UNIVERSITY OF HELSINKI

https://helda.helsinki.fi

Effects of different processing (Paozhi) on structural characterization and antioxidant activities of polysaccharides from Cistanche deserticola

Hou, Shoubu; Tan, Minghui; Chang, Senlin; Zhu, Yuan; Rong, Guang ...

2023-08-01
Elsevier B.V.
http://hdl.handle.net/10138/577619
Hou, S, Tan, M, Chang, S, Zhu, Y, Rong, G, Wei, G, Zhang, J, Zhao, B & Zhao, Q-S 2023, 'Effects of different processing (Paozhi) on structural characterization and antioxidant activities of polysaccharides from Cistanche deserticola', International Journal of Biological Macromolecules, vol. 245. https://doi.org/10.1016/j.ijbiomac.2023.125507

Downloaded from Helda, University of Helsinki institutional repository. https://helda.helsinki.fi
This is an electronic reprint of the original article.
This reprint may differ from the original in pagination and typographic detail.
Please cite the original version.

Journal Pre-proof



Effects of different processing (Paozhi) on structural characterization and antioxidant activities of polysaccharides from Cistanche deserticola

Shoubu Hou, Minghui Tan, Senlin Chang, Yuan Zhu, Guang Rong, Gaojie Wei, Jinyu Zhang, Bing Zhao, Qing-Sheng Zhao

PII: S0141-8130(23)02401-7

DOI: https://doi.org/10.1016/j.ijbiomac.2023.125507

Reference: BIOMAC 125507

To appear in: *International Journal of Biological Macromolecules*

Received date: 3 March 2023

Revised date: 24 May 2023

Accepted date: 20 June 2023

Please cite this article as: S. Hou, M. Tan, S. Chang, et al., Effects of different processing (Paozhi) on structural characterization and antioxidant activities of polysaccharides from Cistanche deserticola, *International Journal of Biological Macromolecules* (2023), https://doi.org/10.1016/j.ijbiomac.2023.125507

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2023 Published by Elsevier B.V.

Journal Pre-proof

Effects of different processing (Paozhi) on structural characterization and antioxidant activities of polysaccharides from *Cistanche deserticola*

Shoubu Hou [a,b], Minghui Tan [c], Senlin Chang [a,b], Yuan Zhu [a,b], Guang Rong [e], Gaojie Wei [a], Jinyu Zhang [d], Bing Zhao [a,b], Qing-Sheng Zhao [a,b] *

[a] *State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, 100190, P. R. China*

[b] *University of Chinese Academy of Sciences, Beijing, 100049, P. R. China*

[c] *College of Food Science and Technology, Guangdong Ocean University, Zhanjiang 524088, P. R. China*

[d] *Inner Mongolia Alashan Cistanche Co. ltd, Alashanzuoqi, Inner Mongolia, 750306, P. R. China*

[e] *HiperCog Group, Department of Education, Faculty of Educational Sciences, University of Helsinki, Helsinki, Finland*

* Corresponding author: Qing Sheng Zhao

E-mail addresses: qszhao@ipe.ac.cn

Address: 1 North 2nd Street, Zhong guan cun, Hai dian District, Beijing, P.R. China

Tel.: +86-010-82622280

ABSTRACT

In this study, five polysaccharides were extracted from processed *Cistanche deserticola*. The processing included crude product, enzymatic hydrolysis, hot air drying, stir-baking with wine and high-pressure steaming, and these polysaccharides

were named as CP-CDPs, EH-CDPs, HAD-CDPs, SBW-CDPs and HPS-CDPs, respectively. The structural characteristics and biological activities were explored. The results showed that processing changed properties of *C. deserticola* polysaccharides. CP-CDPs had the highest brightness value *L**(93.84) and carbohydrate content (61.27%). EH-CDPs had minimum M_w (1531.50 kDa), while SBW-CDPs had maximum M_w (2526.0 kDa). Glucose was major predominant monosaccharide in CP-CDPs (89.82%), HAD-CDPs (79.3%), SBW-CDPs (79.41%) and HPS-CDPs (63.86%), while galactose was major monosaccharide in EH-CDPs (29.44%). According to SEM, SBW-CDPs showed compact structures, while HPS-CDPs and HAD-CDPs had similar looser structure than SBW-CDPs; meanwhile, CP-CDPs showed irregular agglomeration shape and EH-CDPs was dense blocky shape. The AFM showed SBW-CDPs had the largest molecular chain than other polysaccharides. When scavenging activity reaching 50%, the concentrations of CP-CDPs, EH-CDPs, HAD-CDPs, SBW-CDPs, HPS-CDPs are 2.25, 0.25, 0.75, 1.8 and 1.5 mg/mL, respectively. This study sheds light on the effects of traditional Chinese medicine processing on characteristics, bioactivities of *C. deserticola* polysaccharides, and provides the basis for applications in food and pharmaceutical industries.

