were as follows: the ratio of water to material was 50, extraction time was 2h and the extraction temperature was 95°C. The yield of WPs reached 1.15±0.07% under this condition. In addition, the WPs had different scavenging ability on three radicals (hydroxyl > DPPH > superoxide). And the WPs could promote the growth of *LA5*, *BB01* and *LB6*.

Keywords: white hyacinth bean polysaccharides, antioxidant activities, probiotic strains

Introduction

Oxidation is a very important biological process in living organisms for the energy production. However, if the balance between pro-oxidant conditions and the antioxidant defense was broken, the excessive reactive oxygen species (ROS) such as hydrogen radical, superoxide free radical and hydroxyl free radical would be produced (Fukuzawa *et al.*, 2001). Much evidence indicated that the excessive production of free radical would cause various damage to humans such as aging, cancer, atherosclerosis, inflammation, liver injury, heart disease and skin damage (Cadenas & Davies, 2000; Ke *et al.*, 2009). Synthetic antioxidants are widely applied

in the food and cosmetics industries to scavenge free radicals and improve product nutritional quality and increase shelf-life as regards oxidative deterioration (Brawek *et al*., 2010; Tehila *et al*., 2005). However, some synthetic antioxidants are suspected to cause carcinogenesis and liver damage (Qi *et al.*, 2005). Hence, it is necessary to find alternative natural antioxidants to scavenge radicals.

The polysaccharides extracted from several plants showed excellent antioxidant activities with nontoxic property (Kardosova & Machová, 2006; Wang et al., 2014; Ge *et al.*, 2014). White hyacinth bean is a healthy plant, widely cultivated throughout the tropics and subtropics. In many areas, which produce hyacinth beans, the young pods are boiled as vegetables while the dry seeds are cooked with rice (Subagio, 2006). Polysaccharides are important constituents of white hyacinth bean, which may contribute to the antioxidant activity; however, the relative literature is limited.

In our present study, the extraction condition of water-soluble polysaccharides (WPs) from white hyacinth bean was optimized by single-factor and orthogonal experiment. The scavenging activity of WPs to the DPPH radical, hydroxyl radical and superoxide radical were determined by measuring the optical density (OD value) at 590nm. In addition, the effect of WPs on the growth of three selected probiotics including *Lactobacillus bulgaricus LB6, Lactobacillus acidophilus LA5*, and *Bifidobacterium bifidum BB01* was also studied in our present work.

Materials and methods

Materials

The white hyacinth beans were purchased from Xi'an, Shannxi Province, China. The samples were cleaned and dried before extraction. All chemicals were of analytical grade. Lactose, Dibasic Ammonium Citrate, K_2HPO_4, sodium acetate, $MgSO_4$ and MnSO4 were purchased from Tianli Chemical Reagent Co., Ltd. (China). The peptone, yeast extract, beef extract, and Tween 80 were purchased from Beijing Aoboxing Bio-Tech Co., Ltd. (China).

The bacteria used in this study were *Lactobacillus bulgaricus LB6, Lactobacillus acidophilus LA5*, and *Bifidobacterium bifidum BB01*, which were obtained from the School of Food and Biological Engineering, Shaanxi University of Science and Technology.

Extraction of WPs

The WPs were obtained using the water extraction and alcohol precipitation method. The bean samples were pulverized to powder and passed through a 60 mesh sieve before extraction. The dried bean flour was extracted by hot water at 90°C for 3h with the ratio of water volume (mL) to material weight (g) as 30. After extraction, the suspension mixture was separated by centrifugation. The supernatant was concentrated and the protein in the crude polysaccharide was removed by Sevag method (Sevag *et al*., 1938). Afterwards, the ethanol was added to precipitate the polysaccharide. Then the solution was filtered by filter paper, dissolved with distilled water and centrifuged. Finally, supernatant was suitably diluted and the content of

WPs in the extract was evaluated by phenol-sulfuric method (Dubois *et al*., 1956). The yield of WPs in white hyacinth bean was calculated by the following equations:

$$\text{Polysaccharides yield \% (w/w)} = \frac{\text{polysaccharides weight}}{\text{White hyacinth bean weight(1g)}} \times 100\% \quad (1)$$

The orthogonal matrix method was used for optimizing the extracting condition. Confirmatory experiment was conducted three times to verify the results of the orthogonal matrix.

