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Chemical modification, antioxidant and α-amylase inhibitory activities of corn silk polysaccharides

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ABSTRACT

Water-soluble corn silk polysaccharides (CSPS) were chemically modified to obtain their sulfated, acetylated and carboxymethylated derivatives. Chemical characterization and bioactivities of CSPS and its derivatives were comparatively investigated by chemical methods, gas chromatography, gel filtration chromatography, scanning electron microscope, infrared spectroscopy and circular dichroism spectroscopy, scavenging DPPH free radical assay, scavenging hydroxyl radical assay, ferric reducing power assay, lipid peroxidation inhibition assay and α-amylase activity inhibitory assay, respectively. Among the three derivatives, carboxylmethylated polysaccharide (C-CSPS) demonstrated higher solubility, narrower molecular weight distribution, lower intrinsic viscosity, a hyperbranched conformation, significantly higher antioxidant and α-amylase inhibitory abilities compared with the native polysaccharide and other derivatives. C-CSPS might be used as a novel nutraceutical agent for human consumption.

1. Introduction

In normal physiological conditions, reactive oxygen species were produced at low levels, which were necessary for maintaining normal cell functions and the endogenous antioxidant defense systems so that the body had the capacity to avert any harmful damages. However, these systems were insufficient to prevent the harm entirely (Simic, 1988). Free radicals were responsible for aging and causing various human diseases, such as atherosclerosis, diabetes, cancer and cirrhosis. Thus, antioxidant supplements might be used to help the human body reduce oxidative scratch. Recently, there was an extensive interest in the food industry and in the preventive medicine for the development of antioxidants from natural sources. It was reported that polysaccharides, which widely existed in plants, animals and microorganisms, played an important role as free radical scavengers in the prevention of oxidative damage in living organism (Tsiapali et al., 2001).

Corn silk (dried cut stigmata of maize female flowers, *Zea mays* L.) was distributed widely in Asian countries. It was a well known traditional Chinese herbal medicine and functional food, which had significant influence on human health. The bioactivities of corn silk constituents were widely reported in the literatures, including antioxidant capacities (Liu et al., 2011), anti-diabetic activity (Zhao, Yin, Yu, Liu, & Chen, 2012), anti-proliferative effects (Habtemaram, 1998), 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), anti-tumor activity (Habtemaram, 1998) and treating obesity (Du & Xu, 2007). Previous studies confirmed various compounds from corn silk such as proteins, vitamins, alkaloids, tannins, mineral salts (Namba et al., 1993), steroids (Abdel-Wahab, El-Tanbouly, Kassem, & Mohamed, 2002), flavonoids (Liu et al., 2011) and polysaccharides (Zhao et al., 2012). Polysaccharides were one type of the components playing an important role in the growth and development of living organisms (Yang & Zhang, 2009; Zhao et al., 2011). Different kinds of polysaccharides from plants, microorganisms, and animals had been studied owing to their various biological functions such as inhibiting tumor growth, cellular immunoregulatory and anti-diabetic properties (Jia, Dong, Lu, Guo, & Wei, 2009; Shan, Zhang, Diao, Qu, & Zhao, 2010). A β-(1→3)-D-glucan isolated from the fungi, *Lentinus edodes*, was well known for its strong host-mediated anti-cancer activity, *via* activation of the human immune system (Zhang, Li, Wang, Zhang, & Cheung, 2011). 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.

Polysaccharides were widely distributed in animals, plants and microorganisms. In recent years, an increasing number of studies

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had been focused on the structures and biological properties of polysaccharides. It was demonstrated that biological activities of polysaccharides were obviously increased by chemical modification owing to the change of physicochemical properties, structure and conformation improvement. Chemical modification such as sulfation, carboxymethylation, acetylation, acid hydrolysis and $NaIO_4$ oxidation of polysaccharide were studied for preparing custom-made derivatives possessing desirable functional characteristics (Ma, Chen, Zhang, Zhang, & Fu, 2012; Tao, Zhang, & Zhang, 2009; Yang et al., 2005). Some studies showed that the relatively higher bioactivities of the sulfated or carboxymethylated polysaccharides were partially attributed to expanded chain conformation (Tao et al., 2009; Yuan et al., 2005). The activity was strongly dependent on the molecular weight, branch structure and sugar residue types of the polysaccharide and the removal of side chains by acid hydrolysis caused reduction or loss of tumor cell growth inhibition (Yang et al., 2005). The presence of sulfate group of polysaccharides from *Azadirachta indica* leaves appeared to be an important hallmark of anti-viral activity (Saha et al., 2010). However, incorporation of the sulfating group resulted in the decrease of lacquer polysaccharide's activity against leukopenia induced by cyclophosphamide in mice, and this activity greatly decreased with the increase of sulfating group. But the increase of the content of carboxyl groups could result in the increase of the bioactivity (Yang & Du, 2003). The high potential of natural biopolymers with their broad range of structural, functional and physicochemical properties, in various applications has provided the stimulus for the exploration of new or modified polysaccharides. Though there were some studies on corn silk polysaccharide, there are, still no reports on the chemical modifications of polysaccharides from corn silk in the literatures.

In this study, the purpose was to evaluate the chemical modification and explore activities of modified polysaccharides from corn silk. The physicochemical properties and structural characteristics of the native corn silk polysaccharides (N-CSPS) and its derivatives were comparatively analyzed. The in *vitro* antioxidant and α-amylase inhibitory activities between N-CSPS and its derivatives were also compared.

2. Materials and methods

2.1. Materials and chemicals

Corn silk was gathered from corn field in September 2012 in Tianjin, China. 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). All other chemicals and reagents were purchased locally and were of analytical grade.