Keywords: *Cistanche deserticola*; polysaccharides; processing; antioxidant activities

Journal Pre-proof

1. Introduction

Cistanche deserticola is a perennial parasite herb, attaching onto the roots of psammophytes, such as *Haloxylox ammodendron* and *Tamarix*[1]. *C. deserticola* has been used for thousands of years as drug, which is mainly distributed in arid or semi-arid area[2]. Since record, *C. deserticola* is used to treat kidney deficiency[3], including sexual impotence, female sterility, weak waist and knees and constipation. In the folk, *C. deserticola* is also used as food. *C. deserticola* was included in the drug-food homology list several years ago. *C. deserticola* is called "Desert ginseng" in China because of its outstanding medical functions and nourishing effect. Modern medical studies show that *C. deserticola* has many biological activities, such as anti-aging[4], antidepressant efficacy[5], anti-hyperglycemic and hypolipidemic effects[6], hepatoprotective activity[7], antifatigue activity[8], antitumor[9] and anti-osteoporosis[10]. According to component analysis, the biological activity of *C. deserticola* attribute to the complex and varied phytochemical composition. Up to now, several chemical constituents have been isolated and studied. The chemical constituents of *C. deserticola* include phenylethanoid glycosides[11], iridoid glycosides, lignan glycosides, oligosaccharide esters, and polysaccharides[12-15].

Polysaccharides, a natural macromolecule polymer, are composed of ten or more monosaccharides linked by glycosidic bonds [16]. Polysaccharides widely exist in nature, such as animals, plants and micro-organisms[17]. The modern medical studies have shown that polysaccharides have various pharmacological effects, including hypolipidemic[18], hypoglycemic[19], anti-cancer[20], antioxidant[21],

immunomodulatory[22], hepatoprotective[7, 23] and so on. Thus, polysaccharides are an ideal source for medicine and functional food. Polysaccharides have complex structure. The biological activities of polysaccharides are determined by their unique structures [24]. Therefore, understanding the structure of polysaccharides is the basis of studying and utilizing polysaccharides. The structures of polysaccharides in the same material are affected by different processing, which could result in different activities. Liu et al reported that hot-air drying could increase α-configuration in neutral polysaccharides and reduce β-configuration in acidic polysaccharides, while freeze-dried polysaccharides had stronger scavenging ability[25]. Huang et al studied the effects of different extraction techniques on *Dictyophora indusiata* polysaccharides[26], showing that the vitro antioxidant effects of *D. indusiata* polysaccharides obtained by pressurized assisted and ultrasonic-assisted extraction were higher than other methods. Zhou et al found that the different solvents had effects on chemical characterization and bioactivities of *Apocynum venetum* leave polysaccharides[27]. For Chinese herbal medicine, there is a traditional processing, also called Paozhi in Chinese[28]. The different processing may lead to changes in drug efficacy due to changes in composition including polysaccharides[29]. The traditional processing in China for *C. deserticola* includes stir-baking with wine, high-pressure steaming, hot-air drying and so on. Up to now, there has been no report about the effects of different processing on the structural characterization and antioxidant activities of *C. deserticola* polysaccharides. In our previous research, we found that the *C. deserticola* polysaccharides from different processing methods show

different biological activities and other properties. Therefore, in this study, we want to discuss how five processing methods affect the properties of *C. deserticola* polysaccharides. Besides, *C. deserticola* has been included in the food and cosmetics catalog, indicating that it has broader application prospects not limited to medicinal. There are different properties requirements for *C. deserticola* polysaccharides in different applications, such as chroma and biological activity. Therefore, it is helpful for the application of *C. deserticola* polysaccharides by understanding their properties. The results provide basic data for a highly effective application of *C. deserticola* polysaccharides and guide the development of *C. deserticola* polysaccharide food, medicine or cosmetics.