Antioxidant assays

Scavenging hydroxyl radical activity assay

The scavenging ability of WPs on hydroxyl radical was measured according to Fenton method (Smirnoff & Cumbes, 1989) with some modifications. The reaction mixture contained 2 mL phosphate buffer (0.2 mol/L, pH 7.4), 2 mL orthophenanthroline solution (0.1 mmol/L), 1 mL ferrous sulfate (F_eSO_4) solution (0.15 mmol/L), 1 mL hydrogen peroxide (H_2O_2) solution (0.01%) and 1 mL samples. After the incubation at 37°C for 60 minutes, the absorption value of different mixtures was measured at 510 nm with a spectrophotometer (SP-756PC, Shanghai Spectrum Instruments Co., Ltd., Shanghai, China). Ascorbic acid (Vc) was used as reference material and all the tests were carried out in triplicate. The scavenging activity of polysaccharides on the hydroxyl radical was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{A_2 - A_0}{A_1 - A_0} \times 100\% \quad (2)$$

Where A_0 was the absorbance of reaction solution without any sample, A_2 was the absorbance of the sample and A_1 was the absorption value of the sample under identical conditions as A_2 with water instead of H_2O_2 solution.

Superoxide radical scavenging activity

The activity of scavenging superoxide radical was tested by auto-oxidation of pyrogallic acid (Marklund & Marklund, 1974) with a little modification. Pyrogallic acid would produce the O_2 due to auto-oxidation under the weak basic condition. Some colored substance would be produced at the same time, which had the maximum absorbance at 325 nm. In this experiment, the reaction mixture contained 2.5 mL Tris-HCl buffer (0.05 mmo/L, pH 8.2), 0.1 mL pyrogallic acid solution (0.01 mol/L) and 0.4 mL polysaccharide solution. The polysaccharide and Tris-HCl were incubated at 25°C for 20 min, then pyrogallic acid was added to the mixture. After shaking, the absorption value was read at 325 nm using a SP-756PC spectrophotometer. The scavenging activity of polysaccharide on the superoxide radical was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100\% \quad (3)$$

where A_0 was the absorbance of the control (without samples) and A_1 was the absorbance of the mixture containing samples.

DPPH radical scavenging activity

The scavenging activity of polysaccharide on DPPH radicals was measured according to the method of Shimada *et al*. (1992) with a slight modification. An aliquot of 2 ml DPPH solution (0.08 mM alcoholic solution of DPPH radicals) in anhydrous ethanol and 2 ml of sample in different concentrations (1.0 mg/ml, 2.0 mg/ml, 4.0 mg/ml, 6.0 mg/ml and 8.0 mg/ml) were mixed together, standing for 40 minutes after shaking. A solution of 50% (v/v) ethanol was used as control sample. The scavenging ability was measured by determining the absorbance of the mixture at 517 nm and all the tests were carried out in triplicate. The scavenging activity of polysaccharide on DPPH radicals was calculated according to the following formula:

$$\text{Scavenging activity (\%)} = \frac{1-(A_i-A_j)}{A_0} \times 100\% \qquad (4)$$

where A_0 was the absorbance of DPPH solution without the samples, A_i was the absorption value of polysaccharide with DPPH solution and A_j was the absorption value of the sample under identical conditions as A_i with water instead of DPPH solution.

