Carbohydrate Polymers 101 (2014) 332–341

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Enzymolysis-ultrasonic assisted extraction, chemical characteristics and bioactivities of polysaccharides from corn silk

Shuhan Chen, Haixia Chen*, Jingge Tian, Jia Wang, Yanwei Wang, Lisha Xing

Tianjin Key Laboratory for Modern Drug Delivery & High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, PR China

ARTICLE INFO

Article history:
Received 4 July 2013
Received in revised form 25 August 2013
Accepted 14 September 2013
Available online 23 September 2013

Keywords:
Corn silk polysaccharides
Enzymolysis-ultrasonic assisted extraction
Chemical characteristics
Antioxidant and anticancer activities

ABSTRACT

An enzymolysis-ultrasonic assisted extraction (EUAE) procedure of corn silk polysaccharides (CSPS) was established and the physicochemical properties, antioxidant and anticancer activities of CSPS were studied. Orthogonal test and response surface methodology were applied to optimize the extraction parameters. The optimum enzymolysis and ultrasonic conditions were cellulase content of 7.5% for 150 min at 55 °C and liquid–solid ratio of 31.8 for 34.2 min at 66.3 °C, respectively. Under these conditions, the yield of CSPS increased from 4.56% to 7.10%. CSPS obtained by hot water and EUAE were composed of rhamnose, arabinose, xylose, mannose, galactose and glucose with molecular ratios of 4.17:17.33:5.59:18.65:19.11:35.14 and 8.83:15.77:7.92:12.39:11.15:43.94, respectively. Their molecular weight distributions were 10.52×10^4 and 6.88×10^4 Da, respectively. CSPS obtained by EUAE showed morphological and conformation changes and higher antioxidant and anticancer activities compared with CSPS extracted by hot water.

1. Introduction

Corn silk (dried cut stigmata of maize female flowers, *Zea mays* L.) is a well-known functional food and traditional Chinese herbal medicine. It consists of various bioactive constituents which has significant influence on human health. The bioactivities of corn silk were widely reported in the literatures, including antioxidant activities (Liu et al., 2011), anti-proliferative effects on human tumor necrosis factor (TNF)-α and lipopolysaccharide-induced cell adhesion (Habtemaram, 1998), anti-diabetic activity on hyperglycemia rats (Zhao, Yin, Yu, Liu, & Chen, 2012), diuretic activity (Velazquez, Xavier, Batistac, & de Castro-Chaves, 2005), anticoagulant activity (Choi & Choi, 2004), antifungal (Miller, Reid, Butler, Winter, & McGoldrick, 2003; Zeringue, 2000), anti-fatigue (Hu, Zhang, Li, Ding, & Li, 2010) and weight loss activities (Du & Xu, 2007). Corn silk had a long history of application for therapeutic remedy and it was tested non-toxic (Wang et al., 2011).

Polysaccharides are essential biomacromolecules widely exist in the plants, microorganisms, algae and animals. Some polysaccharides isolated from natural sources showed various biological activities such as antidiabetic, antitumor, immunomodulatory and anti-inflammatory effects (Huang, Chow, & Tsai, 2012; Jiang, Wang, Liu, Gan, & Zeng, 2011; Kang et al., 2011; Liang, 2008), which were strongly affected by their chemical structures and chain conformations (Yang & Zhang, 2009). Polysaccharide was one of the main components of corn silk. Previous studies showed that polysaccharides from corn silk (CSPS) could lead to weight loss (Du & Xu, 2007), regulate blood sugar (Zhao et al., 2012) and improve gastrointestinal movement (Du, Xu, & Gao, 2007). So the CSPS might be used as a novel nutraceutical agent for human consumption.

The extraction methods of polysaccharides included traditional water extraction, enzyme, ultrasonic and microwave-assisted methods, which were help to improve the extraction efficacy (Hou & Chen, 2008; Yin, You, & Jiang, 2011; Wang, Zhou, & Wen, 2006; Zhao, Dong, Chen, & Hu, 2010). However, single method usually requires longer extraction time or higher temperature but lower extraction efficiency. Enzymolysis-ultrasonic assisted extraction is a combined extraction method, which is undoubtedly an emerging technology in the food industry since it owns advantages of two extraction methods such as mild extraction conditions, lower investment costs and energy requirements, and simplified manipulation.

Recently, response surface methodology (RSM) has been used increasingly to optimize processing parameters because it allows more efficient and easier arrangement and interpretation of experiments compared to other methods (Box & Behnken, 1960; Gan, Manaf, & Latiff, 2010; Prakash Maran, Manikandan, Thirugnanasambandham, Vigna Nivetha, & Dinesh, 2013). Box–Behnken design (BBD) is a type of response surface design. It is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. In this design the treatment combinations are at the midpoints of edges

* Corresponding author. Tel.: +86 22 27401483; fax: +86 22 27892025.
E-mail address: chennhxx@163.com (H. Chen).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.09.046

of the process space and at the center. These designs are rotatable (or near rotatable) and require 3 levels of each factor (Zou, Chen, Yang, & Liu, 2011). It is more efficient and easier to arrange and interpret experiments comparing with others. It was widely used in many studies on extraction of bioactive constituents (Prakash Maran et al., 2013; Yan et al., 2011; Zhao et al., 2011). Therefore, it was more efficient and easier to arrange and interpret experiments to optimize the extraction parameters for corn silk polysaccharides with an enzymolysis-ultrasonic assisted extraction (EUAE) procedure. Though there were some reports on the extraction of the corn silk polysaccharides (Prakash Maran et al., 2013; Zhao et al., 2011). There is no study on enzymolysis-ultrasonic assisted extraction with BBD on the corn silk polysaccharides till now.

In this study, the enzymolysis-ultrasonic assisted extraction, chemical characteristics and bioactivities of CSPS were investigated to obtain an effective method for CSPS with higher yield and bioactivities. Orthogonal test method with three factors and three levels was designed to optimize the process parameters of enzymolysis pretreatment. With that a three-level, three-variable BBD was used to optimize the ultrasonic extraction parameters of CSPS. Chemical structure, antioxidant and anticancer activities, and the preliminary structure-activity relationship of CSPS were discussed.

2. Materials and methods

2.1. Materials and chemicals

Corn silk was gathered from cornfield in September 2012 in Tianjin, China. The corn silk variety was *Jingke968* as authenticated by associated Professor Haixia Chen. 1,1-diphenyl-2-picrylhydrazyl (DPPH), arabinose, galactose, glucose, rhamnose and mannose were provided by Sigma Chemical Co. (St, Louis, MO, USA). Sephadex G-100 was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Cellulase (10,000 U/g) was obtained from TianJin NuoAo Enzyme Co., Ltd. All other chemicals and reagents were purchased locally and were of analytical grade.

2.2. Experimental design

Orthogonal test method was used to optimize the enzymolysis extraction process. Based on the single factors studies, cellulose content (A), enzymolysis temperature (B) and enzymolysis time (C) were selected as the three factors. CSPS content (Y) was taken as the index, which measured by the phenol–sulfuric acid method with D-glucose as a standard.

Box–Behnken design (BBD) with three independent variables was used for the optimization of extraction conditions. Ultrasonic time ($X1$), liquid–solid ratio ($X2$) and ultrasonic temperature ($X3$) were chosen as key variables based on the results of preliminary experiments. Extraction yield (Y) was taken as the response of the design experiments. Once the experiments were performed, the response variable (extraction yield) was fitted a second-order model in order to correlate the response variable to the independent variable. The complete quadratic equation used was as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \qquad (1)$$

where Y was the estimated response; X_i and X_j were input variables; β_0, β_i, β_{ii}, β_{ij} ($i \neq j$) were the regression coefficient, linear coefficient, quadratic coefficient and the linear-by-linear interaction between X_i and X_j, respectively.