2.2. Extraction and purification of CSPS

Dried ground corn silk (500 g) was extracted with water at 100 °C (1:15 (w/v), 1 h, 3 times). The extract was filtered through a Whatman No.1 filter paper and the filtrate was then concentrated with a rotary evaporator at 50 °C under vacuum, and then was precipitated by the addition of anhydrous ethanol to a final concentration of 80% (v/v). The mixture was maintained overnight at 4 °C to precipitate polysaccharides. The precipitates were collected by centrifugation (3000 × *g*, 10 min), washed by anhydrous ethanol for three times, and then were freezing dried and the crude corn silk polysaccharide was obtained (named as CSPS). CSPS was dissolved in deionized water, centrifuged, and the supernatant was loaded onto a DEAE-52 cellulose column (50 cm × 2.5 cm, i.d.), which was eluted with water, 0.1 M NaCl, 0.2 M NaCl and 0.5 M NaCl solution in order. The elution fractions (5 mL each fraction) were collected and monitored for sugar content based on phenol-sulfuric acid method. CSPS were isolated into 4 collections, then concentrated, dialyzed and lyophilized, named as CSPS-W, CSPS-1, CSPS-2, CSPS-3, respectively. The main fraction with good antioxidant activity was named as N-CSPS for further study.

2.3. Chemical modification of N-CSPS

2.3.1. Preparation of sulfated derivative S-CSPS

The sulfation agent, SO_3 pyridine, was made of 1.0 mL of $HClSO_3$ and 4.0 mL of pyridine under cooling in an ice-water bath. N-CSPS (50 mg) was added to 20 mL of pyridine, stirred at 60 °C for 15 min. Then 5 mL of sulfation agent was added. After keeping for 6 h at 50 °C, the mixture was cooled to room temperature by an ice-water bath, neutralized with 30% NaOH solution and concentrated. The residue was added to 10 mL of dimenthylformamide (DMF) and filtered. The filtrate was then precipitated with acetone. The precipitate was dissolved in distilled water, lyophilized and coded as S-CSPS. Barium sulfate turbidity was used to determine the sulfur contents of S-CSPS (Antonopoulos, 1962). A calibration curve was constructed with sodium sulfate as standard and the degree of substitution (DS) was calculated.

2.3.2. Preparation of acetylated derivative A-CSPS

N-CSPS (50 mg) was dispersed in10 mL of pyridine, and stirred at 60 °C for 30 min, then 10 mL of mixed liquor of pyridine and acetic anhydride (AC_2O) (1:1, v/v) were added. The reaction mixture was stirred at 60 °C for 4 h. Then distilled water (150 mL) was added to react with the excess AC_2O. The mixture was concentrated, dialyzed, precipitated by anhydrous ethanol, lyophilized and named as A-CSPS. The acetyl group (AG) and substitution DS of A-CSPS were determined as described by Das et al. (Das, Singh, Singh, & Riar, 2010).

2.3.3. Preparation of carboxylmethylated derivative C-CSPS

Carboxymethylation of C-CSPS was operated as described by Tao et al. (2009) with a slight modification. N-CSPS (300 mg) was suspended in a mixture of 5 mL of 20% NaOH and 12.5 mL of isopropanol in an ice bath with stirring for 3 h. Then mixtures of 2.63 g chloroacetic acid, 5 mL of 20% NaOH and 12.5 mL of isopropanol were slowly added with stirring. The reaction was continued for 3 h at room temperature, and then at 60 °C for 1.5 h. After the solution was cooled to room temperature, 0.5 mol/L of HCl was added to adjust the pH to 7. The carboxymethylated derivatives were concentrated, dialyzed, precipitated by anhydrous ethanol, lyophilized and named as C-CSPS. Carboxymethyl content was determined by the method of neutralization titration (Regina, Heatley, & Budd, 1998).

2.4. Characterization of N-CSPS and its derivatives

2.4.1. Compositional analysis

Phenol-sulfuric acid method was employed for the measurement of total sugar content of corn silk polysaccharide and its derivatives using D-glucose as the standard. Lowry's method was used to measure protein content 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 were hydrolyzed in a sealed glass tube with 2 M trifluoroacetic acid (TFA) at 105 °C for 10 h. The hydrolysate was 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. Rhamnose, arabinose, xylose, mannose, galactose and glucose were used as the monosaccharide standards.

2.4.2. Molecular weight distribution

Molecular weight was determined by gel filtration chromatography (GFC) on Sephadex G-100 column (60 cm × 2.5 cm, i.d.). The column was eluted by 0.2 M PBS at a flow rate of 10.0 mL/h. Fraction was collected for every 5 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 equation of the standard molecular weight *versus* retention volume plot. The calibration curve was made with dextran standards of different molecular weights (Dextran T-500, T-70, T-40, and T-10) (Chen, Zhang, Qu, & Xie, 2008).

2.4.3. Intrinsic viscosity analysis

Different CSPS samples were dissolved with 0.2 M phosphate buffer solution (PBS, pH 7.0), then centrifuged and filtered through 0.45 μm filter membrane prior to being measured. Ubbelohde glass capillary viscometer (Lunjie, ShangHai, China) was used to determine the passage time of every sample solution flowing through capillary. The viscometer was kept in water bath at 25 °C ± 0.5 °C. The passage time difference between two measuring result should be controlled under ±0.1 s. Intrinsic viscosity [η] was measured according to Ma et al. (2012).

2.4.4. FTIR analysis

FTIR spectroscopy was used to investigate the vibrations of molecules and polar bonds between the different atoms. FTIR spectra (in KBr pellets, 2 mg sample/200 mg KBr) of N-CSPS and its derivatives were measured using the Nicolet 170SX FT-IR spectrophotometers operating at 4 cm^{-1} resolution.

2.4.5. Morphological analysis

Scanning electron microscope (SEM) image of N-CSPS and its derivatives were obtained by means of an environmental scanning electron microscope (ESEM, Philips XL-30, Philips-FEI Co., Eindhoven, the Netherlands). The dried powder samples were placed on a specimen holder with the help of double-sided adhesive tapes and then sputtered with gold powder using an into sputter coater. Each sample was observed with 200- and 1000-fold magnification at an accelerating potential of 20 kV under a high vacuum condition.

2.4.6. Circular dichroism spectroscopy (CD)

Circular dichroism (CD) spectrum was determined on a J-180CD (JASCO, Japan) spectropolarimeter using polysaccharides solution of 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.

