Original Research Paper

The Deproteinization, Antioxidant Acticities and Inhibitory Effect on α-Amylase of Polysaccharides from Corn Silk

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Abstract: An approach of deproteinization of polysaccharides from corn silk (CSPs) was established to improve the purity and activities of CSPs by investigating Sevag method, trichloroacetic acid (TCA) method and HCl method. Deproteinization rate, polysaccharide retention rate, antioxidant activities and inhibition α-amylase effects were used as indicative mark to select suitable deproteinization method of CSPs. The crude CSPs was extracted by the hot water method. Results showed that TCA method had higher deproteinization rate and polysaccharide retention rate than that of Sevag method and HCl method, and the deproteinization optimum concentration of TCA was 4%. Under the optimized conditions, the deproteinization rate and polysaccharide retention rate were 53.68% and 64.13% respectively. In terms of antioxidant and α-amylase inhibitory effects, TCA-deproteinized CSPs (T-CSPs) had stronger activities, followed by Sevag-deproteinized CSPs (S-CSPs) and HCl-deproteinized CSPs (H-CSPs). Form a conclusion, TCA method was a good way to remove protein.

Keywords: Corn Silk, Polysaccharides, Deproteinization, Antioxidant Activities, α-Amylase Inhibition Effect

Introduction

Corn silk (*Stigma maydis*) is the Stigma of *Zea mays* Line. In previous investigations of corn silk, it contains a variety of chemical constituents (He *et al*., 2012), such as volatile oil, saponins, alkaloids, flavonoids, polysaccharide and organic acids. It was reported that the extracts of corn silk also possessed extensive bioactivities including antihyperglycemic (Liu *et al*., 2002), antihypertensive, antioxidant (Chen *et al*., 2005) and immunomodulatory activity (Kim *et al*., 2004). As a natural polysaccharide, the polysaccharides from corn silk (CSPs) possessed significantly higher antioxidant and α-amylase inhibitory abilities and might be used as a novel nutraceutical agent for human consumption (Maksimovic *et al*., 2003; Chen *et al*., 2013).

The existence of protein in polysaccharide fraction is a serious problem (a false conclusion may be led) during the study on characterization, physicochemical properties and biological activity of polysaccharides. Therefore, the proper characterization of these compounds requires deproteinization. And current researches had demonstrated that the pharmacological activities were also closely related to its richness of polysaccharides in the flesh (Wang *et al*., 2006). However, impurities,

especially proteins, are co-precipitated with polysaccharides by ethanol, which poses a serious challenge for subsequent purification steps. It is necessary to find an effective method to remove proteins from crude polysaccharide extracts. For example, the free proteins of polysaccharides from *Laminaria japonica* are usually removed by the Sevage method, the deproteinization rate was 32.21% and the polysaccharide retention rate was 92.82% (Zha, 2012). Two different ways were employed to remove protein from polysaccharides in *Echinops latifolius Tausch*, results showed the protein removal rate of TCA and Sevag methods were 45.13% and 47.47%, and the polysaccharide retention rate were 61.05% and 67.65%, respectively (Yang *et al*., 2014). The deproteinization effects of above three methods were compared by the percentage of the deproteinization, polysaccharide loss and capacity of polysaccharide solution for scavenging DPPH free radical. The results showed that the effective order of these methods is HCl method, then TCA and finally Sevag method. As the pH value of polysaccharide solution is 2 as adjusted by HCl, the results were the protein removal rate 88.9%, the polysaccharide retention rate 85.2% and the capacity of polysaccharide solution scavenging DPPH

free radical 45.6% (Dong *et al.*, 2007). On the basis of guaranteeing the deproteinization rate and the retention rate of polysaccharides, the activities of polysaccharides should also be kept to the maximum extent.

The method used for deproteinization of CSPs should have both high deproteinization rate and polysaccharide retention rate and can't destroy the structure and activity of polysaccharides. Deproteinizing reagents should be safe and cheap. Therefore, in this study, the deproteinization conditions and effects of Sevag method, trichloroacetic acid (TCA) method or hydrochloric acid (HCl) method were investigated. Deproteinization rate, polysaccharide retention rate, antioxidant activities (chelating ferrous ion ability, DPPH free radical scavenging ability, superoxide anions scavenging activity and Ferrous ion reducing power) and α-amylase inhibitory activity were used as indicative mark. An efficient and convenient method for deproteinization of polysaccharides was obtained, which provides a basis for further development and utilization of CSPs.