Effect of WPs on the growth of selected probiotics

MRS culture medium containing 20 g lactose, 10 g peptone, 4 g yeast extract, 8 g beef extract, 2 g dibasic ammonium citrate, 2 g K_2HPO_4, 3 g sodium acetate, 0.2 g $MgSO_4$, 0.05 g $MnSO_4$, 0.5 g L-cysteine hydrochloride, 1 mL Tween 80, was dissolved in 1000 mL distilled water with an initial pH of 6.2, and then the culture medium was sterilized at 121°C for 15 minutes.

L. acidophilus LA5, B. bifidum BB01 and L. bulgaricus LB6 were incubated in MRS culture medium (3% inoculum size) at 37°C. And five different concentrations of WPs (0.05%、0.10%、0.15%、0.20% and 0.25% in volume) were added to the medium. In the blank control group the polysaccharides were absent. The absorbance at 600 nm and pH value of culture solution were determined every 4 h starting with the 14th hour (they were recorded 3 times totally).

Results and discussion

Extraction of polysaccharide

Single-factor experiments of water extraction and alcohol precipitation method

The effect of the ratio of water to material (bean flour) (20, 30, 40, 50 and 60), the extraction time (1.0h, 1.5h, 2.0h, 2.5h and 3.0h) and the extraction temperature (60°C, 70°C, 80°C, 90°C and 95°C) on the polysaccharides yield was analyzed by single-factor experiments. When studied the effect of ratio to extraction, the extraction time and temperature keep invariant (time: 3h, temperature: 90°C). When studied time factor, the ratio and temperature keep invariant (30, 90°C). When studied temperature factor, the other two factors keep invariant (30, 3h). As shown in Figure 1(a), at the initial stage, the polysaccharide yield showed a notable increasing trend as the increasing of the ratio of water : material. Then the speed of increment tended to slow down from 40 to 60, which may be caused by the fact that the solute diffusion would increase as the increasing of the extraction solvent. The

effect of extraction time on the polysaccharide yield was presented in Figure 1(b). The yield of the polysaccharides increased from 0.7 % to 0.9 % with the extraction time from 1 h to 2 h. After that, the polysaccharide yield decreased and tended to be stable, which may be due to a long time of extraction that could cause the decomposition of the polysaccharide based on its instability. A gradually increment was observed with the increasing of the extraction temperature, which was shown in Figure 1(c). The yield of polysaccharide reached maximum (1.11%) at 95℃, which may be caused by the fact that the polysaccharide diffusion speed increased due to the increasing of the temperature. According to Figure 1, the optimum parameters of single-factor test were: ratio of water to material: 50, extraction temperature: 95°C, and extraction time: 2h, respectively.