2.3. Hot water extraction of crude polysaccharides

All the corn silk raw materials were immersed in 80% (v/v) ethanol at 78 °C for three times to remove colored materials, monosaccharides and liposoluble constituents. The organic solvent was volatilized and the pretreated corn silk was obtained to crush. Dried ground corn silk (100 g) was extracted with water at 100 °C (1:15 (w/v), 1 h, 3 times). The extraction solutions were combined, filtered and concentrated, precipitated by the addition of anhydrous ethanol to a final concentration of 80% (v/v). Precipitates were collected by centrifugation (3000 × *g*, 10 min), then dissolved with water, and subjected to the Sevag method (chloroform:butyl alcohol, 4:1) to remove free proteins. The deproteinized solution was re-precipitated in 80% (v/v) ethanol. The precipitates were collected and successively washed with anhydrous ethanol and acetone. After freezing dried, the hot water extraction corn silk polysaccharides (PS) were obtained.

2.4. Enzymolysis-ultrasonic assisted extraction (EUAE) of crude polysaccharides (EUPS)

Dried ground corn silk samples (100 g) were soaked with cellulase, which could destroy cell wall to promote the dissolving of active ingredients at the corresponding conditions. After inactivating the cellulose in boil water for 5 min, ultrasonic was applied to the extraction procedure at the corresponding conditions. The water extraction solutions were filtered, concentrated and precipitated by the addition of anhydrous ethanol to a final concentration of 80% (v/v). Precipitates were collected by centrifugation (3000 × *g*, 10 min), removed free proteins by Sevag method, washed by anhydrous ethanol and acetone for three times. After freezing dried, the corn silk polysaccharides extracted by enzymolysis-ultrasonic assisted extraction (EUPS). Polysaccharide yield (%) was calculated using the following equation:

$$\text{Polysaccharide yield}\,(\%) = \frac{C \times N \times V}{W \times 1000} \times 100 \qquad (2)$$

where C was the concentration of polysaccharide calculated from the standard curve equation (mg/mL); N was the dilution factor; V was the total volume of extraction solution (mL); and W was the weight of raw material (g).

2.5. Separation and purification of PS and EUPS

The crude polysaccharides were dissolved in deionized water, centrifuged and the supernatant was loaded onto a DEAE-52 cellulose ion exchange column (3 cm × 50 cm). Then the column was eluted with water, 0.1 M NaCl, 0.2 M NaCl and 0.5 M NaCl solution in proper order. Every elution fraction (5 mL) was collected and monitored for sugar content based on phenol–sulfuric acid method. Polysaccharides PS and EUPS were divided into 4 fractions, respectively. The main fractions of PS and EUPS were concentrated, dialyzed, lyophilized and named as PS-2 and EUPS-2, respectively.

2.6. Molecular weight distribution of PS-2 and EUPS-2

Molecular weight distribution of PS-2 and EUPS-2 was determined by gel filtration chromatography (GFC) on Sephadex G-100 column (2.5 cm × 60 cm, i.d.). The column was eluted by 0.2 M PBS (pH 6.8) at a flow rate of 8 mL/h. Fraction was collected for every 4 mL. The total carbohydrate of each fraction was determined by using phenol–sulfuric acid method. The molecular weight of polysaccharides was obtained from the regression line of the standard molecular weight compared with fraction number plot. The calibration curve was made with dextran standards of

different molecular weighs (Dextran T-500, T-70, T-40, and T-10) (Chen, Zhang, Qu, & Xie, 2008).

2.7. Composition analysis of PS-2 and EUPS-2

Using D-glucose as the standard, phenol–sulfuric acid method was employed for the measurement of total sugar content of polysaccharide. Bradford's method was used to measure protein content by using bovine serum albumin as the standard (Lowry, Rosebrough, Farr, & Randall, 1951). Uronic acid content was determined by m-hydroxydiphenyl method with galacturonic acid as standard (Bitter & Muir, 1962).

The composition of neutral monosaccharide was measured by gas chromatography (GC) after converting them into acetylated derivatives. Briefly, 10 mg of polysaccharide was hydrolyzed in a sealed glass tube with 2 M trifluoroacetic acid (TFA) at 105 °C for 10 h. The hydrolysates were evaporated to dryness. The acid was removed under reduced pressure by repeated co-evaporations with methanol. The hydrolysates were then converted into alditol acetates according to conventional procedures. Gas chromatography was performed on a Shimadzu GC-14B instrument with capillary column (HP-5, 30 m × 0.32 mm × 0.5 μm). The operation was performed in the following conditions: injection temperature: 250 °C; detector temperature: 260 °C; column temperature programmed: 150–210 °C increasing at 10 °C/min for 6 min; then increasing to 255 °C at 15 °C/min for 3 min; and finally increasing to 260 °C at 1 °C/min for 5 min. N_2 was used as the carrier gas and maintained at 1.0 mL/min. Monosaccharides rhamnose, arabinose, xylose, mannose, galactose and glucose were used as the standards.

2.8. FTIR analysis of PS-2 and EUPS-2

FTIR spectra of PS-2 and EUPS-2 (in KBr pellets, 2 mg sample/200 mg KBr) were measured using the TENSOR 27 (Bruker, Germany) infrared spectrometer operating at 4 cm^{-1} resolution.

2.9. Morphological analysis of PS-2 and EUPS-2

Scanning electron microscope (SEM) image of the polysaccharides PS-2 and EUPS-2 were obtained by means of an environmental scanning electron microscope (ESEM, Philips XL-30 TMP, Philips-FEI Co., Eindhoven, and the Netherlands). The dried powder PS-2 and EUPS-2 were placed on a specimen holder with the help of double-sided adhesive tapes and sputtered with gold powder using an into sputter coater. Each sample was observed with magnification of 1000 at an accelerating potential of 20 kV under a high vacuum condition.

2.10. Circular dichroism spectroscopy (CD) of PS-2 and EUPS-2

Circular dichroism (CD) spectra of PS-2 and EUPS-2 were determined on a J-810CD (JASCO, Japan) spectropolarimeter using sample solutions at concentration 0.5 mg/mL. Each CD spectrum was the accumulation of three times scans at 100 nm/min with a 1 nm slit width and a time constant of 1 s. Data was collected from 190 nm to 350 nm at 1 nm interval (Ma, Chen, Zhang, Zhang, & Fu, 2012).

2.11. Antioxidant activity analysis in vitro

2.11.1. Inhibitory activity against hydroxyl radicals

The inhibitory activity against hydroxyl radicals was quantified by the method described by Halliwell, Gutteridge, and Aruoma (1987) with minor modification. Briefly, the reaction mixture, containing different polysaccharides content (0.5–1.5 mg/mL), was incubated with 0.1 mL of deoxyribose (60 mM), EDTA (1.04 mM), ascorbic acid (2.0 mM), H_2O_2 (10.0 mM), and $FeCl_3$ (2.0 mM) in 0.4 mL of KH_2PO_4–KOH buffer solution (50 mM, pH 7.4) for 60 min at 37 °C. The reaction was terminated by adding 1 mL of HCl (25%, v/v) and 1 mL of TBA (1%, w/v) and then heating the tubes in a boiling water bath for 15 min. The contents were cooled to room temperature and the absorbance of the mixture was measured at 532 nm against a blank using UV–vis spectrophotometer (UV-2450, Shmadzu, Japan). The capability of inhibition to hydroxyl radical was calculated. Each test was conducted in triplicate.

2.11.2. Measurement of ferric reducing power (FRP)

FRP potential of CSPS was determined according to the modified method by Yen and Chen (1995). 100 μL of reaction mixture, containing different concentrations of samples were mixed with 0.7 mL of PBS (0.2 M, pH 6.6) and 2 mL of aqueous potassium hexacyanoferrate $K_3[Fe(CN)_6]$ solution (30 mM). After incubating at 50 °C for 20 min, 2 mL of TCA (10%, w/v) was added into the mixture and kept for 10 min. Then mix 1 mL of reaction solution with 3 mL of aqueous $FeCl_3$ (1.7 mM). The absorbance of the mixture was measured at 700 nm. A higher absorbance of the reaction mixture indicated a higher reducing power. Each test was conducted in triplicate.