Materials and Methods

Materials

Corn silk was obtained from the suburb of Jilin city, Jilin Province, China in 2017. Ascorbic acid (VC) were bought from Sinopharm Chemical Reagent Co. (Beijing). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (USA). Trichloro acetic acid, ferrous sulfate, potassium dihydrogen phosphate, sodium hydroxide, Ethylenediamine Tetraacetic Acid (EDTA), sulfuric acid, phenol and ethanol were purchased from Chemical Reagent Factory (Tianjin, China). All other reagents and chemicals used in the study were of analytical grade.

About 752N UV visible spectrophotometry meter (Shanghai Chaojing science and Technology Co., Ltd.); HHV-6 digital thermostat water bath (Guohua Electrical Appliance Co., Ltd.); electronic balance (Shanghai Precision Scientific Instrument Co., Ltd.); pHs-3c digital pH meter (Shanghai Lida instrument factory); TGL-20M benchtop high speed refrigerated centrifuge (Hunan Xiangyi laboratory instrument Development Co., Ltd.).

Methods

Extraction of the Crude CSPs

The 100 g of corn silk was extracted with 3 L of water at 90°C for 1 h and then extracted for 3 times. The extraction solution was concentrated to 1/5 of original volume, which was then precipitated by slowly adding ethanol (95%, v/v) until the ethanol content dropped to 80% (v/v) and then the solution was keeping the solution for 12 h at 4°C. The precipitates obtained by centrifugation at 4000 rpm for 10 min were lyophilized to obtain the crude CSPs (Cai *et al.*, 2018).

Determination of Polysaccharide and Protein Content of CSPs

Glucose is the main component of CSPs (Chen *et al.*, 2014), so the content of CSPs was determined by using glucose as the standard. Polysaccharide content of CSPs was determined by phenol sulfuric acid method (Georgiou *et al.*, 2018; Cuesta *et al.*, 2003). The sample solution (0.5 mL) and the phenol solution (1.0 mL) were added to test tube, which were shocked. Then 3.5 ml of concentrated sulfuric acid was added either directly against the liquid surface in 2 or 10 s, or slowly down the side of the tube. The tubes were then shock for 5 s and incubated in water at 40°C for 30 min. All tubes were allowed to cool down to room temperature before reading the absorbances at 490 nm using distilled water as blank in an Ultraviolet-Visible Spectrophotometer.

Protein content was determined by Coomassie brilliant blue G-250 staining with calf serum as standard (Xia *et al.*, 2012). The sample solution (1.0 mL) and the Coomassie brilliant blue G-250 solution (5.0 mL) were added to test tube and kept away from light for 5 min. The absorption was measured at 595 nm.

$$\text{The retention rate polysaccharide (\%)} = \frac{M_1}{M_0} \times 100\%$$

$$\text{Deproteinized rate (\%)} = \frac{M_2 - M_3}{M_2} \times 100\%$$

Where:
M_0 = Polysaccharide mass before deproteinized
M_1 = Polysaccharide mass after deproteinized
M_2 = Protein mass before deproteinized
M_3 = Protein mass after deproteinized

Three Methods of Deproteinization of CSPs

Deproteinization by Sevag Method

50 mL of the crude CSPs solution (2 mg/mL) was transferred into a 250 mL conical flask and 20 mL of Sevag reagent (chloroform: *n*-buthanol, 5:1 (v/v)) was added. The mixed solution was continually stirred for 2 min and centrifuged at 4000 rpm for 10 min. As a result, the solution was divided into three layers, in order, CSPs solution after deproteinization, denatured protein and redundant Sevag reagent from top to bottom. The supernatant was repeatedly deproteinized with the same amount of Sevag reagent and the other two layers were recovered and disposed. After the last

deproteinization step, the supernatant was collected and dried and Sevag-deproteinized CSPs (S-CSPs) were obtained (Xiong *et al.*, 2017).