In this study, five kinds of *C. deserticola* polysaccharides from fresh or dried *C. deserticola* were investigated. The chroma, chemical composition, monosaccharide composition, molecular weight, triple-helical structure and biological activities of polysaccharides were systematically compared. This study reveals different structure and properties of *C. deserticola* polysaccharides obtained from different processing methods.

2. Materials and methods

2.1. Materials and reagents

The *C. deserticola* were provided by Inner Mongolia Alashan Cistanche Co. ltd (Alxa League, China). The monosaccharide standards, including fucose, arabinose, rhamnose, galactose, glucose, xylose and mannose, were purchased from Sino Pharm Chemical Reagent Co., Ltd., while glucuronic acid and galacturonic acid were

purchased from Sigma Co., Ltd., USA. The other reagents, such as phenol, sulfuric acid, carbazole, bovine serum albumin, Coomassie blueG-250, phosphoric acid, ethanol (95%), were purchased from Sinopharm Chemical Reagents co., LTD (Shanghai, China).

2.2. Processing of *C. deserticola*

2.2.1. Crude product

The surface sediment of *C. deserticola* was removed by washing. The cleaned sample was cut into 3-5 mm thick slices. The slices were then placed in the prepared preservative and rinsed three times after soaking for 30 minutes to preserve the primary color.

2.2.2. Enzymatic hydrolysis

The enzymatic hydrolysis was carried out according to previous method[30]. The steps for pretreatment homogenate were consistent with 2.2.1. Then, cellulase and pectinase were added to *C. deserticola*. All of the content of enzyme were 0.1% (Ratio to dry basis). The mixture was stirred for 2 h at 50℃, and heated for 20 min at 90℃ to inactivate the enzyme.

2.2.3. Hot air drying

The fresh *C. deserticola* was cleaned and cut into 3-5 mm thick slices. The slices were put into oven at 80℃ until constant weight. Drying samples were crushed.

2.2.4. Stir-baking with wine

The fresh *C. deserticola* was cleaned and cut into 3-5 mm thick slices. Then, slices were immersed into Shaoxing Hua Tiao Chiew (a traditional Chinese yellow rice wine,

and the concentration of ethanol is 12%) and moistened for 4 h at sealed condition. The soaked samples were steamed for 3 h at 121℃ (0.1 Mpa). The steamed samples were dried to constant weight.

2.2.5. High-pressure steaming

The fresh *C. deserticola* was cleaned and cut into 3-5 mm thick slices. The slices were put into bain-marie and steamed for 3 h (pressure: 0.1 MPa, temperature: 121℃). Then, the samples were dried to constant weight.

2.3. Polysaccharide extraction and purification

The polysaccharides were extracted according to previous method[31]. All of the processed *C. deserticola* were crushed. Water was added at the ratio of 1:20(w/v, Ratio to dry basis). The mixture was kept at 80℃ for 2 h to extract polysaccharides and repeated three times. Centrifugation and filtration were used to remove insoluble residue, and the supernatant was concentrated at 50℃. The enzymatic hydrolysate from 2.2.2 was directly filtrated and concentrated. All of the concentrates were mixed with absolute ethanol at the ratio of 1:4(v/v). Then, the mixture was allowed to stand for 24 h at 4℃, and centrifuged (3000×g, 10 min) to obtain precipitates. The precipitates were washed with ethanol and acetone, respectively, and lyophilized using a freeze-dryer to get crude polysaccharides.

The crude polysaccharides redissolved in distilled water. The free proteins were removed according to the method reported by SEVAG [32]. Specifically, trichloromethane and 1-butanol were mixed at the ratio of 4:1(v/v). The polysaccharide solution was then mixed with Sevage reagent and shaken for 10

minutes to extract protein. The mixture was centrifuged, and the supernatant was extracted repeatedly while the bottom liquid was discarded. After ten repetitions of the process, no intermediate layer was visible any longer and the preparation was ready. Quadruple absolute ethanol was added into the final supernatant to get purified polysaccharides. The five purified polysaccharide fractions were named CP-CDPs (crude product), EH-CDPs (enzymatic hydrolysis), HAD-CDPs (hot air drying), SBW-CDPs (Stir-baking with wine) and HPS-CDPs (high-pressure steaming), respectively.

2.4. Chemical composition analysis

The carbohydrate contents were measured by phenol-sulfuric acid method[33], using glucose as the standard. Bradford method was used to measure the protein content[34]. Uronic acid contents were determined by a modified carbazole method[35].