2.11.3. Inhibitory effect on lipid peroxidation induced by Fe^{2+}/ascorbate

The assay was performed by using the method described by Chen et al. (2008) with a slight modification. The mice livers were cut into small pieces and homogenized in physiological saline at 4 °C. The reaction mixture was composed of 0.5 mL of tissue homogenate, 0.9 mL of physiological saline, 0.25 mL of 0.01 mM $FeSO_4$, 0.25 mL of 0.1 mM ascorbic acid, and 0.1 mL of ample aqueous solutions. The reaction mixture was incubated at 37 °C for 30 min. The reaction was terminated by adding 1 mL of TCA (20%, w/v) and 1 mL of TBA (0.67%, w/v) and then heating the tubes in a boiling water bath for 15 min. The contents were cooled to room temperature. After centrifugation at 3000 × g/min for 10 min, the absorbance of the supernatant was measured at 532 nm against a blank. Each test was conducted in triplicate. The antioxidant capacity of the inhibition of lipid peroxide formation was calculated.

2.12. Anticancer activity against human cancer cell lines

The human prostatic carcinoma cell lines PC3, LNcap and breast carcinoma cell lines MCF-7 (obtained from the American Type Culture Collection, Rockville, MD, USA) were used for the anticancer assays by MTT-based colorimetric method (Mosmann, 1983). These cell lines were maintained and cultured at 37 °C under humidified air, with 5% CO_2 atmosphere in RPMI1640 (GIBCO Invitrogen Corporation, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, 100 mg/mL streptomycin and 1.176 g/L sodium bicarbonate. The cells were pipetted into 96-well plates at the density of 1×10^4 cells/well and allowed to adhere at 37 °C under 5% CO_2 for 24 h. Then, 100 μL of test sample were added into each well. After 72 h incubation, MTT reagent (5.0 mg/mL) was added to each well and incubated for another 4 h at 37 °C. After incubation, media were removed and DMSO was added to dissolve purple precipitates. The absorbance of each well was read at 570 nm using an EMax microplate reader (Molecular Devices, Sunnyvale, CA, USA) and the inhibitory rate was calculated.

2.13. Statistical analysis

Values were expressed as means ± standard deviation (SD) of three replicates. Differences between groups were analyzed by a one-way analysis of variance (ANOVA) and student's test was used

Table 1
L9 $(3)^4$ result of orthogonal test.

Run	A (%)	B (°C)	C (min)	Y (yield %)
1	6.0	45	150	1.683 ± 0.001
2	6.0	50	180	3.249 ± 0.003
3	6.0	55	210	4.193 ± 0.012
4	7.5	45	180	3.013 ± 0.008
5	7.5	50	210	3.836 ± 0.001
6	7.5	55	150	4.893 ± 0.002
7	9.0	45	210	2.250 ± 0.017
8	9.0	50	150	2.729 ± 0.010
9	9.0	55	180	4.679 ± 0.005
$K1$	4.652	3.473	5.528	
$K2$	6.753	4.900	5.470	
$K3$	4.822	7.765	5.140	
R	2.191	4.292	0.388	

$R^2 = 0.9961$, $R^2_{adj.} = 0.9845$, CV % = 4.02.

for the statistical analysis and a p-value of less than 0.05 was considered significant.

3. Result and discussion

3.1. Enzymolysis pretreatment

The orthogonal test with three factors and three levels was designed to analyze the optimal parameters of the enzymolysis treatment. From the range analysis (Table 1), the polysaccharide yield was increased from 1.683% to 4.893%, the sequence of influential significance of the three factors was $B > A > C$, the optimum combination was $A_2B_3C_1$, so the optimum conditions were as follows, the cellulase content was 7.5%, the enzymolysis temperature was 55 °C, the enzymolysis time was 150 min. Under these conditions, the verified experiment yield was 4.86 ± 0.012%.

At the same time, the variable with the largest influence on polysaccharide yield was enzymolysis temperature ($P < 0.01$). The coefficient of variation (CV) and value of adjusted determination coefficient (adj. R^2) was 4.023 and 98.45%, respectively (Table 1), which indicated a high degree of precision of reliability of the experimental values and a high degree of correlation between the observed and predicted values.

3.2. Statistical analysis and model fitting

RSM optimization has many advantages over the traditional single factor optimization, which is more time-saving, material-saving and high efficient. In this study, a total of 17 experimental runs were performed for optimizing the three individual parameters ($X1$, $X2$, and $X3$) in the BBD. Table 2 shows the experimental conditions and the results of yield of polysaccharides according to the factorial design. By applying multiple regression analyses on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation:

$$Y = 6.873333 + 0.755 * X1 + 0.785 * X3 - 0.756667 * X1 * X1 - 0.716667 * X2 * X2 - 0.946667 * X3 * X3 \quad (3)$$

The analysis of variance was shown in Table 3. P-values were used as a tool to check the significance of each coefficient. The smaller the P-value, the more significant was the corresponding coefficient. It could be seen that the variables with the largest influence were $X1$, $X3$, $X1{*}X1$, $X2{*}X2$, $X3{*}X3$ ($P < 0.01$). Meanwhile, the coefficient, liquid–solid ratio ($X2$) and the interactive effect of ultrasonic time and ultrasonic temperature ($X1{*}X3$) also showed remarkable effects ($P < 0.05$). While the interactive effect of ultrasonic time and liquid–solid ratio ($X1{*}X2$), liquid–solid ratio and ultrasonic temperature ($X2{*}X3$) had less significant influence on the yield of crude polysaccharide from corn silk ($P > 0.05$).

Table 2
The Box–Behnken design matrix and the results for extraction yield of crude polysaccharides.

Run	X_1 (min)	X_2 (mL g^{-1})	X_3 (°C)	Y (yield %)
1	20	20	60	4.54 ± 0.007
2	20	40	60	5.18 ± 0.008
3	40	20	60	5.58 ± 0.001
4	40	40	60	6.30 ± 0.002
5	30	20	40	4.20 ± 0.001
6	30	20	80	5.70 ± 0.005
7	30	40	40	4.98 ± 0.018
8	30	40	80	5.96 ± 0.001
9	20	30	40	2.86 ± 0.009
10	40	30	40	5.58 ± 0.006
11	20	30	80	5.54 ± 0.005
12	40	30	80	6.70 ± 0.010
13	30	30	60	6.80 ± 0.005
14	30	30	60	6.92 ± 0.001
15	30	30	60	6.90 ± 0.006
16	30	30	60	6.90 ± 0.005
17	30	30	60	6.85 ± 0.003

The closer the value of R^2 to the unity, the better the empirical model fits the actual data (Lee, Yusof, Hamid, & Baharin, 2006). In this case, from Table 3, the R^2 value of 97.07% indicated that the response model could explain 97.07% of the total variations. In general, a regression model having a R^2 value higher than 0.9 was considered to have a very high correlation. The value of the adjusted R^2 (adj. R^2 = 93.30%) was also high enough to indicate the significance of this model.

3.3. Effects of ultrasonic time, liquid–solid ratio, and ultrasonic temperature on the yield of CSPS

Interactions between the variables and the relationship between responses and experiment levels of each variable are illustrated by the 3-D response surface and 2-D contour plots. A circular contour plot indicated that the interactions between the corresponding variables were negligible, while elliptical contour plot indicated otherwise (Muralidhar, Chirumamila, Marchant, & Nigam, 2001). In the present study, significance analysis results (Table 3) showed that interactions between the variables were insignificant ($P > 0.05$). It was in agreement with previous investigation (Yan et al., 2011), which had reported that the interaction between ultrasonic time and liquid–solid ratio, and liquid–solid ratio and ultrasonic temperature caused no significant effect on the extraction yield. The ultrasonic time ($X1$) and liquid–solid ratio ($X2$) both had positive effects on the yields. There was an increase in the CSPS yield when the yield reached its maximum at a fixed ultrasonic temperature, no significant further improvement appeared thereafter (Fig. 1A). The liquid–solid ratio and extraction temperature also had a similar effect on the CSPS yields when the extraction time was fixed (Fig. 1B). As shown in Fig. 1C, longer or shorter times led to a decrease in the CSPS yield.

By analyzing these three-dimensional plots and their respective contour plots, the predicted values (7.18%) of the extracted polysaccharides lay in the following conditions: ultrasonic time for 34.2 min, liquid–solid ratio at 31.8, and ultrasonic temperature at 66.3 °C. To validate the adequacy of the model equations, a verification experiment was carried out under the optimal conditions mentioned above. Under those conditions, the experimental yield was 7.10 ± 0.15% ($n = 3$), which was well-matched with the predicted value obtained from real experiments.