Deproteinization by the TCA Method

About 20 mL of the crude CSPs solution (2 mg/mL) was mixed with TCA solution 10 mL, which the mass fractions were 2.0%, 4.0%, 6.0%, 8.0% and 10.0% (m/m) respectively. After sonication for 10 min, the mixed solution was centrifuged at 4,000 rpm for 10 min and the supernatant was collected and dried to provide the TCA-deproteinized CSPs (T-CSPs) (Huang *et al.*, 2011).

Deproteinization by the HCl Method

About 20 mL of the crude CSPs solution (2 mg/mL) was adjusted to the designed pH (1, 1.5, 2.0, 2.5, 3.0) with HCl solution (0.2 mol/L). After shaking, the mixed solution was centrifuged for 10 min at 4,000 rpm. The HCl-deproteinized CSPs (H-CSPs) was collected (Dong *et al.*, 2007).

Antioxidant Activity in Vitro of CSPs

Determination of Ferrous Ion Chelating Ability

The test mixture included 1 mL sample solution (0.2, 0.4, 0.6, 0.8, 1.0, 2.0 mg/mL), 1mL ferrous sulfate solution (0.1 mmol/L) and 1mL Ferro Zine methanol solution (0.25mmol/L) and the mixture was then shaken vigorously and left to stand in test tubes in the dark for 10 min, the absorbance at 562 nm was then measured. Vitamin C (Vc) was used as a positive control (Qiao *et al.*, 2009). The ferrous ion chelating ability was calculated according to the following equation:

$$\text{Chelating rate (\%)} = \frac{A_0 - A_j}{A_0} \times 100\%$$

A_0 = The absorbance of the blank control
A_i = The absorbance of the sample

Determination of DPPH Free Radical Scavenging Ability

About 100 μL of sample solution (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) were mixed with DPPH-methanol solution (100 μL, 0.5 mM). The mixture was then shaken vigorously and left to stand in test tubes in the dark at room temperature for 30 min, the absorbance at 517 nm was then measured (Liu *et al.*, 2017). Vitamin C (Vc) was used as a positive control. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{The scavenging rate (\%)} = \left(1 - \frac{A_i - A_j}{A_0}\right) \times 100\%$$

where, A_i is the absorbance value of the DPPH-ethanol solution (2 mL) plus samples (2 mL) at different concentrations, A_j is the absorbance value of absolute ethanol (2 mL) plus samples (2 mL) at different concentrations, A_0 is the absorbance value of the DPPH-ethanol solution (2 mL) plus absolute ethanol (2 mL).

Determination of Superoxide Anions Scavenging Activity

About 200 μL of 50 mmol/L Tris-HCl buffer solution (pH 8.2), 20μL of sample solution (contrast with distilled water) with different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL) were added into the test tubes, respectively. The temperature was kept at 25°C for 20 min in the water bath. 20 μL of the same pre-warm pyrogallic acid solution (0.03 mol/L) was added and reacted for 5 min. The absorbances at 325 nm were quickly measured respectively. Tris-HCl buffer solution was used as a blank and Vc was as the control. Each group was done for 3 times (Song *et al.*, 2010). The calculation formula of clearance rate (E) was as follows:

$$\text{The scavenging rate (\%)} = \frac{A_0 - A_j}{A_0} \times 100\%$$

A_0 = Was the absorbance value of the Tris-HCl buffer solution and the pyrogallic acid solution (the unsampled system)
A_i = Was the absorbance value of sample

Determination of Total Reduction Activity

The test mixture included 2.5 mL of 0.2 mol/L sodium phosphate buffer (pH 6.6), 2.5 mL of 1% potassium hexacyanoferrate ($K_3Fe(CN)_6$) solution (w/v) and 1.0 mL sample solution. The samples were tested using a series of concentrations (0.2, 0.4, 0.6, 0.8, 1.0mg/mL). These mixtures were heated in water bath at 50°C for 20 min. After cooling, 2.5 mL of trichloroacetic acid (10%, w/v) was added and the resulting mixture was centrifuged (3000 rpm, 10 min). 2.5 mL the supernatant was collected and mixed with 2.5mL deionized water and then the color was developed by adding 0.1% ferric chloride (0.5 mL). The absorbance at 700 nm was measured with spectrophotometer. Higher absorbance was indicative of greater reducing ability (Lan *et al.*, 2018).