2.5. Color test

Color parameters were determined using a colorimeter (model 10QC, 3nh Inc., Shenzhen, China). The color parameters include L^*, a^* and b^*. L^* value represents brightness (100=white, 0=black), while a^* and b^* values represent chroma (a^*, positive=redness, negative=greenness; b^*, positive=yellowness, negative=blueness, respectively). Reference chromaticity values were L_0^*=94.26, a_0^* =-0.87, b_0^*=3.56. Each sample was measured for three times. ΔE was calculated according to the following formula:

$$\Delta E = \sqrt{(L^* - {L_0}^*)^2 + (a^* - {a_0}^*)^2 + (b^* - {b_0}^*)^2} \quad (1)$$

Where ΔE represents the color changes from the reference color.

2.6. Monosaccharide composition and molecular weight

The monosaccharide composition was carried out according to the method reported by Tan[36]. Specifically, 5 mg polysaccharides were dissolved in 2 mL of trifluoroacetic acid (2 M), and hydrolyzed for 2 h at 121℃. The mixed liquid was dried, washed for 3 times with methanol and redissolved in purified water. The sample solution was filtered through 0.22 μm microporous filtering film for measurement. Monosaccharides fucose, arabinose, rhamnose, galactose, glucose, xylose, mannose, galacturonic acid, guluronic acid and glucuronic acid were used as references. Test conditions: Column: C18 (250 mm×4.6 mm, 5 μm). Mobile phase B: acetonitrile, and used at 82% A and 18% B. Detection wavelength: 245 nm. The column temperature was 25℃. Flow rate: 1.0 mL/min. Injection volume: 10 μL.

Molecular weight (M_W) was determined according to the method reported by Zhao[37]. High performance size exclusion chromatography (HPSEC, TSKgel G4000PW_{XL}, TOSOH, Japan) was used in a HPLC system (Agilent 1260, USA) to measure molecular weight. Approximately 5 mg sample was dissolved in 1 mL of deionized water, and filtered by 0.22 μm filter membrane before injection. Test conditions: temperature (25℃), eluent (purified water), flow rate (0.6 mL/min), injection volume (5 μL). A series of dextrans with different molecular weights were used as control. Plot the curve with the logarithm of the relative molecular weight and the peak time as coordinates.

2.7. UV–Vis and FT-IR spectroscopic analysis

The polysaccharides samples were dissolved in water and the final concentration was 2 mg/mL. Then, spectrums were obtained from a spectrophotometer (UV-2802, Unico, USA) at the wavelength range of 190-800 nm at room temperature. Pure water was used as reference. The FT-IR spectra were obtained from a FT-IR spectroscopy analysis (Nicolet iS5, Thermo Fisher Scientific, Waltham, USA). Around 1.0 mg polysaccharides sample was mixed with 200 mg KBr sufficiently and tableted. The resolution was 4 cm^{-1} and the wavenumber was in the range of 4000 to 400 cm^{-1}.

2.8. Congo red analysis

The Congo red test was measured according to previously reported method[31]. 2 mg of polysaccharides were dissolved in 1 mL distilled water. The solution was mixed with 1 mL Congo red (0.1 mmol/L), and 0.5 mL sodium hydroxide with different concentrations to the final concentrations of 0-0.5 mol/L. Then, spectrum was obtained at the wavelength range of 400-800 nm.

2.9. Scanning electron microscopy

Scanning electron microscopy (SEM) (ZEISS Gemini 300) was used to observe the appearances of polysaccharides. After being sprayed with gold powder, polysaccharides samples were placed in high vacuum to record images.

2.10. Atomic force microscopy

The molecular morphologies of polysaccharides samples were obtained by atomic force microscope (AFM). About 1 mg of polysaccharides was dissolved to 10 μg/mL and filtered by membranes (0.22 μm). Then, 5 μL polysaccharide solution was dropped on the surface of freshly peeled mica tablets and dried with nitrogen.

2.11. X-ray diffraction

XRD spectra of polysaccharides were researched by using an X-ray diffractometer (X'PERT-PRO MPD, PANalytical B.V, Netherlands). The 2θ was ranged from 5 to 90 ° at room temperature and the scanning rate was 5 °/min.