Table 3
Regression coefficients estimate and their significance test for the quadratic polynomial model.

Model term	Sum of squares	*df*	Mean square	*F*-value	Probability (*P*) > *F*	Significance
Model	20.2025	9	2.244723	25.76249	0.0001	***
*X*1 (ultrasonic time)	4.5602	1	4.5602	52.33703	0.0002	***
*X*2 (liquid–solid ratio)	0.72	1	0.72	8.263379	0.0238	*
*X*3 (ultrasonic temperature)	4.9298	1	4.9298	56.5789	0.0001	***
*X*1*X2	0.0016	1	0.0016	0.018363	0.8960	
*X*1*X3	0.6084	1	0.6084	6.982555	0.0333	*
*X*2*X3	0.0676	1	0.0676	0.775839	0.4076	
$X1\hat{}$	2.412838	1	2.412838	27.69194	0.0012	**
$X2\hat{}$	2.164585	1	2.164585	24.84276	0.0016	**
$X3\hat{}$	3.776038	1	3.776038	43.33727	0.0003	**

R^2 = 0.9707, $R^2_{adj.}$ = 0.9330, CV % = 5.15, and mean = 5.73.

3.4. Purification of PS and EUPS

PS and EUPS were isolated from hot water and enzymolysis-ultrasonic assistant extraction with a yield of 4.56% and 7.10%, respectively, and then fractionated on DEAE-52 cellulose column chromatography. Eight main fractions were then obtained, and termed as PS-1, PS-2, PS-3, PS-4 (EUPS-1, EUPS-2, EUPS-3, EUPS-4,), respectively (Fig. 2). PS-1 (EUPS-1) was from the water elute fraction, PS-2, PS-3, PS-4 (EUPS-2, EUPS-3, EUPS-4,) were from the 0.1 M, 0.2 M, 0.5 M NaCl elute fractions, respectively. The main fractions PS-2 and EUPS-2 remained to be further studied (Fig. 2).

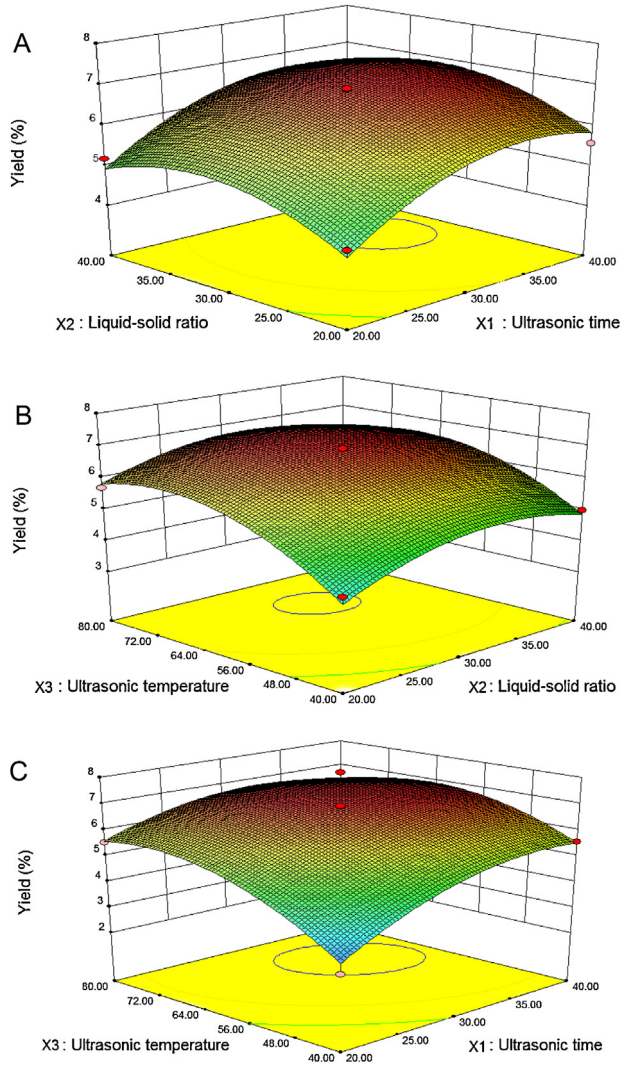

Fig. 1. Response surface plots for EUPS yield. (A) Effect of ultrasonic time and liquid–solid ratio on EUPS yield. (B) Effect of liquid–solid ratio and ultrasonic temperature on the yield on EUPS yield. (C) Effect of ultrasonic time and ultrasonic temperature on EUPS yield.

3.5. Chemical composition analysis

The neutral sugar content of PS-2 and EUPS-2 were 58.75 ± 0.03% and 76.73 ± 0.07%, respectively. The uronic acid contents of those were 30.87 ± 0.05% and 13.18 ± 0.02%. There was hardly any protein in PS-2 and EUPS-2. GC results showed that both PS-2 and EUPS-2 were found to be composed of rhamnose, arabinose, xylose, mannose, galactose and glucose with molecular ratio of 4.17:17.33:5.59:18.65:19.11:35.14 and 8.83:15.77:7.92:12.39:11.15:43.94, respectively. The results showed that ultrasonic treatment during the extraction process induced the changes of molecular ratio of monosaccharides. The results were different from the study of Li, Cui, and Zhao (2008), in which corn silk polysaccharide detected by HPLC was composed of

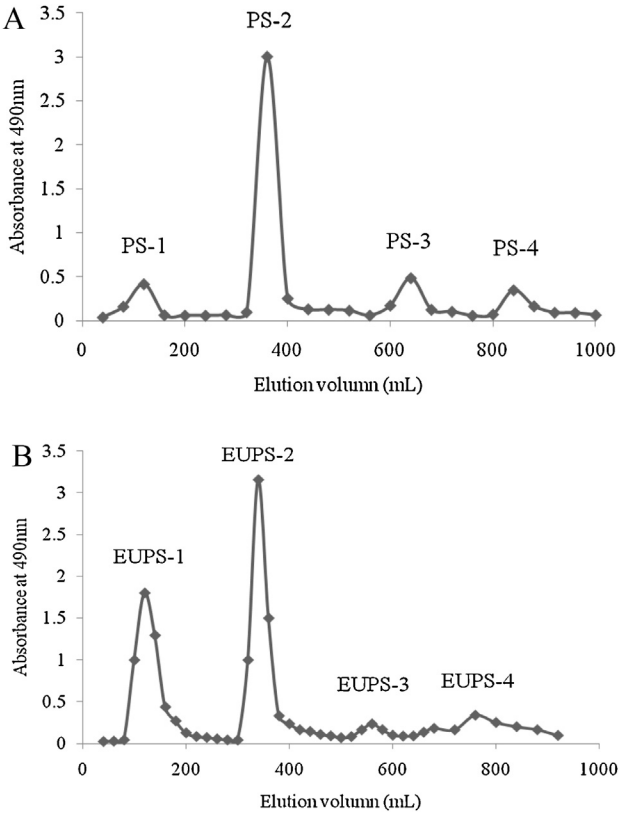

Fig. 2. Elution curves of PS (A) and EUPS (B) by DEAE-52 cellulose column.