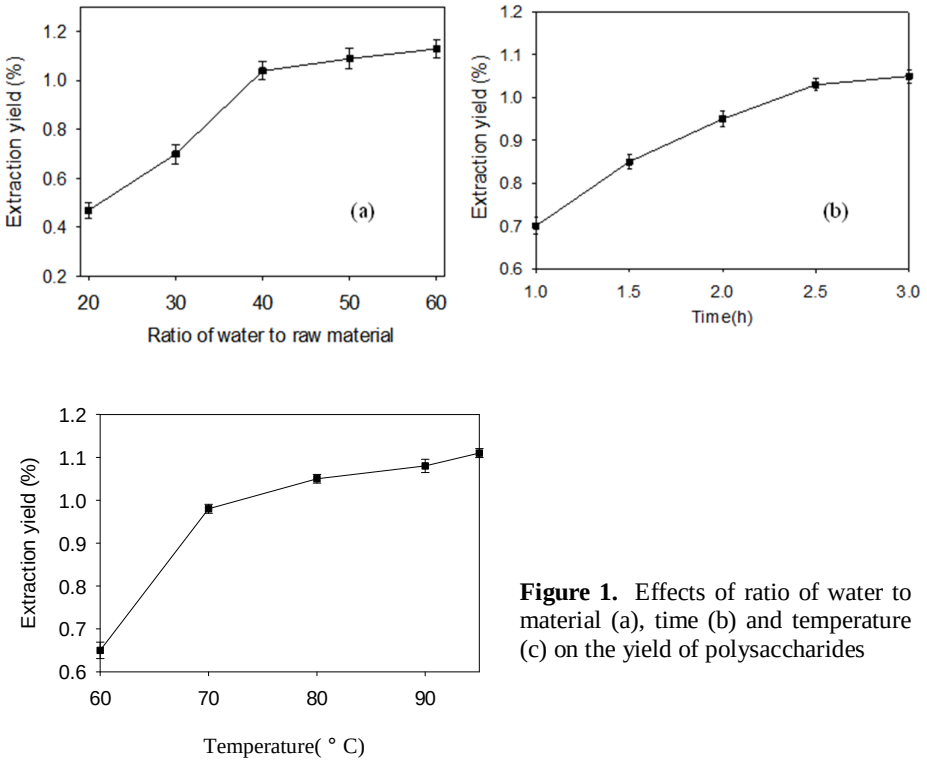

Figure 1. Effects of ratio of water to material (a), time (b) and temperature (c) on the yield of polysaccharides

The orthogonal matrix method of polysaccharides extraction

In order to obtain the optimum parameters of polysaccharide extraction, the orthogonal experiment was conducted to optimize the extraction condition based on the single-factor test. The four-factor, three-level experiment design was shown in Table 1 and the results of the orthogonal experiment are presented in Table 2.

Table 1. Factors and levels of polysaccharides extraction orthogonal experiment

Variable	Level		
	1	2	3
A - ratio of water to raw material	45	50	55
B - extraction time (h)	1.5	2	2.5
C - extraction temperature (°C)	85	90	95

Table 2. Results of polysaccharides extraction orthogonal experiment

Run	Ratio of water to material	Time (h)	Temperature (°C)	Yield of polysaccharide (%)
1	1	1	1	0.86
2	1	2	2	0.91
3	1	3	3	0.94
4	2	1	2	0.85
5	2	2	3	1.18
6	2	3	1	0.95
7	3	1	3	0.89
8	3	2	1	0.77
9	3	3	2	0.86
K1	0.840	0.917	0.873	
K2	0.983	0.947	0.897	
K3	0.887	0.847	0.940	
R	0.143	0.100	0.067	

From Table 2, the optimum condition was A2B2C3 (the ratio of water to material: 50, the extraction time: 2h, the extraction temperature: 95°C). The yield of polysaccharide reached 1.18% under this condition, which was the highest value in the experiment. According to R1>R2>R3, it can be also suggested that the ratio of water to material was the most important parameter, followed by extraction time while the extraction temperature had relatively weak influence on the polysaccharide yield. The yield of polysaccharide was 1.15±0.07% (N=5), which was closed to the optimum result in the orthogonal experiment.

Antioxidant activities of WPs from white hyacinth bean

Scavenging hydroxyl radical activity

The results of scavenging hydroxyl radical activities of WPs and Vc were shown in Figure 2 (a). We can see clearly that the WPs and Vc both had the ability to scavenge hydroxyl radical, and the scavenging ability was stronger with the increasing of the WPs and Vc concentration. It can be suggested that the WPs had an obvious scavenging ability toward the hydroxyl radical. From Figure 2(a), the scavenging activity reached 35.71% when WPs was 10mg/mL, the percentage of

polysaccharides scavenging activity with Vc scavenging activity (at the same concentration) was 39.61%.