Determination of α-Amylase Inhibition Effects

About 0.5 mL of sample solution (0.8 mg/mL) was added to 0.5 mL of α-amylase before incubation at 37°C for 10min, followed by the addition of soluble starch solution (1 mL). After further incubating at 37°C for 15 min, the reaction was terminated by adding 3mL of hydrochloric acid solution (2 mol/L). The reaction

solution was diluted with 4 mL of distilled water and immediately added to a test tube containing 0.2 mL of dilute iodine solution and shaken. The absorbance was measured at 660 nm (Zhang *et al.*, 2015). The percentage inhibition of α-amylase was calculated as follows:

$$\alpha - \text{Amylase inhibition rate (\%)} = \frac{A_{sample-A_{background}}}{A_{contral} - A_{background}} \times 100\%$$

where, $A_{control}$, A_{sample} and $A_{background}$ are defined as the absorbance at 100% enzyme activity (only for that solvent without the enzyme), a test sample (with the enzyme) and a blank (only for that solvent without the sample but with enzymes), respectively.

Statistical Analysis

The data are represented as mean ± SD. The experimental data were statistically analyzed by ANOVA, $p<0.05$ and $p<0.01$ were considered statistically significant.

Results

Polysaccharides and Protein Content of Crude CSPs

The standard curve equation of glucose: $y = 7.4352x+0.0071$ ($R^2 = 0.9998$). The content of crude CSPs was 20.97%. The standard curve equation of Bovine Serum Protein: $y = 5.4954x-0.2806$ ($R^2 = 0.9997$). According to the formula, the protein content of crude CSPs was 10.78%.

Deproteinization Rates and Polysaccharide Retention Rate of Different Methods

Sevag Method

Sevag is a kind of organic solvent, which affects the dielectric constant between protein and the medium, thereby changing the stability of the protein, so as to achieve the purpose of separating the protein from polysaccharide. As can be seen from Table 1, with the increase of the number of times, the polysaccharides retention rate decreased and the deproteinization rates increased. After two times deproteinizations, the deproteinization rate reached 43.14% and the polysaccharide retention rate reached 48.93%, so it is too low.

TCA Method

Table 1 showed that with the increase of TCA concentration, the deproteinization rate increased first and then decreased and the polysaccharide retention rate decreased gradually. It might be that with the increase of TCA, the isoelectric point of protein was deviated and the polysaccharide was hydrolyzed by high concentration TCA, which resulted in the decrease of protein deproteinization rate and polysaccharide retention rate.

Table 1: Comparison of deproteinization rate (%) and polysaccharide retention rate (%) by different methods in removing proteins

Method	Deproteinized rate (%)	Polysaccharide retention rate (%)
Sevag method to remove proteins		
1 (times)	39.61±1.32	60.61±2.39
2 (times)	43.14±1.89	48.93±1.89
TCA method to remove proteins		
2.0%	47.69±3.52	68.56±3.19
4.0%	53.68±2.89*	64.13±2.28**
6.0%	46.64±3.45	60.08±2.94
8.0%	38.66±2.32	50.67±2.89
10.0%	35.42±1.96	55.69±2.78
HCl method to remove proteins		
pH1.0	49.68±2.84	48.42±2.89
pH 1.5	49.21±2.83	50.36±2.84
pH 2.0	45.50±2.86[ns]	58.06±2.72*
pH 2.5	32.67±2.90	59.18±2.38
pH 3.0	11.15±1.48	61.27±1.36

Compared with Sevag method, TCA method and HCl method had significant differences in protein removal rate and polysaccharide retention rate. (*: P<0.05; **: P<0.01). Ns means that there was no significant difference.

The results showed that the optimum concentration of TCA was 4%. The deproteinization rate was 53.68% and polysaccharide retention rate was 64.56%.