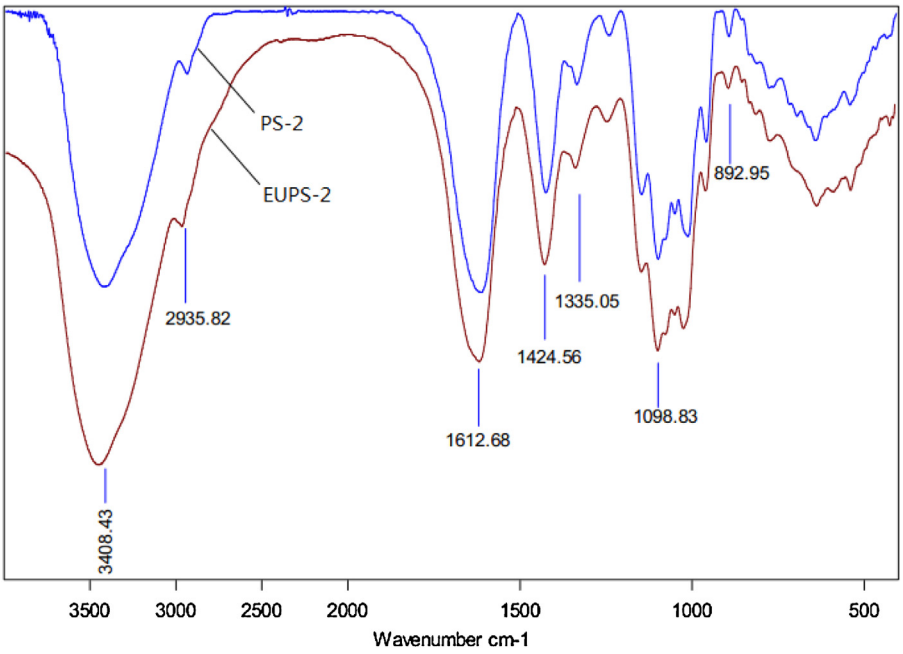

Fig. 3. FTIR spectrum of polysaccharides from corn silk.

glucose, galactose, arabinose, galacturonic acid, mannose, xylose with molar ratio of 1:0.89:0.48:0.39:0.34:0.26. Wang, Liu, Qiu, Cai, and Xie (2009) reported that corn silk polysaccharide obtained by microwave-assisted extraction was composed of rhamnose, arabinose, and mannose with a ratio of 1.505:1.000:1.120. The difference might be due to the different resource of corn silk, different extraction, purification and analysis methods of the polysaccharides.

3.6. Molecular weight distribution

Molecular weight of PS-2 and EUPS-2 was determined by gel filtration chromatography (GFC) on Sephadex G-100 column. The molecular weight distribution of the main wide peak of PS-2 was 10.52×10^4 Da. While the molecular weight distribution of the main wide peak of EUPS-2 was 6.88×10^4 Da. The results showed that the molecular weight of the polysaccharides dealing with enzymolysis and ultrasonic treatment was lower than that of extracted by hot water, which might be due to two reasons. One reason might be the cellulase can hydrolyze plant cell wall to promote polysaccharides dissolution. At the meantime, cellulase may decompose a part of polysaccharides to small ones. The other reason might be the ultrasound could decrease molecular weight by splitting the most susceptible chemical bond (Li, Li, Guo, & Li, 2005). Degrading polysaccharide using ultrasound to enhance its physicochemical properties and ever to change its activity function has been reported (Zhou & Ma, 2006).

3.7. FTIR analysis

FTIR spectroscopy is typically used for the qualitative analysis of organic functional groups, especially for O—H, N—H, and C═O. The FTIR spectra of PS-2 and EUPS-2 were illustrated in Fig. 3. No significant difference was detected in the FTIR of the two polysaccharides, which indicated that the two polysaccharides had the same chemical groups. The strong absorption band appeared at 3000–3500 cm^{-1} was due to stretching vibration of saccharides O—H and protein N—H. The peak at 2935 cm^{-1} was assigned to CH stretching of the CH_2 and CH_3 groups. And the bands in the region 1310–1450 cm^{-1} were corresponded to symmetrical deformations of CH_2 and C—OH groups. The band at 1612 cm^{-1} was assigned to ring stretching of glucose appeared (de Paula Regina, Heatley, & Budd, 1998). Thus carbohydrates were preliminarily identified from the infrared spectra, and group compositions of the two polysaccharides were almost the same. The absorption band at 1424 cm^{-1} was inferred to be variable angle

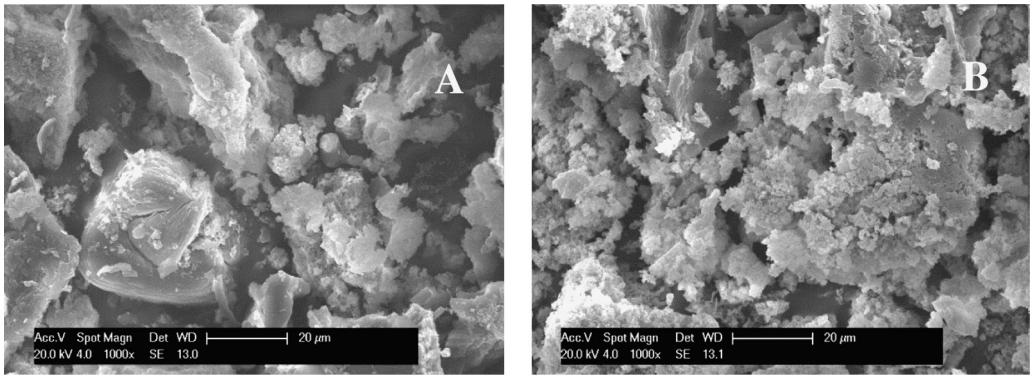

Fig. 4. SEM images of PS-2 and EUPS-2, ×1000. (A) PS-2, the polysaccharide obtained from corn silk by hot water. (B) EUPS-2, the polysaccharide extracted from corn silk at the enzymolysis-ultrasonic treatment condition.

vibration of N—H group, indicating that the existence of protein. An absorbance at nearly 892 cm^{-1} suggested the linkage of β-glycosides in the molecular structure of the two polysaccharides (Wu, Zhu, Zhang, Yang, & Zhou, 2012). The bands attributed to primary alcoholic —CH_2OH stretching mode and C—O—C stretching vibrations appearedat about 1098 cm^{-1}.

3.8. Morphological analysis

Fig. 4A and B presented the scanning electron micrographs (SEM) of PS-2 and EUPS-2 at magnification of 1000×. The surface morphology of the two polysaccharides showed significant difference in size and shape. PS-2 exhibited a lumpish appearance with a size of 10–90 μm (Fig. 4A). Refer to EUPS-2, most of the polysaccharides exhibited a granular shape with a size ranged from 3 to 50 μm (Fig. 4B). After ultrasonic treatment, a significant effect on the morphological changes caused by ultrasonic cavitation was observed. EUPS-2 contains clear and small particles distributed discretely (Fig. 4B). These results were in step with those of the molecular weight size.

3.9. Circular dichroism (CD) analysis

Circular dichroism was an effective method to investigate the three-dimensional structure of compounds (Kwaambwa & Maikokera, 2008). Due to the low uronic acid content in PS-2 and EUPS-2, PS-2 and EUPS-2 belonged to neutral polysaccharide. Owing to lacking spectral characteristic in ultraviolet band, it is hard to determine structure characteristic of polysaccharides or neutral polysaccharides according to CD. Thus the conformation of polysaccharides could be obtained by molecular modification or complexing with Congo red (Bystricky, Szu, Gotoh, & Kovac, 1995). After complexing with Congo red, polysaccharide PS-2 showed positive Cotton effect in 210 nm, indicating that PS-2 was ordered and formed complex with Congo red. At the same time, strong negative Cotton effect appeared in 194 nm and 200 nm, which suggested that PS-2 had ordered helical structure in water solution (Fig. 5A). After complexing with Congo red, EUPS-2 showed positive Cotton effect at 202 nm, indicating that EUPS-2 was ordered and formed complex with Congo red. Strong negative Cotton effect appeared at 200 nm, indicating that EUPS-2 exhibited ordered helical structure in water solution (Fig. 5B).

In order to investigate the polysaccharide conformation changes influenced by ionic strength, Ca^{2+} was added into polysaccharide solution. CD spectrum of PS-2 and EUPS-2 added with Ca^{2+} appeared weak negative peak, and peak intensity became strong. It was suggested that Ca^{2+} had interaction with some carboxyl groups in polysaccharide chains, which led to a part of chain polymerization of CSPS (Zhang, 1998). Thus the structure of PS-2 and EUPS-2 altered and an increasing asymmetry appeared as the existence of Ca^{2+}. Other positive and negative characteristic signals remained unchanged (Fig. 5A and B), illustrating that molecules of CSPS built the bridge connection by Ca^{2+} or complexing with Ca^{2+}, and the basic conformation of CSPS remained unchanged (Kwaambwa & Maikokera, 2008).