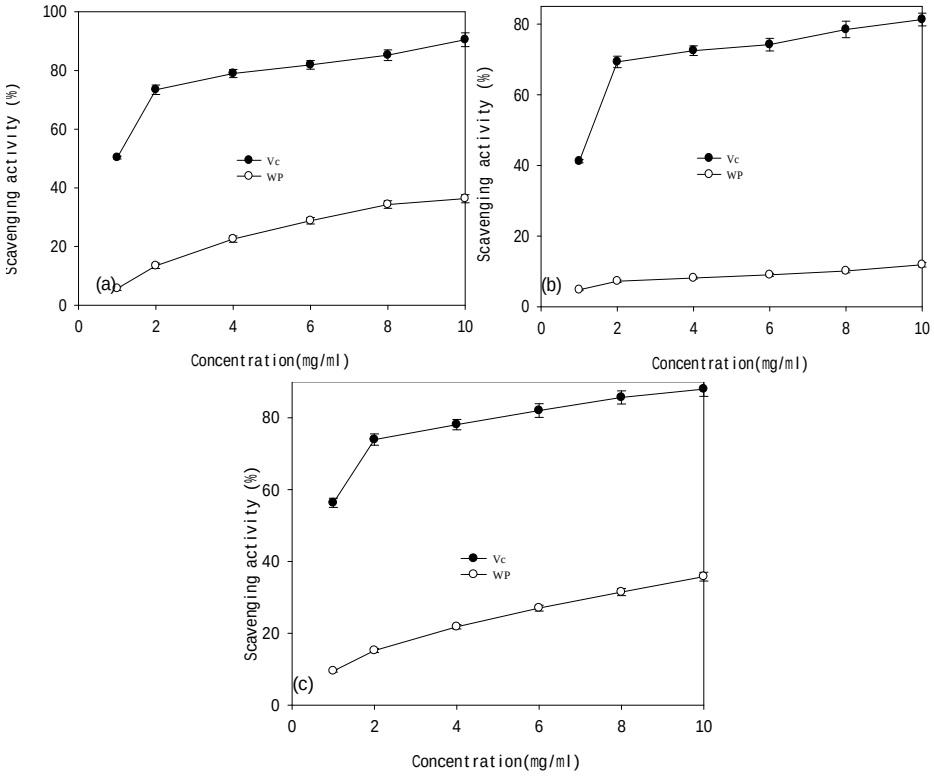

Figure 2. Scavenging activities of WPs and Vc to the hydroxyl radical (a), superoxide radical (b) and DPPH radical (c)

Superoxide radical scavenging activity

Figure 2(b) showed the results of scavenging superoxide radical activities of polysaccharide and ascorbic acid. Similar to the scavenging hydroxyl radical activities, the scavenging activities of polysaccharide and ascorbic acid on superoxide radical increased with concentration. When the concentration of polysaccharide was 10 mg/mL, the scavenging activity reached 11.89%, which was about 14.63% of Vc scavenging activity (at the same concentration).

DPPH radical scavenging activity

The results of scavenging DPPH radical activities of polysaccharide and ascorbic acid were presented in Figure 2(c). It can be suggested that both the polysaccharide and the ascorbic acid could scavenge DPPH radical, and there was a positive correlation between the scavenging activity and polysaccharide concentration. When the polysaccharide was 10 mg/mL, the scavenging activity on DPPH radical reached

33.89%, the percentage of polysaccharides scavenging activity with Vc scavenging activity (at the same concentration) was 38.51%.

Both polysaccharide and ascorbic acid had scavenging activity on the hydroxyl radical, superoxide radical and DPPH radical, but the extent of their scavenging ability was different. The scavenging hydroxyl radical activity was the strongest, followed by the scavenging activity on DPPH, and the superoxide radical scavenging activity was the lowest. It can be also suggested that the scavenging activity of polysaccharide was lower compared to the Vc.

Effect of polysaccharide on the growth of probiotics

Effect of polysaccharide on the growth of L. acidophilus LA5

Considering the results concerning the effect of polysaccharide on the growth of *L. acidophilus LA5*, shown in Figure 3, it can be suggested that the promotion effect of polysaccharide on *L. acidophilus LA5* was relative to the addition of polysaccharide. Within 0.2% concentration, the promotion effect of polysaccharide on *L. acidophilus LA5* increased with the addition of polysaccharide. When the polysaccharide addition was 0.20%, the OD value and pH value tended to be stable. This may be caused by the change of osmotic pressure and pH value due to the high-concentration of polysaccharide; these variations and the accumulation of metabolites would limit the growth of *L. acidophilus LA5*.