HCl Method

As can be seen from Table 1, with the increase of pH, the deproteinization rate decreases gradually, while the polysaccharide retention rate increases gradually and finally tends to balance. The result showed that 2 was the optimum pH value of HCl method for deproteinization.

The results were shown in Table 1, TCA method is more suitable for mass purification of CSPs considering the deproteinization rate, the retention rate of polysaccharides and the practicability of reagents.

Antioxidant Activities of CSPs

Antioxidant Activities of Crude CSPs

The ferrous ion chelating activity of crude CSPs and EDTA were presented in Fig. 1a. CSPs had a reducing ability to ferrous ions, the results indicated that with increasing concentration of polysaccharides, the ferrous ion chelating activity was also enhanced. The IC_{50} of crude CSPs and EDTA were 1.34 mg/mL and 0.03 mg/mL, respectively, so the reduction ability was weaker than EDTA.

DPPH radical rcavenging rctivity of crude CSPs and Vc were shown in Fig. 1b. The scavenging ability of DPPH free radicals was strong and the clearance rate was linear with the concentration of polysaccharide, the IC_{50} of crude CSPs and VC were 0.45 mg/mL and 0.05 mg/mL, respectively. The results indicated that crude CSPs had a strong ability to remove DPPH free radicals, but the removal effect was less than Vc.
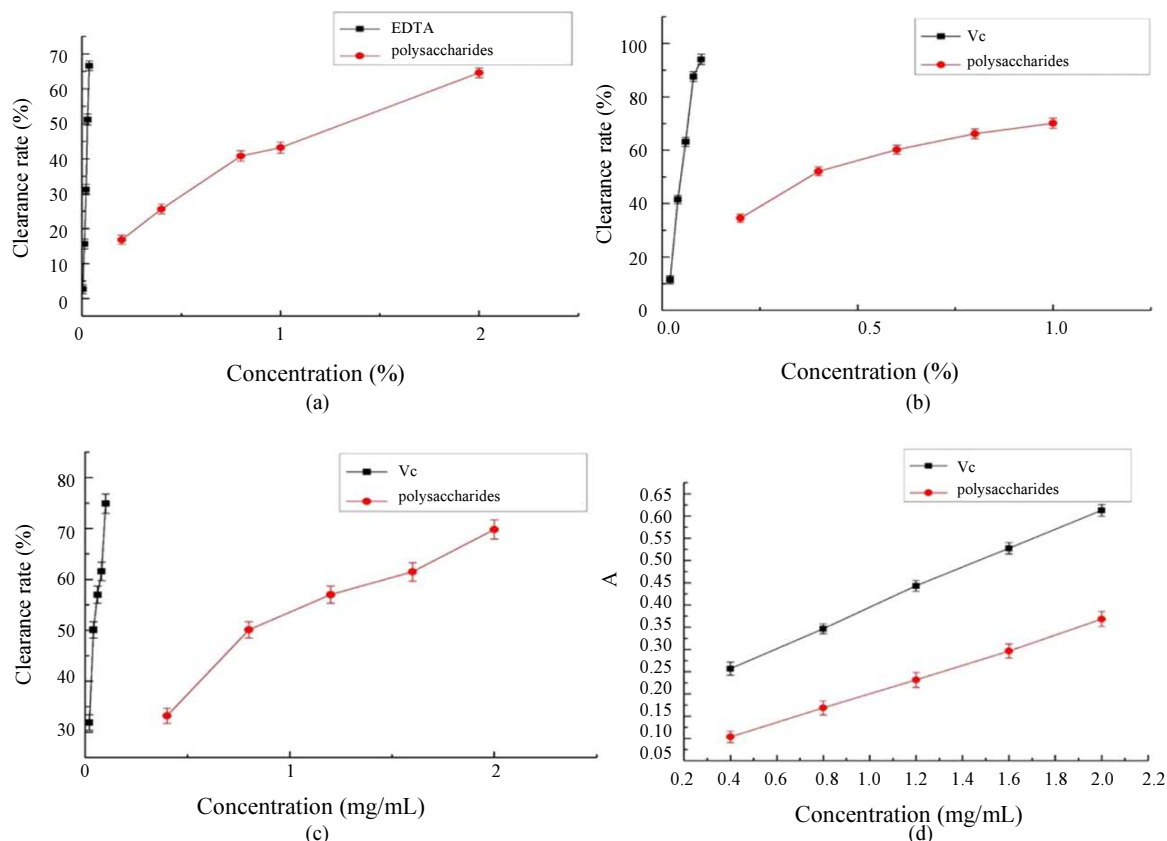
Fig. 1: Antioxidant activities of crude CSPs. Ferrous ion chelating ability (a) DPPH radical scavenging activity (b) Superoxide anion radical scavenging activity (c) Total reduction activity (d) Each value is the means ± standard deviation (SD) of triplicate measurements