3.10. Antioxidant activities analysis

3.10.1. Inhibitory activity against hydroxyl radicals

Hydroxyl radicals, generated by reaction of iron–EDTA complex with H_2O_2 in the presence of ascorbic acid, attack deoxyribose to form products that, upon heating with 2-thiobarbituric acid under acid conditions, yield a pink tint. Added hydroxyl radical scavengers compete with deoxyribose for the resulted hydroxyl radicals and diminish tint formation (Cheng, Ren, Li, Chang, & Chen, 2002). The above-mentioned theory was used to measure inhibitory effect

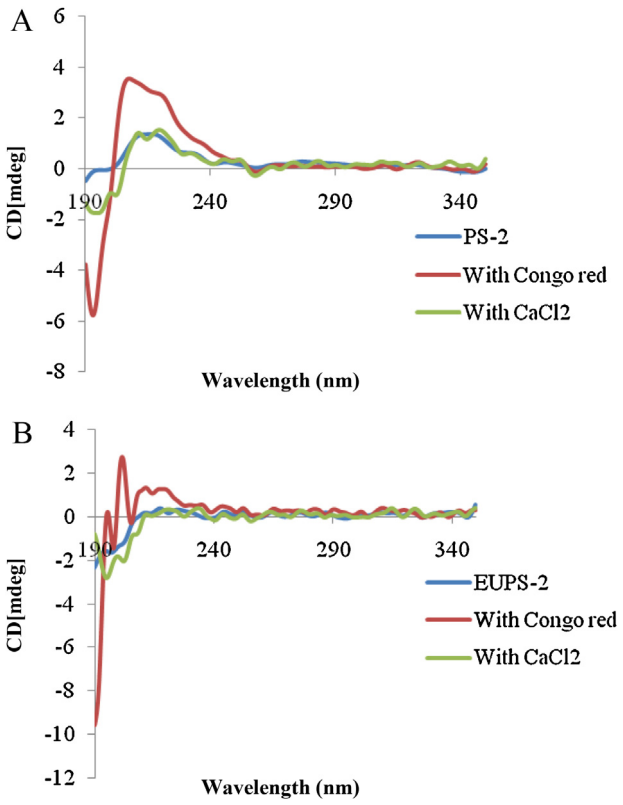

Fig. 5. CD spectra results of PS-2 and EUPS-2. (A) Adding Congo red and $CaCl_2$ to PS-2 solution. (B) Adding Congo red and $CaCl_2$ to EUPS-2 solution.

of CSPS on hydroxyl radicals. Fig. 6A showed that the inhibitory effect of EUPS-2 (0.30–1.50 mg/mL) on hydroxyl radicals were more evident as compared with PS-2. At 0.90 mg/mL, EUPS-2 exhibited strong ability to quench hydroxyl radical (52.6%), which was relatively higher than that of PS-2 (30.5%). The IC_{50} values of EUPS-2 and PS-2 were 0.89 and 1.36 mg/mL. It was reported that scavenging effect on hydroxyl radical of Vitamin C was between 50.0% and 60.0% at 1.63 mg/mL (Qi et al., 2005), which suggested that the scavenging ability on hydroxyl radical of CSPS was more pronounced than that of Vitamin C. In the meantime, the corn silk polysaccharides obtained from enzymolysis and ultrasonic treatment demonstrated preferable scavenging capability.

Qi et al. (2005) reported two types of antioxidant mechanism: the effect of the metal complexes which lead to the suppression against hydroxyl radical generation, and the direct clearance of the generated hydroxyl radical. The mechanism of CSPS on cleaning hydroxyl radicals needs to be further investigated. However, our present results suggested that polysaccharides obtained by different extraction methods demonstrated different activities, which, in some respects, had some relationship with their different molecular structures.

3.10.2. Measurement of ferric reducing power (FRP)

The current literatures reported that many different *in vitro* methods are being used to evaluate antioxidants of substances from food and biological systems (Frankel & Meyer, 2000). In some of these methods, antioxidant assays were performed in alcoholic solutions, however, on these conditions polysaccharides would precipitate. Thus, the antioxidant power of polysaccharides from corn silk was estimated by the FRP assay in aqueous solution.

As shown in Fig. 6B, the reducing power values of EUPS-2 and PS-2 were 0.155 and 0.099, respectively, when the concentration was 31.25 μg/mL. The reducing capacity of the two samples ascended

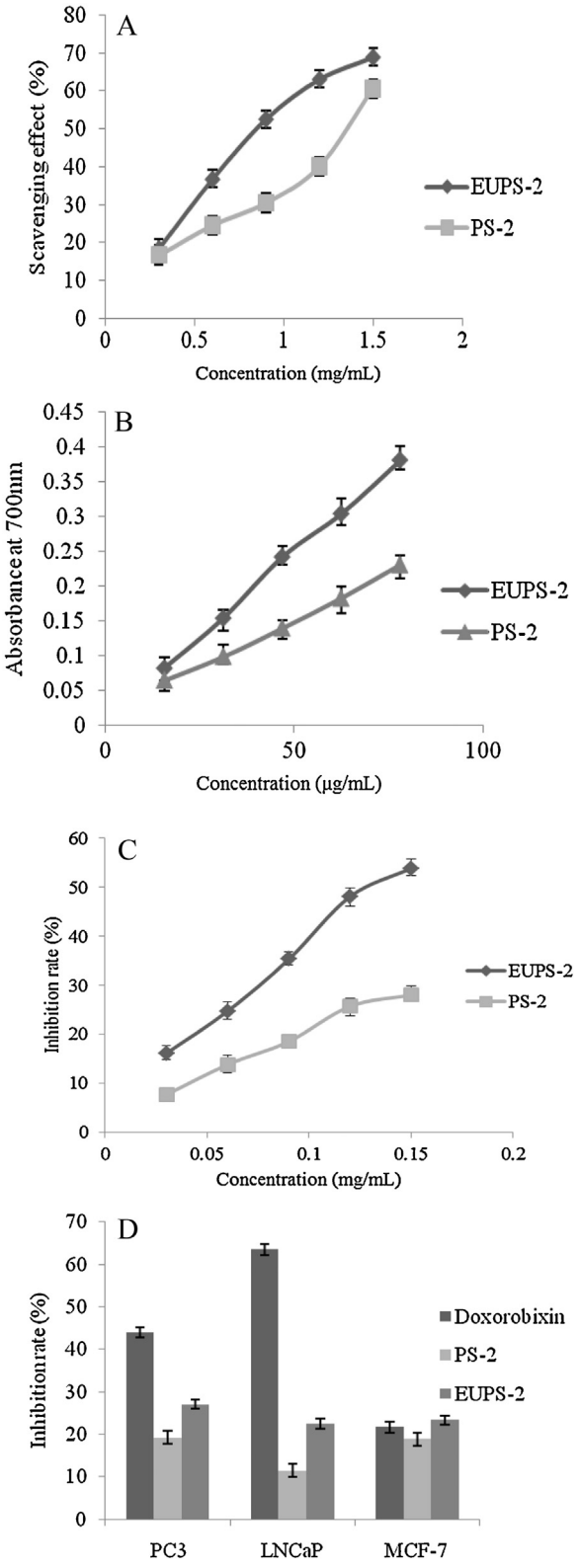

Fig. 6. Antioxidant and anticancer activities results of PS-2 and EUPS-2. (A) Scavenging hydroxyl radical activity of PS-2 and EUPS-2. (B) Ferric reducing power activity of PS-2 and EUPS-2. (C) Lipid peroxidation inhibition activity of PS-2 and EUPS-2. (D) Inhibitory effect on human cancer cells of PS-2 and EUPS-2. Results are means ± SD of three measurements.

with increasing concentration, which indicated that EUPS-2 and PS-2 were electron donors and could react with Fe^{3+}/ferricyanide to convert them into Fe^{2+}. In the detected concentration, EUPS-2 showed pronounced advantage compared with PS-2 ($P < 0.05$). The results were in accordance with the results of scavenging hydroxyl radical. Both of them indicated dose-dependent inhibitory effects with the coefficient $R^2 = 0.9979$ and 0.9962, respectively.