2.5. Antioxidant activity analysis

2.5.1. Scavenging activity against DPPH free radical

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity was determined as described by Bersuder, Hole, & Smith (1998). 100 μL of N-CSPS and its derivatives were mixed with 2.9 mL of DPPH solution (120 μM). The mixtures were shaken and then incubated for 30 min in dark at 37 °C. The absorbance of the mixture was measured at 517 nm. A lower absorbance of the reaction mixture indicated a higher DPPH scavenging activity. DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity}\,(\%) = \left(\frac{1 - A_{\text{sample 517 nm}}}{A_{\text{blank 517 nm}}}\right) \times 100$$

2.5.2. Inhibitory activity against hydroxyl radical

The inhibitory activities against hydroxyl radical of N-CSPS and its derivatives were quantified by the method described earlier by Halliwell, Gutteridge, & Aruoma (1987) with minor modification. Briefly, the reaction mixture, containing different polysaccharides content (2.0–10.0 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, pH7.4) 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 absorbance of the mixture was measured at 532 nm against a blank. The capability of inhibition to hydroxyl radical was calculated using the following equation:

$$\text{Hydroxyl radical inhibitory effect}(\%) = \left(1 - \frac{A_{\text{sample 532 nm}}}{A_{\text{blank 532 nm}}}\right) \times 100$$

2.5.3. Measurement of ferric reducing power (FRP)

FRP potential was determined according to the modified method by Yen and Chen (Yen & Chen, 1995). 100 μL of reaction mixture, containing different concentrations of N-CSPS and its derivatives 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 1 mL of reaction solution was mixed with 3 mL of aqueous $FeCl_3$ (1.7 mM). The absorbance of the mixture was measured at 700 nm using UV–vis spectrophotometer (UV-2450, Shimadzu, Japan). A higher absorbance of the reaction mixture indicated a higher reducing power.

2.5.4. 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 slight modification. The mice livers were cut into small pieces and homogenized in physiological saline at 4 °C. The reaction mixtures were 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, and then 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, then cooling to room temperature. After centrifugation at 3000 r/min for 10 min, the absorbance of the supernatant was measured at 532 nm against a blank. The antioxidant capacity of the inhibition of lipid peroxide formation was expressed as follows:

$$\text{Inhibitory rate}\,(\%) = \left(1 - \frac{A_{\text{sample 532 nm}}}{A_{\text{blank 532 nm}}}\right) \times 100$$

Table 1
Yield and antioxidant activities results of different fractions.

	CSPS-W	CSPS-1	CSPS-2	CSPS-3
Yield (%)	1.12 ± 0.14	19.2 ± 0.15	4.75 ± 0.29	2.31 ± 0.18
IC_{50} (mg/mL)[a]	3.56 ± 0.05	1.31 ± 0.02	2.78 ± 0.12	1.89 ± 0.03
Abs (700 nm)[b]	0.064 ± 0.01	0.128 ± 0.03	0.066 ± 0.01	0.086 ± 0.02

All data are means ± SD (n = 3).
[a] Means IC_{50} for scavenging DPPH free radical capacity.
[b] Means absorbance of ferric reducing power at 700 nm, concentration of all samples was 46.88 μg/mL. Higher absorbance indicates higher reducing power.

2.6. α-Amylase inhibitory activity assay

The α-amylase inhibitory activity was measured according to Ademiluyi (Ademiluyi & Oboh, 2013) with slight modification. 0.1 mL of 0.1 M sodium phosphate buffer (pH 5.6) containing porcine pancreatic α-amylase (2 mg/mL) mixed with1.8 mL of 0.5% starch solution were added to the polysaccharides solutions at 40 °C for 30 min. The reaction was stopped by the addition of 2.0 mL of DNS reagent (1% dinitrosalicylic acid, 12% potassium sodium tartrate). Then the mixture was incubated in a boiling water bath for 15 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water and the absorbance was measured at 540 nm.

2.7. Statistical analysis

Values were expressed as means ± standard deviation (SD) of three replicates. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA) and Student's t-test using SPSS statistical software (version 16.0 for Windows, SPSS Inc., Chicago, IL, USA), the statistical significance of mean differences was based on a p value of <0.05.

3. Results and discussion

3.1. Extraction and purification of CSPS

CSPS was isolated from the hot-water extract of corn silk with a yield of 4.53%, and then fractionated on DEAE-52 cellulose column. Four main fractions were obtained and termed as CSPS-W, CSPS-1, CSPS-2, and CSPS-3, respectively. CSPS-W was from the water elution fraction, CSPS-1, CSPS-2, and CSPS-3 were from the 0.1 M, 0.2 M, and 0.5 M NaCl elution fractions, respectively. The yield of CSPS-1 was the highest (19.2%) compared with the other fractions (Table 1). From the antioxidant results, CSPS-1 showed the preferable scavenging DPPH free radical capacity with the IC_{50} value of 1.31 mg/mL and the prominent ferric reducing power with the highest absorbance value of 0.128 at 700 nm (Table 1). Owing to the highest yield and best antioxidant properties, CSPS-1 was selected for further study and named as N-CSPS.