2.12. Thermal stabilities analysis

The thermal properties of the polysaccharide were measured by TG/DTA7300 simultaneous analyzer (HITACHI, Japan). The samples were heated from 30 to 800℃ at the rate of 10℃/min under nitrogen atmosphere.

2.13. Antioxidant activities

2.13.1. DPPH radical scavenging activity

DPPH(2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was measured by the method reported by Parhat Rozi[31]. 100 μL polysaccharide solution with different concentrations and 100 μL DPPH-ethanol solution were mixed in 96 well plate. The absorbances were obtained at 517 nm after being kept for 30 min in the dark. Ascorbic acid of the same concentration with polysaccharides solution was used as a positive control. The DPPH radical scavenging activity is calculated by the following formula:

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

Where A_2 represents the absorbance of mixture of DPPH-ethanol solution and distilled water, A_1 represents the absorbance of mixture of DPPH-ethanol solution and sample solution, A_0 represents the absorbance of ethanol and sample solution.

2.13.2. ABTS radical scavenging activity

The ABTS(2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity was determined using the method[38]. 10 mL ABTS solution (7 M) was mixed with 176 μL potassium persulfate solution (140 M) and kept for 12 h in the dark to get $ABTS^+$ solution. The solution was diluted with deionized water to get basic absorbance of 0.70±0.02 at 734 nm. Then, 1 mL of polysaccharide solution with different concentrations was mixed with 4 mL $ABTS^+$ solution, and kept for 6 min in the dark to obtain the absorbance at 734 nm. Ascorbic acid of the same concentration with polysaccharides solutions was used as a positive control. The ABTS radical scavenging activity is calculated according to the following formula:

$$\text{ABTS scavenging activity (\%)} = \frac{A_2 - (A_1 - A_0)}{A_2} \times 100 \quad (3)$$

Where A_2 represents the absorbance of the control; A_1 represents the absorbance of samples; A_0 represents the absorbance of the distilled water with sample.

2.13.3. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity also was measured by the method reported by Parhat Rozi[31]. Specifically, polysaccharide solution with different concentrations, ferrous sulphate solution (9 M), salicylic acid-ethanol solution (9 M) and hydrogen peroxide solution (8.8 M) were blended at the ratio of 1:1:1:1. The mixture reacted for 30 min at 37℃. Then, the absorbance was got at 510 nm. Ascorbic acid of the same concentration with polysaccharides solution was used as a positive control. The hydroxyl radical scavenging activity is calculated according to the following formula:

$$\text{Hydroxyl radical scavenging activity (\%)} = \frac{A_2 - (A_1 - A_0)}{A_2} \times 100 \quad (4)$$

Where A_2 represents the absorbance of the blank control; A_1 represents the

absorbance of samples; A_0 represents the absorbance of the solution which H_2O_2 was replaced by distilled water.

2.14. Statistical analysis

All experiments were carried out in triplicate, and data were expressed as mean ± standard errors. Statistical significance was calculated by one-way ANOVA using Origin software (2019). $P < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Chemical composition of *C. deserticola* polysaccharides

The chemical compositions of *C. deserticola* polysaccharides by five different processing were listed in Table 1. The carbohydrate contents with different processing were significantly different as the following order: CP-CDPs (61.27%) > SBW-CDPs (58.42%) > HPS-CDPs (57.07%) > HAD-CDPs (54.09%) > EH-CDPs (35.27%). The possible reason may be the composite structure of cellulose, hemicellulose, pectin and polysaccharide was swell in fresh tissue, resulting polysaccharides was easily to be separated. The composite structure was dense in dry samples, which led to difficulties in polysaccharide separation. Tiny amounts of protein fractions (around 1.0%) were determined in all polysaccharide samples, but UV spectrum did not have obvious absorption peaks at about 280 nm. It shows that all polysaccharides sample may include very little binding protein, because Sevage reagent basically removes free proteins. Uronic acid is an important component of pectins and carragenans from organism[39]. The content of uronic acid in different polysaccharides was significantly different. The HAD-CDPs has the lowest content of uronic acid

(16.87%), while SBW-CDPs has the highest content of uronic acid (22.9%).

Table 1. Chemical composition and the molecular weight of different *C. deserticola* polysaccharides.