Effect of polysaccharide on the growth of B. bifidum BB01

The effect of polysaccharide on the growth of *B. bifidum BB01* was shown in Figure 3c,d. The growth of B. bifidum BB01 showed an increasing trend with the increment of polysaccharide addition. When the addition was 0.20%, the OD value and the pH value tended to be stable.

Effect of polysaccharide on the growth of L. acidophilus LB6

Figure 3 e, f shows the effect of polysaccharide on the growth of L. acidophilus LB6. It can be suggested that the promotion effect of polysaccharide on L. acidophilus LB6 was relative to the addition of polysaccharide. Similar to *L. acidophilus LA5* and *B. bifidum BB01*, the promotion effect had an obvious enhancement with the increase of polysaccharide addition.

Taking into account the results presented in Figure 3 it can be stated that the polysaccharide from white hyacinth bean had promoting effect on the three investigated probiotics, *L. acidophilus LA5*, *B. bifidum BB01* and *L. bulgaricus LB6*. This may be due to the fact that the polysaccharide contains stachyose and raffinose which can promote the growth of probiotics. And these two functional oligosaccharides can also inhibit the propagation of harmful bacteria and build a healthy intestinal environment.

In the present study, the scavenging activity of WPs on three different radicals (hydroxyl radical, superoxide radical and DPPH radical) were evaluated to present the antioxidant activity of white hyacinth bean. DPPH is one of the important compounds that possess a proton free radical and has a characteristic absorption value at 517nm (its color is purple at 517nm) (Matsukawa *et al*., 1997). The reason why WPs can scavenge DPPH activity may be that the hydroxyls in the

polysaccharide molecule could donate and transfer electron to DPPH (Leong & Shui, 2002). When proton radical from DPPH is scavenged by polysaccharide, its purple would fade rapidly (Yamaguchi *et al.*, 1998), which would change the absorption value at 517 nm.