3.2. Characterization of N-CSPS and its derivatives

3.2.1. Chemical composition analysis

Neutral sugar contents, uronic acid contents and protein contents of N-CSPS and its derivatives were summarized in Table 2. After chemical modification of CSPS, significant changes were found in the contents of chemical compositions in different derivatives. Total neutral sugar contents of S-CSPS, A-CSPS and C-CSPS were increased significantly ($P < 0.05$), while the protein contents were decreased relatively. Uronic acid content of C-CSPS showed an evident increase compared with the slight changes of those of S-CSPS and A-CSPS. The reasons might be the degradation of CSPS occurred during the chemical modification process. The obvious difference between C-CSPS and N-CSPS might be due to the β-elimination reaction during the carboxymethylation modifications under alkali condition (Saha et al., 2010). Under the sulfation and acetylation conditions, the substitution degrees of S-CSPS and A-CSPS were low and there were moderate degradations occurred in the process of S-CSPS and A-CSPS preparation. The results were in accordance with the findings of Ma et al. (2012). The monosaccharide compositions were determined by gas chromatography (GC). Sugar compositional analysis revealed the presence of arabinose, mannose, galactose and glucose as the major neutral sugar, together with a small amount of rhamnose, xylose, which indicated that β-glucan probably was the prominent active composition of polysaccharides (Rhee, Cho, Kim, Cha, & Park, 2008). Proportion of glucose was the highest in N-CSPS and its derivatives (Table 2). N-CSPS and its derivatives 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, 8.83:15.77:7.92:12.39:11.15:43.94, 2.79:10.59:3.06:15.89:14.89:52.78 and 3.70:22.25:4.86:17.48:24.00:27.71, respectively. The various compositions of N-CSPS and its derivatives might be due to the degradation during the process of derivatization reaction (Ren, Sun, & Peng, 2008). On the other hand, the group

Table 2
Chemical composition of N-CSPS and its derivatives.

	N-CSPS	S-CSPS	A-CSPS	C-CSPS
Neutral sugar (w%)	28.75 ± 0.03	37.35 ± 0.27[a]	36.85 ± 0.07[a]	35.28 ± 0.15[a]
Uronic acid (w%)	30.87 ± 0.05	31.12 ± 0.05	29.66 ± 0.10	50.62 ± 0.02[a]
Protein (w%)	2.13 ± 0.00	4.13 ± 0.02	4.63 ± 0.00	4.12 ± 0.01
Monosaccharide composition (mol%)				
Rha	4.17	8.83[a]	2.79	3.70
Ara	17.33	15.77[b]	10.59[a]	22.25[a]
Xyl	5.59	7.92	3.06	4.86
Man	18.65	12.39[b]	15.89	17.48
Gal	19.11	11.15[a]	14.89	24.00[a]
Glc	35.14	43.94[b]	52.78[a]	27.71[b]

All data are means ± SD (n = 3).
Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.
[a] Means there was very significant difference between N-CSPS and its derivatives.
[b] Means there was significant difference between N-CSPS and its derivatives.

Table 3
Degree of substitution (DS), intrinsic viscosity [η], and molecular weight (M_w) of Un-PS and its derivatives.

Samples	N-CSPS	S-CSPS	A-CSPS	C-CSPS
DS	–	1.35	0.17	1.22
[η] (mL/g)	8.87	2.06	3.26	1.88
M_w ($\times 10^4$ Da)	10.52	6.44, 4.67	8.27	3.68

substitute of sulfate, acetyl and carboxymethyl (Sun et al., 2009) in polysaccharides had also been reported an interesting fact that the carbohydrate content decrease with the increase in the degree of substitution. But the derivatives still retained the main component of the original polysaccharides. The degrees of substitution of S-CSPS, A-CSPS and C-CSPS were 1.35, 0.17, and 1.22, respectively.

3.2.2. Molecular weight distribution analysis

The molecular weight distribution of N-CSPS and its derivatives were determined by gel filtration chromatography (GFC) on Sephadex G-100 column (2.0 cm × 60 cm, i.d.). Size exclusion chromatography of N-CSPS showed that the polysaccharide was polydisperse. Based on calibration with dextrans, it showed a broad molecule-mass distribution pattern ($M_w \sim 6.50–13.35 \times 10^4$ Da) with a dominance of 10.52×10^4 Da molecule-mass polysaccharide (Table 3). The M_w value of sulfated and carboxymethylated polysaccharide decreased to 6.44×10^4 Da and 4.67×10^4 Da, and 3.68×10^4 Da respectively (Table 3), as a result of the degradation of biopolymers during the derivative process (Tao et al., 2009). During carboxymethylation process, the O-glycosidic bonds in polysaccharide-protein complex were apt to break in alkali condition, leading to the decrease of M_w. The results were in accordance with the findings in Tao et al. (2009). Change of the M_w value of acetylated polysaccharides was slighter than that of sulfated and carboxymethylated polysaccharide (Table 3).

3.2.3. Intrinsic viscosity analysis

Intrinsic viscosity [η] was a characteristic property of polysaccharide, which was determined by Ubbelohde glass capillary viscometer. From Table 3, intrinsic viscosity of the four polysaccharide samples were decreased in the order of N-CSPS (8.87 mL/g), A-CSPS (3.26 mL/g), S-CSPS (2.06 mL/g), C-CSPS (1.88 mL/g), indicating that intrinsic viscosity of modified corn silk polysaccharides had a tendency of decrease, which might be due to the decrease of M_w (Table 3).

3.2.4. FTIR analysis

The FTIR spectra of N-CSPS and their derivatives were illustrated in Fig. 1. Compared with the FTIR spectra of the native samples, two new absorption peaks appeared at 819 cm^{-1} and 1236 cm^{-1} for the sulfated derivatives S-CSPS that was due to the presence of the stretching vibration of C–S–O bonds and asymmetrical stretching vibration of S=O bond, respectively. The results were strongly suggested the conversion of hydroxyl groups to O-sulfate groups, and indicating that sulfation had actually occurred (Saha et al., 2010). Two new absorption peaks appeared at 1616 cm^{-1} and 1336 cm^{-1} for the carboxymethylated derivatives C-CSPS, which were the results of the existence of [$\gamma_{sym}(COO^-)$] and [$\gamma_{as}(COO^-)$], respectively. And the bands in the region 1310–1450 cm^{-1} were corresponded to symmetrical deformations of CH_2 and C—OH groups. The strong absorption band appeared at 3000–3500 cm^{-1} was due to stretching vibration of 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.