Index	Samples				
	CP-CDPs	EH-CDPs	HAD-CDPs	SBW-CDPs	HPS-CDPs
Carbohydrate (wt%)	61.27±0.006[a]	35.27±0.013[b]	54.09±0.005[c]	58.42±0.013[d]	57.07±0.001[d]
Protein(wt%)	0.47±0.001[a]	0.69±0.0007[b]	0.77±0.001[b]	1.27±0.005[c]	0.84±0.0009[b]
Uronic acid(wt%)	20.43±0.002[a]	19.83±0.009[a]	16.37±0.003[b]	22.90±0.008[c]	20.85±0.010[a]
Molecular weight parameters					
M_w (kDa)	1764.41	1531.50	1767.21	2526.00	1851.05
M_n (kDa)	1634.78	172.71	1677.22	2097.86	1813.17
M_w/M_n	1.08	8.32	1.05	1.20	1.02

Note: Values are expressed as mean ± SD (n = 3).

Means within a row with different letters indicate significant differences ($P \leq 0.05$).

3.2. Color analysis

The chromaticity parameters of different *C. deserticola* polysaccharides were presented in Table 2. The appearance of different *C. deserticola* polysaccharides are shown in Fig.3(A). It can be seen in Table 2 and Fig.3 that there are significantly differences among the samples. Specifically, the CP-CDPs is nearly white, while the other samples are brown or black-brown-colored. The CP-CDPs has highest *L** value (93.84) and the lowest color difference ΔE value (4.1), while the lowest *L** value

(43.17) and the highest ΔE value (54.54) were found in EH-CDPs. The other three samples were similar in chromaticity. The primary cause of color change in fresh-cut fruits and vegetables is enzymatic browning [40]. The membranolysis occurred during processing, which lead to intracellular enzyme and substrate binding, producing colored substances. However, the CP-CDPs was obtained from inactivated-enzyme fresh *C. deserticola*, and had better exterior than other samples.

Table 2. The color characteristics of different *C. deserticola* polysaccharides.

Chromaticity parameter	Samples				
	CP-CDPs	EH-CDPs	HAL-CDPs	SBW-CDPs	HPS-CDPs
*L**	93.84±0.33^{a}	43.17±0.43^{e}	61.7±0.29$^{c.d}$	62.33±0.68^{d}	60.85±0.55^{c}
*a**	-0.39±0.035^{a}	6.32±0.24^{b}	6.35±0.17^{b}	3.46±0.29^{c}	5.0±0.21^{d}
*b**	7.60±0.60^{a}	21.26±0.37^{b}	23.0±0.68^{c}	16.08±0.41^{d}	20.13±0.90^{b}
ΔE	4.1±0.63^{a}	54.54±0.41^{b}	38.61±0.53^{c}	34.57±0.73^{d}	37.76±0.74^{c}

Note: Values are expressed as mean ± SD (n = 3).

Means within a row with different letters indicate significant differences (P≤ 0.05).

3.3. Molecular weight analysis and monosaccharide composition

Previous studies have proved that the molecular weight of polysaccharide is related to their bioactivities[41]. Therefore, in this study, the effects of different processing on molecular weights were analyzed. As shown in Table.1, there are significant differences in molecular weights of five polysaccharides. The EH-CDPs has the smallest weight-average molecular weight value and number-average molecular

weight value (1531.50 kDa, 173.71 kDa, respectively), indicating that cellulase and pectinase hydrolyzed polysaccharide chain in *C. deserticola*. The weight-average molecular weight value of SBW-CDPs(2526.00kDa) was much higher than other polysaccharides, one possible reason may be the polysaccharide chain occurred condensation under the action of wine. M_w/M_n (degree of dispersion) is used to describe the polydispersity of polysaccharides molecular weight. According to Table 1, EH-CDPs have higher polydispersity value than other four polysaccharides, indicating that they have wider molecular weight distribution.

The monosaccharide compositions of the five CDPs are listed in Table 3. There are marked differences of monosaccharide content among the five CDPs. Glucose, arabinose and galactose existed with different molar ratios in CP-CDPs (89.82%. 3.47% and 3.98%), HAD-CDPs (79.3%, 10.43% and 5.59%), SBW-CDPs (59.41%, 19.94% and 7.56%) and HPS-CDPs (63.85%, 24.52% and 8.43%), while galactose, rhamnose, glucose and arabinose are major predominant monosaccharide in EH-CDPs (29.44%, 26.31%, 10.49% and 10.21%, respectively). It shows that enzymes have obviously changed the monosaccharide compositions of CDPs. Comparing with CP-CDPs, the content of glucose is obviously reduced in HAD-CDPs, SBW-CDPs and HPS-CDPs, indicating that processing could change the monosaccharide compositions of CDPs. Arabinose and galactose may form the main chain of the pectin molecule[42], and EH-CDPs has the highest galacturonic acid content. It indicates that EH-CDPs may contain certain pectin polysaccharide. Different monosaccharide compositions are the basis of different structures, which cause significant differences in biological activity.