Table 2: Antioxidant Acticities and Inhibitory Effects on α-amylase of polysaccharides after protein removal by different methods (Polysaccharides concentration was 0.8 mg/mL)

Method	Ferrousion chelating ability (%)	DPPH radical scavenging activity (%)	Superoxide anion radical scavenging activity (%)	Total reduction activity	Inhibitory Effects on α-amylase (%)
crude CSPs (0.8 mg/mL)	40.80±1.67	66.16±1.26	50.12±1.43	0.17±0.01	71.94±1.24
Sevag method (times)					
1	30.41±1.32	77.30±1.15	54.50±1.59	0.12±0.01	66.31±0.68
2	26.24±1.15−−	68.04±1.13ns	43.60±1.73−	0.11±0.01−−	50.13±0.84−−−−
TCA method (concentrations)					
2.0%	60.62±1.36	73.46±0.83	66.50±1.43	0.27±0.01	70.08±0.94
4.0%	55.09±1.54++++	75.91±0.96++++	64.04±1.50+++	0.29±0.01++++	68.01±0.73−
6.0%	49.29±149	79.08±0.89	58.99±1.63	0.23±0.01	60.92±0.68
8.0%	47.57±1.59	77.59±0.97	47.60±1.54	0.21±0.01	59.04±0.87
10.0%	46.83±1.25	75.29±1.18	50.20±1.39	0.21±0.01	57.98±0.67
HCl method (pH)					
1.0	21.45±1.67	77.05±0.94	33.10±1.49	0.16±0.01	47.84±0.56
1.5	32.63±1.49	68.14±0.85	20.00±1.52	0.11±0.01	55.07±0.62
2.0	33.04±1.57−−	62.04±0.79−	17.11±1.73−−−−	0.10±0.01−−	60.42±0.91−−−−
2.5	22.76±1.38	70.25±0.94	12.64±1.63	0.09±0.01	62.8 ± 0.58
3.0	16.48±1.63	74.36±1.17	38.83±1.39	0.10±0.01	63.33±0.87

Compared with crude CSPs, there were significant differences in antioxidant activities and α-amylase inhibitory activities of S-CSPs, T-CSPs and H-CSPs under the optimal conditions of the three methods. "+" represented positive significant difference and "-" represented negative significant difference. ("+" or "-": P<0.05; "+ +" or "- -": P<0.01; "+ + +" or "- - -": P<0.001; "+ + + +" or "- - - -": P<0.0001). Ns means that there was no significant difference.

Superoxide Radical Scavenging Activity of the crude CSPs and Vc were shown in Fig. 1c. The crude CSPs had strong ability to scavenge superoxide anion radicals and the clearance rate was dose-dependent with the concentration of polysaccharide. The IC_{50} of crude CSPs and Vc were 1.00 mg/mL and 0.50 mg/mL, respectively. The results indicated that the crude CSPs has a strong ability to remove superoxide anion free radicals, but the removal effect is less than Vc.

Total antioxidant capacity of the crude CSPs and Vc were shown in Fig. 1d. The crude CSPs had a certain ability to restore. The reduction abilities of crude CSPs and Vc were dose-dependent and increased with the increase of concentration, but the reducing ability of polysaccharide was less than that of Vc.