3.2.5. Morphological analysis

Fig. 2 presents the scanning electron micrographs (SEM) of N-CSPS and their derivatives at magnifications of 200 and 1000. The results showed that the different chemical modification methods induced different physical changes in size and shape. N-CSPS exhibited a sheet appearance with a size of 30–60 μm (Fig. 2A). The chemical modified polysaccharides S-CSPS showed small lumpish particles (Fig. 2B), whereas the surface of A-CSPS was rough, taking on irregular shape and plenty of pores (Fig. 2C), which might be due to the branches and network structures of the polysaccharides. C-CSPS was demonstrated relatively regular and homogeneous shapes (Fig. 2D). The diameter of N-CSPS, S-CSPS, A-CSPS and C-CSPS were about 45 μm, 15 μm, 40 μm and 8 μm, respectively, which were in agreement with the results of molecular weight distributions.

3.2.6. Circular dichroism spectroscopy (CD)

Circular dichroism was an effective method to investigate the three-dimensional structure of organic compounds (including biomacromolecule), which can provide the information of compounds' absolute configuration, conformational changes (Kwaambwa & Maikokera, 2008). Owing to lacking spectral characteristic in ultraviolet band, it was hard to determine structure characteristic of polysaccharides or neutral polysaccharides according to CD. Thus the conformation of polysaccharides could

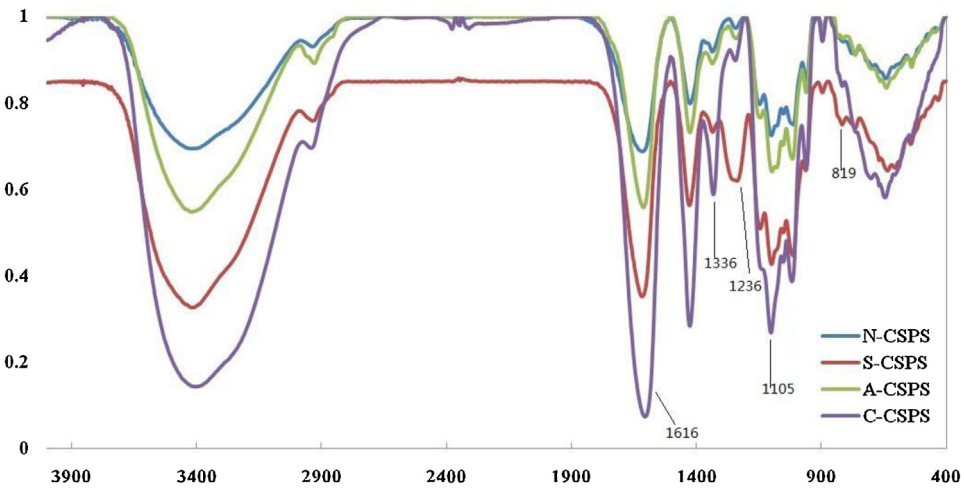

Fig. 1. FTIR spectra of the native polysaccharide N-CSPS and its derivatives.

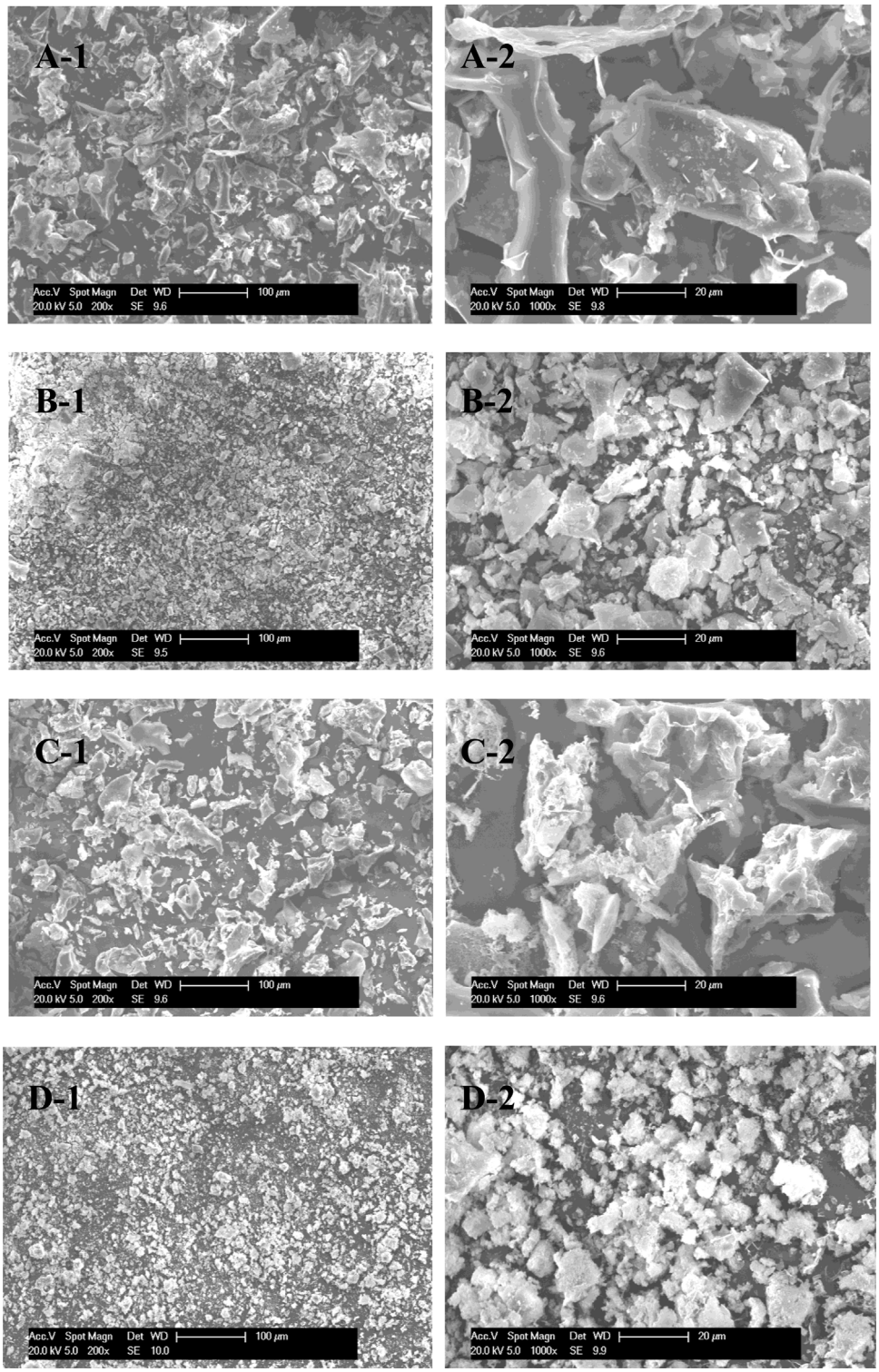

Fig. 2. SEM images of the native polysaccharide N-CSPS and its derivatives (A–D represent N-CSPS, S-CSPS, A-CSPS and C-CSPS, respectively. A-1, B-1, C-1 and D-1: ×200; A-2, B-2, C-2 and D-2: ×1000).

be obtained by molecular modification or complexing with Congo red (Ojinnaka et al., 1996).