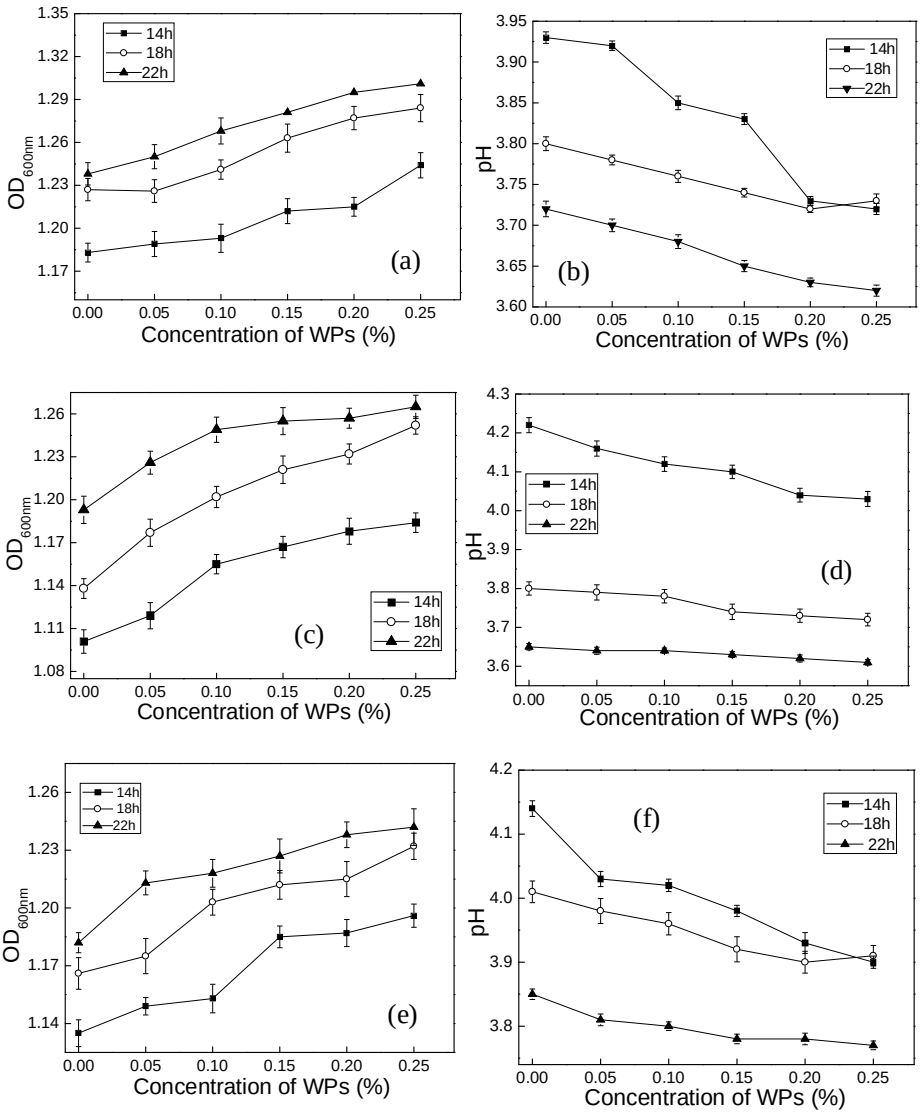

Figure 3. Effects of the extracted polysaccharide on the growth of LA5 (a, b), BB01 (c, d) and LB6 (e, f)

Superoxide anion radicals could be generated by some enzyme systems, such as peroxidase, NADPH oxidase and xanthine oxidase, which play crucial roles in the

production of some damage free radicals, such as hydrogen peroxide and hydroxyl radical. When superoxide anion radicals were scavenged by antioxidant, the absorbance at 320nm increased due to the color changed from purple to yellow (Chen *et al.*, 2008b, Sun *et al*., 2004), which could represent the antioxidant ability of polysaccharides. The hydroxyl radical would produce severe damage to the biomoleculars (Boligon *et al*., 2009, Erel, 2004). It is necessary to scavenge or decrease the content of hydroxyl radical.

L. acidophilus LA5, *B. bifidum BB01* and *L. bulgaricus LB6* are three common probiotics, which have been used in many foods, especially in yogurt. The WPs have positive effect on these three probiotics strains, most probably because the WPs contain oligosaccharide which can promote the growth of probiotics. Some oligosaccharides are prebiotics and are widely used in yogurt. The prebiotics could be hydrolyzed by the enzyme in the probiotics, such as α-glucosidase, β-glucosidase, α-galactosidase and β-galactosidase. Hence, probiotics could hydrolyze and utilize the carbohydrate prebiotics to produce energy and promote their growth.

It has been proved that the polysaccharide obtained from plants has excellent antioxidant activity (Kardosova & Machová, 2006). However, this mechanism has not been very clearly understood yet. The scavenging ability of polysaccharide on radicals could be affected by many different factors, such as chemical structure, molecular and isolation method (Chen *et al*., 2008a). The polysaccharides from white hyacinth bean were just crude polysaccharides, and it is necessary and essential to conduct further experiments to purify the extracted polysaccharide.

Conclusions

In this study, we successfully obtained the WPs from white hyacinth bean. The optimum parameters of WPs extraction were as follows: ratio of water to material: 50, extraction time: 2h, extraction temperature: 95°C. The extraction rate was 1.10% below the optimum conditions. In addition, the WPs obtained with the water extraction and alcohol precipitation method had scavenging activity on the hydroxyl radical, superoxide radical and DPPH radical. However, the scavenging activity was different; the scavenging activity to hydroxyl radical was the strongest, being followed by DPPH radical, whereas the scavenging activity to superoxide radical was the weakest. The scavenging ability of WPs on the three radicals was lower than that of Vc. Furthermore, the results indicated that the WPs from white hyacinth bean could promote the growth of *L. acidophilus LA5*, *B. bifidum BB01* and *L. bulgaricus LB6*.

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