Similar studies have also reported that drying pretreatments could affect monosaccharide compositions[25].

Table 3. The monosaccharide composition of *C. deserticola* polysaccharides.

Sugar components	Samples				
(%, molar ratio)	CP-CDPs	EH-CDPs	HAD-CDPs	SBW-CDPs	HPS-CDPs
Fucose	0.19	0.32	0.2	0.13	0.16
Arabinose	3.47	10.21	10.43	19.94	24.52
Rhamnose	0.0	26.31	0.0	4.24	0.0
Galactose	3.98	29.44	5.59	7.56	8.43
Glucose	89.82	10.49	79.3	59.41	63.86
Xylose	0.8	1.01	1.23	0.58	0.62
Mannose	0.28	3.5	2.17	0.29	0.52
Galacturonic Acid	0.21	16.53	0.29	6.76	0.84
Guluronic Acid	0.6	0.46	0.4	0.42	0.51
Glucuronic Acid	0.66	1.73	0.39	0.68	0.55

3.4. UV–Vis and FT-IR spectroscopic analysis

The UV absorption of different *C. deserticola* polysaccharides was shown in Fig.1(A). The maximum absorption peaks are not concentrated, implying that the polysaccharide composition is not homogeneous[43]. The curves of UV absorption are very different, the reason might be that the internal structures and composition of *C. deserticola* polysaccharides are different. The minor peak between 260 and 280 nm represents protein or nucleic acid, while SBW-CDPs has a clear peak, indicating more

protein than other samples. The results were consistent with the protein assay analysis in Table 1. Meanwhile, the EH-CDPs, SBW-CDPs and HPS-CDPs have unknown peaks at the wavelengths of 410 nm, showing the differences among the polysaccharides.

FT-IR spectroscopy is one useful technique to identify the functional groups of polysaccharides[44]. The FT-IR spectra of different polysaccharides are basically same according to Fig.1(B), and the all-characteristic polysaccharide bands are included. The strong absorption peaks at 3400 cm^{-1} were derived from the O-H stretching vibration[45], while the peaks at around 2940 cm^{-1} were attributed to the C-H stretching[46]. The peaks at approximately 1620 cm^{-1} and 1420 cm^{-1} were due to the asymmetric and symmetric stretching of the carboxylate anion groups ($–COO^–$), respectively[47]. The characteristic absorption peaks at 1150, 1081, 1021 and 926 cm^{-1} were related to the stretching vibrations of the C-O-H side groups, the C-O-C glycosidic band vibration and expansion vibration of asymmetric ring of a pyranose ring[48]. However, there are some differences among the FT-IR spectra of different polysaccharides. The EH-CDPs has a weak peak at around 1150 cm^{-1}, indicating C-O-C bond was destroyed by cellulase. The CP-CDPs shows strong peaks at around 930 and 854 cm^{-1}, which belong to characteristic peaks of α-D-glucopyranose, and it was not showed in others samples. It shows that processing changes the structure of α-D-glucopyranose. The peak at around 762 cm^{-1} may belong to symmetric stretching vibration peak of C-O-C bond, which could be broken by cellulase. According to the results of FT-IR, comparing with CP-CDPs, the structure of *C. deserticola*

polysaccharides could be changed by processing.

3.5. Congo red analysis

The triple helix conformations of polysaccharides are related to their bioactivity, which can be analyzed by Congo red method[49]. The polysaccharides with triple-helix conformation combine with Congo red dye causing the red shift in the λ_{max}, and the λ_{max} decrease with the increase in the concentration of NaOH[38]. Fig.2 shows the λ_{max} of *C. deserticola* polysaccharide-Congo red complexes in different concentrations of NaOH. Specifically, among the five polysaccharides, CP-CDPs and EH-CDPs have red shifts in λ_{max}, while there is no red shift shown in other three polysaccharides. It shows that CP-CDPs and EH-CDPs have triple helix conformations, while HAD-CDPs, SPW-CDPs and HPS-CDPs have non-three helical structure. Besides, the previous study had reported monosaccharide composition could inhibit polysaccharides from producing triple-helix conformation[50].