Corn silk polysaccharides belonged to neutral polysaccharide and their CD spectra lacked obvious CD signals (Fig. 3A–C). Different chemical groups were introduced into the structure of polysaccharide N-CSPS by different derivatization methods, thus the corresponding group signals could appear in the CD spectra. When modified by sulfation, the obvious sulfate group signals of polysaccharides were appeared in 190–220 nm regions. Positive Cotton effect appeared in 200 nm and 215 nm regions, indicating that S-CSPS was ordered. At the same time, strong negative Cotton effect appeared in 207 nm, indicating that S-CSPS existed by ordered helical structure in water solution (Fig. 3A). Significant changes appeared in 190–210 nm of polysaccharides modified by acetylation (Fig. 3B). An obvious positive Cotton effect appeared in 206 nm region, and strong negative Cotton effect appeared in

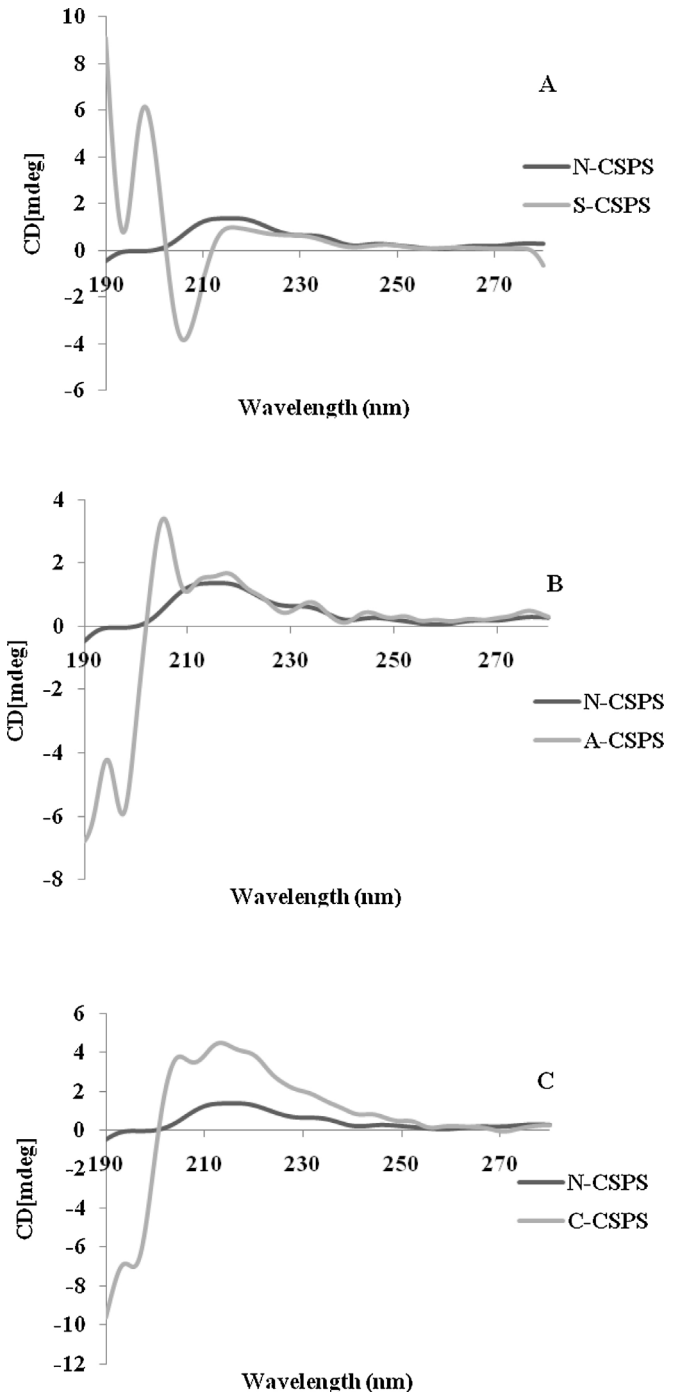

Fig. 3. The CD spectra of the native polysaccharide N-CSPS and its derivates (A, CD results comparison between N-CSPS and S-CSPS; B, CD results comparison between N-CSPS and A-CSPS; C, CD results comparison between N-CSPS and C-CSPS).

198 nm region, indicating an n → π transition of acetyl group. For C-CSPS (Fig. 3C), negative Cotton effect appeared in 196 nm, illustrating the n → π transition of carboxymethyl group (Ma et al., 2012). All these findings were in according to the FTIR results.

3.3. Antioxidant activity analysis

3.3.1. Scavenging activity against DPPH free radical

The proton radical scavenging action was known to be one of the various mechanisms for antioxidation. DPPH was one of the compounds that possessed a proton free radical and showed a characteristic absorption at 517 nm and was commonly used as a substrate to evaluate antioxidant activity. When DPPH encountered proton radical scavengers, its purple color would fade rapidly (Yamaguchi, Takamura, Matoba, & Terao, 1998). For further insight into the activation mechanism, we examined whether the protective effect of N-CSPS and its derivatives was associated with DPPH radical.