3.10.3. Inhibitory effect on lipid peroxidation induced by Fe^{2+}/ascorbate

Malondialdehyde (MDA) is the secondary byproduct, which is released during the lipid peroxidation. A decrease in the production of MDA in turn symbolizes the inhibition of lipid peroxidation. The liver peroxidation inhibition activity of PS-2 and EUPS-2 was summarized in Fig. 6C, showing that the generation of MDA was inhibited owing to the existence of CSPS. It was showed that EUPS-2 had an obviously stronger inhibition activity than that of PS-2 ($P < 0.05$). Both of them indicated dose-dependent inhibitory effects. IC_{50} value of EUPS-2 was 0.13 mg/mL, which was close to that of Vitamin E, 0.15 mg/mL. However, IC_{50} value of PS-2 could not be read. Although the antioxidant capacity of corn silk polysaccharide had been proven, the relationships between structure of corn silk polysaccharides and antioxidant mechanisms had not yet been elucidated. One of the reasons was that the huge structural diversity of these polysaccharides had given a major hindrance in the establishment of structure–activity relationship. According to Xue, Yu, Hirata, Terao, and Lin (1998), differences in antioxidant abilities of marine polysaccharides might be related to sulfate content and molecular weight. It had been proved that different molecular weight and neutral sugar composition of polysaccharides would also play a role inhibiting human LDL oxidation *in vitro* (Xue et al., 2001). In this study, ultrasound process resulted in the changes of the structure of corn silk polysaccharides, including molecular weight, neutral sugar composition, the content of neutral sugar and uronic acid. It was concluded that the effect of the above-mentioned factors on the bioactivity might not be neglected.

3.11. Anticancer activity

The anti-tumor activity of CSPS against human prostate cancer cell lines PC3, LNCaP and breast cancer cell lines MCF7 was investigated *in vitro*. The colorimetric MTT-based assay was a rapid and sensitive spectrophotometric assay for determining viability in monolayer culture cell lines. The anticancer activity results of CSPS was illustrated in Fig. 6D. Using doxorobixin as the positive control, in the concentration of 200 μg/mL, inhibition rates of EUPS-2 on PC3, LNCaP and MCF7 were 27.1%, 22.5% and 23.4%, respectively. Comparative results of PS-2 were 19.3%, 11.5%, 18.9%. The inhibitory effects of EUPS-2 on PC3, LNCaP and MCF7 demonstrated a slight advantage over PS-2 ($P < 0.05$). The results were in accordance with the antioxidant analysis, in which EUPS-2 showed higher antioxidant activities. It was reported that the anticancer activity of polysaccharides was probably a consequence of the stimulation of the cell-mediated immune response (Ooi & Liu, 2000). Some literatures reported that the immunostimulatory effect might be the main mechanism for the anti-tumor activities of polysaccharides extracted from *Cyclina sinensis* (Jiang et al., 2011) and *Ganoderma lucidum* (Cao & Lin, 2004). The mechanism of CSPS acted on human cancer cells needs to be further studied.

The levels of anti-oxidation and reactive oxygen species had deep relationship with the generation and malign transformation of cancer cells. If compounds could enhance the level of anti-oxidation and clear the reactive oxygen species in cancer cells, they might inhibit the cells growth (Leng, Liu, & Chen, 2005). In the present antioxidant assay, it was obviously demonstrated that EUPS-2 showed stronger antioxidant activity than PS-2 *in vitro*.

Therefore, the higher anticancer activity of EUPS-2 might be related to their relatively stronger antioxidant activity.

4. Conclusion

An efficient enzymolysis-ultrasonic assisted extraction procedure (EUAE) was employed to extract polysaccharides from corn silk (CSPS) in this study for the first time. The orthogonal test and Box–Behnken design (BBD) were applied to optimize the process parameters of enzymolysis (cellulase content of 7.5% for 150 min at 55 °C) and ultrasonic pretreatment (liquid–solid ratio of 31.8 for 34.2 min at 66.3 °C) and a maximum CSPS yield of (7.10%) could be obtained. Furthermore, ultrasonic treatment could result in the changes of the chemical composition, morphological feature and conformation of CSPS and the decrease of the molecular weight distribution of CSPS. Polysaccharides obtained by EUAE possessed lower molecular weight (6.88×10^4 Da), uronic acid content (13.18%) and high neutral sugar content (76.73%), presented more remarkable antioxidant properties (scavenging hydroxyl radical and inhibition effect on lipid peroxidation) and inhibitory effects on the proliferation of human prostate and breast cancer cells than polysaccharides from hot water extraction. CSPS might be a new source of natural antioxidants with potential value for health food and therapeutics.

Acknowledgements

The authors are grateful for the financial support of this research from the National High Technology Research and Development Program ("863" Program) of China (Grant No. SS2013AA100207), the financial support of Project of National Key Technology Research and Development Program for the 12th Five-year Plan (No. 2012BAD33B08).