Antioxidant Activities of CSPs After Deproteinized

Results as shown in Table 2, when the concentration of polysaccharide was 0.8 mg/mL, the antioxidant activities of T-CSPs was stronger than those of crude CSPs, S-CSPs and H-CSPs. The antioxidant activity of T-CSPs decreased with the increase of TCA concentration, which might be that too much TCA would destroy the structure of polysaccharides. The low deproteinization rate and polysaccharide retention rate of S-CSPs resulted in the lower purity of S-CSPs than that of T-CSPs, so the antioxidant activities of S-CSPs was lower than that of T-CSPs. The structure of H-CSPs might be severely damaged by HCl, resulting in the decrease of their activities. Compared with the antioxidant activities of CSPs, the antioxidant activiies of T-CSPs was improved, which indicated that TCA was one of the effective ways to improve the purity and antioxidant activity of polysaccharides.

Amylase Inhibitory Activities of CSPs

From Table 2, it could be seen that the α-amylase inhibitory activities of S-CSPs decreased with the increase of deproteinization times. The α-amylase inhibitory activity of T-CSPs reduced with the increase of TCA concentration, it might be that excessive TCA destroyed the structure of CSPs and leaded to the decrease of their activity. The α-amylase inhibitory activity of H-CSPs strenghten with the increase of pH. The amylase inhibitory activity of H-CSPs was lower than that of crude CSPs, it might be that the structure of polysaccharides had changed or that proteins with α-amylase inhibitory activity had been removed. However, compared with crude CSPs, when the concentration of TCA was 4%, the α-amylase inhibitory activity of T-CSPs decreased less than those of S-CSPs and H-CSPs. The results showed that the activity of CSPs could be preserved when 4% TCA was used for deproteinization. The inhibitory activity of amylase of T-CSPs was stronger than those of S-CSPs and H-CSPs.

Discussion

The most typical depropeinization method was Sevag method, however, it involved excessive consumption of chloroform and *n*-buthanol, which resulted in massive amount of organic toxicities. Furthermore, there was a dramatic loss of polysaccharide after several times Sevag treatment (Xiong *et al.*, 2015). Therefore, Sevag method is not suitable for mass purification of CSPs. The results of deproteinization by HCl method showed that with the increase of pH value, the concentration of HCl decreased, which could not destroy the protein sufficiently, leading to the decrease of protein removal rate. However, the hydrolysis of CSPs could decrease, resulting in a corresponding increase in the retention rate of CSPs, but HCl destroys the structure of CSPs, resulting in a sharp decrease in antioxidant activity and α-amylase inhibition effect of H-CSPs. So HCl method is not suitable for deproteinization of CSPs (Qiang *et al.*, 2010). The principle of TCA method is that the protein cation can bind the TCA to form an insoluble salt for precipitation at pH<pI (isoelectric point). The strong acidity of TCA could cause the degradation of CSPs and consequently the loss of CSPs, so the concentration of TCA should not be too high (Teng *et al.*, 2013). The antioxidant activity of T-CSPs was stronger than that of crude CSPs and it's α-amylase inhibition effect was also higher than those of S-CSPs and H-CSPs. The results showed that the antioxidant activity of CSPs was improved after deproteinization by TCA method. According to the deproteinization rate, polysaccharide retention rate, antioxidant activity and α-amylase inhibition effect. Compared with the other two methods, TCA method is an efficient and inexpensive deproteinization method, which was suitable for mass purification of CSPs.

Conclusion

The results demonstrated that the TCA method offers higher deproteinization rate and polysaccharides retention rate than the Sevag method and HCl method and the antioxidant and α-amylase inhibitory effects of T-CSPs were stronger than those of S-CSPs and H-CSPs. T-CSPs had stronger the antioxidant activity than that of crude CSPs and the α-amylase inhibitory activity of T-CSPs was close to that of crude CSPs. Therefore, TCA method was suitable for mass purification of CSPs. However, further studies on the possible loss of glycoproteins, deproteinization efficiency, effect on the biological activity of polysaccharides and mechanism of protein removal, should be carried out in the near future.

Acknowledgments

Author's Contributions

Yaping Li: Antioxidant activity and α-amylase inhibitory activity of CSPs before and after deproteinization and composing of thesis

Songbiao Shi: The research on the deproteinization of CSPs.

Xiudong Yang: The revision and proofreading of the thesis.

Hongli Zhou: The design and guidance of research.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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