In the present study, scavenging capability of the three modified polysaccharides was improved in different extents. An obvious enhancement of scavenging capability on DPPH radical at a dosage of 0.5 mg/mL was found with the C-CSPS (53.2%) and as compared with S-CSPS (35.7%) and A-CSPS (23.5%) (Fig. 4A). In contrast, the untreated polysaccharides demonstrated least scavenging capability (20.8%) at the concentration of 0.5 mg/mL. The polysaccharides modified by carboxymethylation demonstrated preferable scavenging capability. Moreover, the inhibitory effects of these polysaccharides on DPPH radical were found to be dose-dependent in the concentrations detected (Fig. 4A). The results revealed that the modified polysaccharides from corn silk were potent scavenger and their antioxidant activity might be attributed to their proton-donating ability (Shimada, Fujikawa, Yahara, & Nakamura, 1992). Whether the structures can be related to scavenging capacity for DPPH radicals remains to be determined in future studies.

3.3.2. Inhibitory activity against hydroxyl radical

Hydroxyl radical, 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-thiobarbituricacid 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). Fig. 4B shows that the inhibitory effects of S-CSPS and C-CSPS (0.1–0.5 mg/mL) on hydroxyl radicals were more evident and dose-dependent comparing with N-CSPS and A-CSPS. The ability to quench hydroxyl radical of S-CSPS was 83.7% at the concentration of 0.5 mg/mL, which was relatively higher than that of C-CSPS (71.5%). The maximal inhibitory activity of N-CSPS was 42.9%. In the molecule of high sulfate content polysaccharides, part of -OH groups were substituted by $-OSO_3H$ groups, so the inhibitory effect of S-CSPS had obvious differences. The results were in accordance in the studies of Qi et al. (2005), in which two types of antioxidation mechanism were stated. One mechanism was that the effect of the metal complexes led to the suppression against hydroxyl radical generation, and the other was the direct clearance of the generated hydroxyl radical. The mechanism of C-CSPS on cleaning hydroxyl radicals needed to be further investigated.

3.3.3. Measurement of ferric reducing power (FRP)

The current literatures reported that many difference *in vitro* methods were 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 hydrophobic solutions, which would be a limit for water soluble samples. The antioxidant power of polysaccharides from corn silk was detected by the FRP assay in aqueous solution. The mechanism of reducing property of reducer was that the presence of the antioxidant substances in the samples caused the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form Fe^{2+}. And the Fe^{2+}could be monitored by measuring the absorption of the Prussian blue at 700 nm. As shown in Fig. 4C, the reducing power values of N-CSPS, S-CSPS, A-CSPS and C-CSPS were 0.098, 0.115, 0.100 and 0.157, respectively, when the concentration was 31.25 μg/mL. All of the samples showed dose-dependent inhibitory effects with the coefficient R^2 value of 0.9959, 0.9919, 0.9972 and 0.9955, respectively. The reducing power of all samples were ascended with the increase of concentrations (15.63–78.13 μg/mL), which

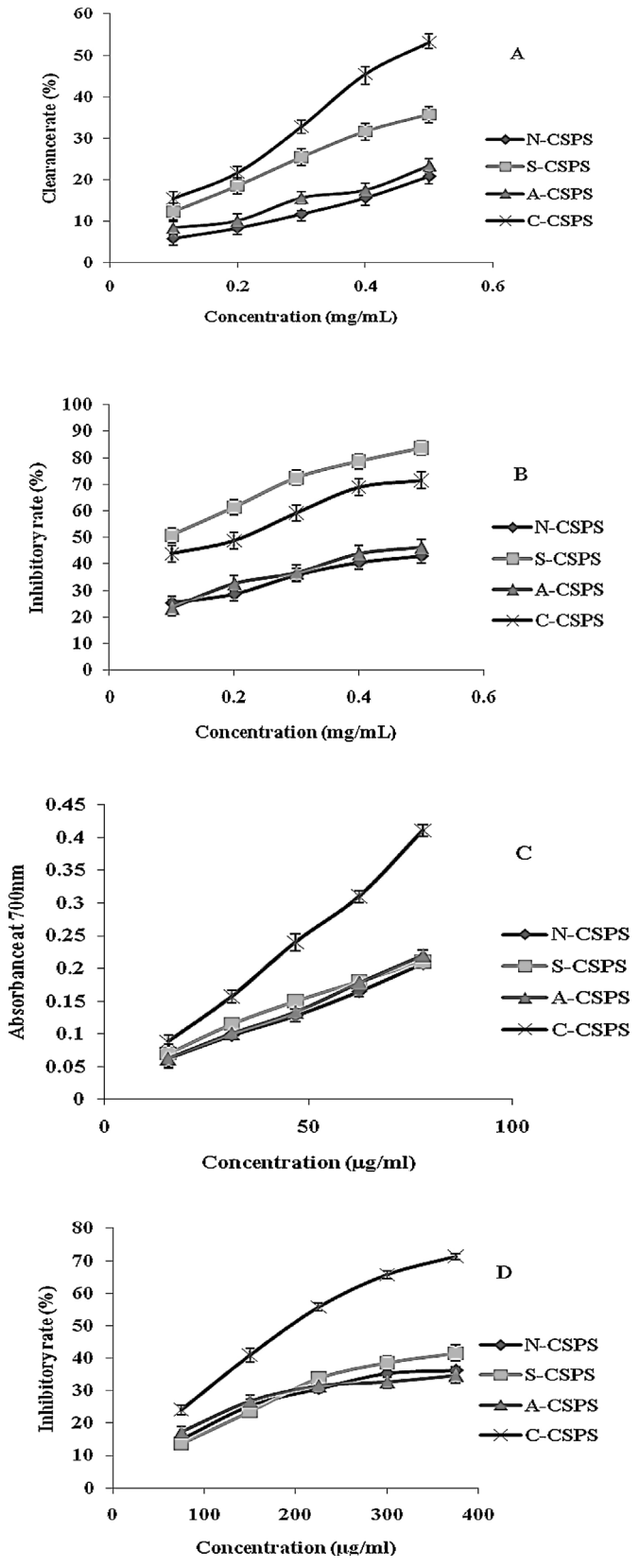

Fig. 4. Antioxidant activities of the native polysaccharide N-CSPS and its derivates (A, scavenging activity against DPPH free radical; B, inhibitory activity against hydroxyl radical; C, ferric reducing power activity; D, lipid peroxidation inhibition activity. Results are means ± S.D. of three measurements.).