References

Bitter, T., & Muir, H. M. (1962). A modified uronic acid carbazole reaction. *Analytical Biochemistry*, *4*, 330–334.
Box, G. E. P., & Behnken, D. W. (1960). Some new three level designs for the study of quantitative variables. *Technometrics*, *2*, 455–475.
Bystricky, S., Szu, S. C., Gotoh, M., & Kovac, P. (1995). Circular dichroism of the O-specific polysaccharide of *Vibrio cholerae* O1 and some related derivatives. *Carbohydrate Research*, *270*, 115–122.
Cao, Q. Z., & Lin, Z. B. (2004). Antitumor and anti-angiogenic activity of *Ganoderma lucidum* polysaccharides peptide. *Acta Pharmacologica Sinica*, *25*, 833–838.
Chen, H. X., Zhang, M., Qu, Z. S., & Xie, B. J. (2008). Antioxidant activities of different fractions of polysaccharide conjugate from green tea (*Camellia sinensis*). *Food Chemistry*, *106*, 559–563.
Cheng, Z. Y., Ren, J., Li, Y. Z., Chang, W. B., & Chen, Z. D. (2002). Study on the multiple mechanisms underlying the reaction between hydroxyl radical and phenolic compounds by qualitative structure and activity relationship. *Bioorganic and Medicinal Chemistry*, *10*, 4067–4073.
Choi, S. K., & Choi, H. S. (2004). Purification and characterization of an anticoagulant from corn silk. *Han'guk Sikp'um Yongyang Kwahak Hoechi*, *33*, 1262–1267.
Du, J., & Xu, Q. T. (2007). A study on mechanisms of stigma maydis polysaccharide on weight loss in experimental animals. *Chinese Pharmacological Bulletin*, *23*, 816–820.
Du, J., Xu, Q. T., & Gao, X. H. (2007). Effects of stigma maydis polysaccharide on gastrointestinal movement. *Journal of Traditional Chinese Medicine*, *32*, 1203–1206.
Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture*, *80*, 1925–1941.
Gan, C. Y., Manaf, N. H. A., & Latiff, A. A. (2010). Optimization of alcohol insoluble polysaccharides (AIPS) extraction from the *Parkia speciosa* pod using response surface methodology (RSM). *Carbohydrate Polymers*, *79*, 825–831.
Habtemaram, S. (1998). Extract of silk (stigma of *Zea mays*) inhibits the tumour necrosis factor-α- and bacterial lipopolysaccharide-induced cell adhesion and ICAM-1 expression. *Planta Medica*, *64*, 314–318.
Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: A simple 'test-tube' assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry*, *165*, 215–219.
Hou, X. J., & Chen, W. (2008). Optimization of extraction process of crude polysaccharides from wild edible BaChu mushroom by response surface methodology. *Carbohydrate Polymers*, *73*, 67–74.
Hu, Q. L., Zhang, L. J., Li, Y. N., Ding, Y. J., & Li, F. L. (2010). Purification and anti-fatigue activity of flavonoids from corn silk. *International Journal of Physical Sciences*, *5*, 321–326.
Huang, Y. L., Chow, C. J., & Tsai, Y. H. (2012). Composition, characteristics, and in-vitro physiological effects of the water-soluble polysaccharides from Cassia seed. *Food Chemistry*, *134*, 1967–1972.
Jiang, C. X., Wang, M. C., Liu, J., Gan, D., & Zeng, X. X. (2011). Extraction, preliminary characterization, antioxidant and anticancer activities in vitro of polysaccharides from *Cyclina sinensis*. *Carbohydrate Polymers*, *84*, 851–857.
Kang, S. M., Kim, K. N., Lee, S. H., Ahn, G., Cha, S. H., & Kim, A. D. (2011). Anti-inflammatory activity of polysaccharide purified from AMG-assistant extract of *Ecklonia cava* in LPS-stimulated RAW 264.7 macrophages. *Carbohydrate Polymers*, *85*, 80–85.
Kwaambwa, H. M., & Maikokera, R. (2008). Infrared and circular dichroism spectroscopic characterization of secondary structure components of a water treatment coagulant protein extracted from *Moringa oleifera* seeds. *Colloids and Surfaces B: Biointerfaces*, *64*, 118–125.
Lee, W. C., Yusof, S., Hamid, N. S. A., & Baharin, B. S. (2006). Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM). *Journal of Food Engineering*, *73*, 55–63.
Leng, B., Liu, X. D., & Chen, Q. X. (2005). Inhibitory effects of anticancer peptide from Mercenaria on the BGC-823 cells and several enzymes. *FEBS Letters*, *579*, 1187–1190.
Li, B., Cui, Z. K., & Zhao, Y. C. (2008). Study on the preparation and composition of polysaccharide in corn silk. *Academic Periodical of Farm Products Processing*, *136*, 34–36.
Li, Y., Li, J., Guo, S., & Li, H. (2005). Mechanochemical degradation kinetics of high-density polyethylene melt and its mechanism in the presence of ultrasonic irradiation. *Ultrasonics Sonochemistry*, *12*, 183–189.
Liang, R. J. (2008). Orthogonal test design for optimization of the extraction of polysaccharides from *Phascolosoma esulenta* and evaluation of its immunity activity. *Carbohydrate Polymers*, *73*, 558–563.
Liu, J., Wang, C. N., Wang, Z. Z., Zhang, C., Lu, S., & Liu, J. B. (2011). The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (*Zea mays* L.) and related flavone glycosides. *Food Chemistry*, *126*, 261–269.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry*, *193*, 265–275.
Ma, L. S., Chen, H. X., Zhang, Y., Zhang, N., & Fu, L. L. (2012). Chemical modification and antioxidant activities of polysaccharide from mushroom *Inonotus obliquus*. *Carbohydrate Polymers*, *89*, 371–378.
Miller, S. S., Reid, L. M., Butler, G., Winter, S. P., & McGoldrick, N. J. (2003). Long chain alkanes in silk extracts of maize genotypes with varying resistance to *Fusarium graminearum*. *Journal of Agricultural and Food Chemistry*, *51*, 6702–6708.
Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*, 55–63.
Muralidhar, R. V., Chirumamila, R. R., Marchant, R., & Nigam, P. (2001). A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. *Biochemical Engineering Journal*, *9*, 17–23.
Ooi, V. E., & Liu, F. (2000). Immunomodulation and anti-cancer activity of polysaccharide–protein complexes. *Current Medicinal Chemistry*, *7*, 715–729.
de Paula Regina, C. M., Heatley, F., & Budd, P. M. (1998). Characterization of *Anacardium occidentale* exudate polysaccharide. *Polymer International*, *45*, 27–35.
Prakash Maran, J., Manikandan, S., Thirugnanasambandham, K., Vigna Nivetha, C., & Dinesh, R. (2013). Box–Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk polysaccharide. *Carbohydrate Polymers*, *92*, 604–611.
Qi, H. M., Zhang, Q. B., Zhao, T. T., Chen, R., Zhang, H., Niu, X. Z., et al. (2005). Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) in vitro. *International Journal of Biological Macromolecules*, *37*, 195–199.
Velazquez, D. V. O., Xavier, H. S., Batistac, J. E. M., & de Castro-Chaves, C. (2005). *Zea mays* L. extracts modify glomerular function and potassium urinary excretion in conscious rats. *Phytomedicine*, *12*, 363–369.
Wang, C. N., Zhang, T. H., Liu, J., Lu, S., Zhang, C., Wang, E. L., et al. (2011). Subchronic toxicity study of corn silk with rats. *Journal of Ethnopharmacology*, *137*, 36–43.
Wang, L., Liu, X. F., Qiu, F. P., Cai, J. X., & Xie, R. L. (2009). Extraction of corn silk polysaccharides by microwave-assisted technology. *Journal of Food Science and Biotechnology*, *28*, 72–75.
Wang, Q., Zhou, J., & Wen, Q. B. (2006). Extraction of polysaccharide from Ginkgo Biloba seed by ultrasonic wave. *Food and Fermentation Industry*, *32*, 126–128.
Wu, W. L., Zhu, Y. T., Zhang, L., Yang, R. W., & Zhou, Y. H. (2012). Extraction, preliminary structural characterization, and antioxidant activities of polysaccharides from *Salvia miltiorrhiza* Bunge. *Carbohydrate Polymers*, *87*, 1348–1353.
Xue, C., Fang, Y., Lin, H., Chen, L., Li, Z., Deng, D., et al. (2001). Chemical characters and antioxidative properties of sulfated polysaccharides from *Laminaria japonica*. *Journal of Applied Phycology*, *13*, 67–70.
Xue, C., Yu, G., Hirata, T., Terao, J., & Lin, H. (1998). Antioxidative activities of several marine polysaccharides evaluated in a phosphatidylcholine-liposomal suspension and organic solvents. *Bioscience, Biotechnology and Biochemistry*, *62*, 206–209.
Yan, Y. L., Yu, C. H., Chen, J., Li, X. X., Wang, W., & Li, S. Q. (2011). Ultrasonic-assisted extraction optimized by response surface methodology, chemical composition and antioxidant activity of polysaccharides from *Tremella mesenterica*. *Carbohydrate Polymers*, *83*, 217–224.

Yang, L. Q., & Zhang, L. M. (2009). Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. *Carbohydrate Polymers*, *76*, 349–361.

Yen, G. C., & Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry*, *43*, 27–32.

Yin, X. L., You, Q. H., & Jiang, Z. H. (2011). Optimization of enzyme assisted extraction of polysaccharides from *Tricholoma matsutake* by response surface methodology. *Carbohydrate Polymers*, *86*, 1358–1364.

Zeringue, H. J. (2000). Identification and effects of maize silk volatiles on cultures of *Aspergillus flavus*. *Journal of Agricultural and Food Chemistry*, *48*, 921–925.

Zhang, Y. S. (1998). Conformational study of polysaccharide. *Journal of Northeast Normal University*, *2*, 55–60.

Zhao, L. Y., Dong, Y. H., Chen, G. T., & Hu, Q. H. (2010). Extraction, purification, characterization and antitumor activity of polysaccharides from *Ganoderma lucidum*. *Carbohydrate Polymers*, *80*, 783–789.

Zhao, W. Z., Yin, Y. G., Yu, Z. P., Liu, J. B., & Chen, F. (2012). Comparison of anti-diabetic effects of polysaccharides from corn silk on normal and hyperglycemia rats. *International Journal of Biological Macromolecules*, *50*, 1133–1137.

Zhao, W. Z., Yu, Z. P., Liu, J. B., Yu, Y. D., Yin, Y. G., Lin, S. Y., et al. (2011). Optimized extraction of polysaccharides from corn silk by pulsed electric field and response surface quadratic design. *Journal of the Science of Food and Agriculture*, *91*, 2201–2209.

Zhou, C., & Ma, H. (2006). Ultrasonic degradation of polysaccharide from a red algae (*Porphyra yezoensis*). *Journal of Agricultural and Food Chemistry*, *54*, 2223–2228.

Zou, Y. F., Chen, X. F., Yang, W. Y., & Liu, S. (2011). Response surface methodology for optimization of the ultrasonic extraction of polysaccharides from *Codonopsis pilosula* Nannf. var. modesta L.T. Shen. *Carbohydrate Polymers*, *84*, 503–508.