indicated that N-CSPS and its derivatives were electron donors and could react with Fe^{3+}/ferricyanide to convert them into ferrous form Fe^{2+}. In the detected concentration, C-CSPS showed pronounced advantage compared with N-CSPS ($P < 0.05$), while no obvious change could be detected between S-CSPS, A-CSPS and N-CSPS. Xu et al. (2009) reported that reducing power of carboxylmethylated polysaccharide (C-GLP) from *Ganoder malucidum* was greatly improved in comparison with GLP. The activities of antioxidants were attributed to various mechanisms, such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Zou et al., 2008). The results obtained in our study could possibly be related to the introduction of carboxymethyl group, which could enhance the electron cloud density of active hydroxyl groups. Thus the electron-donating activity was increased and the reducing power of the polysaccharides was improved.

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

Lipid peroxidation was one major cause of food deterioration, affecting color, flavor, texture, and nutritional value. Oxidative modification of low-density lipoproteins (LDLs) might play a role in the development of atherosclerosis. The generated lipid peroxides further acted on the cell/cellular components, leading to both structural and functional damage of the biomolecule as well as the cellular structure (Puttaraju, Venkateshaiah, Dharmesh, Urs, & Somasundaram, 2006). Malondialdehyde (MDA) was the secondary byproduct, which was released during the lipid peroxidation. A decrease in the production of MDA in turn symbolized the inhibition of lipid peroxidation.

The liver peroxidation inhibition activity of the four samples was summarized in Fig. 4D, showing that the generation of MDA was inhibited owing to the existence of N-CSPS and its derivatives at the concentration from 75 to 375 μg/mL. All of them indicated dose-dependent inhibitory effects. It was showed that C-CSPS had a distinctly stronger inhibitory activity than that of N-CSPS, S-CSPS and A-CSPS ($P < 0.05$). Inhibitory rate of C-CSPS was 71.3% when the concentration was 375 μg/mL. It was the highest one when compared with those of N-CSPS (36.2%), S-CSPS (41.5%) and A-CSPS (34.7%), respectively. Thus carboxymethylation modification for corn silk polysaccharides could be used as an efficient way to enhance physiochemical properties and antioxidant activity of polysaccharides. In addition, it should not be neglected that the effects of introduction of ionic groups, the content of protein and uronic acid, and M_w on the bioactivity of the modified polysaccharides (Tao et al., 2009).

3.4. *α-Amylase inhibitory activity assay*

The α-amylase inhibitors could slow down starch digestion rate of food and restrain the increase of post-meal blood sugar. And thus α-amylase inhibitors have been one of the research hotspots as oral hypoglycemic agents for diabetic (Wang, Yang, & Wei, 2010). The inhibitory effect of N-CSPS and its derivatives on α-amylase were compared with the IC_{50} values (Fig. 5). The IC_{50} value of positive control arcabose was 2.51 mg/mL. The inhibitory effects order of N-CSPS, S-CSPS, A-CSPS and C-CSPS were determined as C-CSPS > S-CSPS > N-CSPS > A-CSPS with the IC_{50} values of 5.33 mg/mL, 8.54 mg/mL, 10.07 mg/mL and 10.31 mg/mL, respectively. The carboxymethylated CSPS, C-CSPS showed the highest inhibitory effect among the four polysaccharides samples but it was still weaker than the positive control arcabose. The reasons might be due to the different conformation between arcabose and the polysaccharides. As an enzyme, α-amylase could play the optimal enzyme activity in a certain pH range. Lower or higher pH

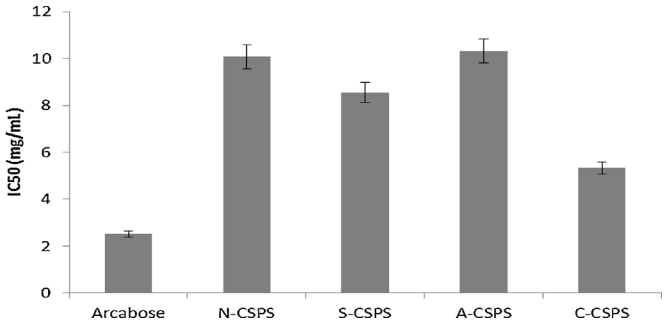

Fig. 5. α-Amylase inhibitory activity of the native polysaccharide N-CSPS and its derivatives.

value might result in the conformation transition and inactive of the active center of the enzyme. In order to ensure the optimal activity of α-amylase and assay the inhibitory effects of the samples, the α-amylase was dissolved in the PBS with the pH of 5.6 in the test, which was the optimal pH of alpha-amylase. The results in this study were in accordance with the findings from Gourgue, Champ, Lozano, & Delort-Laval (1992), in which it was described that the polysaccharides had a large amount of free carboxylic groups isolated from fruit would inhibit the enzyme activity. From Table 1 and Fig. 5, there was no obvious relationship found between the inhibitory effect on α-amylase and the content of neutral polysaccharides in CSPS. However, the inhibition rates ascended with the content of acid polysaccharides. The results were not agreement with the findings in Wang et al. (2010). This might be due to the different inhibitory mechanism of different polysaccharides, which will need further study.

4. Conclusion

Corn silk polysaccharides were firstly modified by three methods including suflation, acetylation and carboxymethylation in this study. Among the three derivatives, carboxylmethylated polysaccharide (C-CSPS) showed higher solubility, narrower molecular weight distribution, lower intrinsic viscosity, a hyperbranched conformation, significantly higher antioxidant and α-amylase inhibitory abilities compared with the native polysaccharide and other derivatives. C-CSPS might be used as effective antioxidant with potential value for healthy food as well as therapeutic agent.

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