3.6. Scanning electron microscopy

The microstructures of *C. deserticola* polysaccharides were observed by scanning electron microscopy. According to Fig.3(B), there are significant difference of microstructures in different *C. deserticola* polysaccharides. EH-CDPs has completely different microstructure with other samples. The other samples are irregular network structures, while EH-CDPs is dense structure with holes. It may be because enzymes destroy the chain structure of macromolecular polysaccharides, resulting the molecular chain to collapse. The other four polysaccharide samples have similar microstructures with minor differences, they exhibited network structures by

numerous spherical particles piled with different tightness. To be specific, HAD-CDPs has similar structural features with HPS-CDPs, including certain pore structures. SBW-CDPs, on the other hand, have the densest microstructure, almost without any pore, which could be due to their highest molecular weight, resulting in larger interaction polysaccharide chains. In CP-CDPs, there was a significant reunion with different particle size. According to SEM image, the junction in CP-CDPs is ambiguous and the components are not uniform. One possible reason is that CP-CDPs contains much complex components. This result was in accordance with the molecular weight result.

3.7. Atomic force microscopy

The chain conformation of five different *C. deserticola* polysaccharides was obtained by atomic force microscopy (AFM). Fig.3(C and D) shows 2- and 3-dimensional images of *C. deserticola* polysaccharides molecular morphologies, respectively. The results demonstrate that the five polysaccharide fractions show significant differences in molecular morphologies. Therein, HAD-CDPs and HPS-CDPs have similar molecular morphologies. According to Table1, the M_w of HAD-CDPs and HPS-CDPs are close, and the microstructure also are similar according to Fig.3(B). However, it seems that the diameters of a single polysaccharide strand in HAD-CDPs (more than 200 nm) were larger than HPS-CDPs (around 200 nm), one possible reason is that polysaccharide chains overlapped in HAD-CDPs. Besides, HAD-CDPs and HPS-CDPs have many short side chains. The SBW-CDPs has the largest polysaccharide chains (around 800 nm), and height: 0.2-3.6 nm, which

is higher than a single polysaccharide chain (0.1~1 nm)[51]. It shows that the molecular aggregation of SBW-CDPs occurred, which is consistent with previous studies: the polysaccharide with higher M_w is easily to aggregate[52]. The EH-CDPs has multiple irregular protuberances and short polysaccharide chain, showing many complex fractions existing in EH-CDPs. The result is consistent with that EH-CDPs has the largest M_w/M_n value. It is supposed that the polysaccharide chain was cut into short chains by enzymes, and the remaining components (such as glycoprotein) may branch and tangle. There were no polysaccharide chains were observed in CP-CDPs and surface morphology was rough. According to Fig 3(D), 3-D image consists of rod shape and irregular lumps. This phenomenon shows that molecular aggregation occurred in CP-CDPs, which was similar to the result obtained by SEM.

3.8. X-ray diffraction

XRD is a technology to analyze the crystalline and non-crystalline materials of polysaccharides. As shown in Fig.4, all polysaccharides have a diffraction peak at around 20 °, indicating that all samples have both crystalline and amorphous nature[53]. However, the diffraction curves are slightly different. Firstly, the height of diffraction peak is significantly different, CP-CDPs has the highest peak and the other samples have similar heights, showing that CP-CDPs has higher crystallinity than other samples. Secondly, CP-CDPs has a weak diffraction peak at approximately 36 °, while other diffraction curves do not contain this weak diffraction peak. The result indicated crystalline structures of polysaccharides could be affected by processing method.

3.9. Thermal stabilities analysis

Thermal stability is an important characteristic for polysaccharides. In this study, thermodynamic properties were investigated by TG, DTG and DTA of polysaccharides. According to Fig.5(A), the TG curves demonstrate relatively similar shape with slight differences. All samples have three mass losses, the first mass loss occurred at around 30-220℃, which was due to water loss (absorbed and bound water)[54]. The second mass loss was attributed to thermal degradation of polysaccharide chains, decarboxylation of acid groups, and decomposition of the C–C linkages on the pyran ring[55]. The third mass loss was due to the pyrolysis of polysaccharide skeletons. The mass loss trends are different, EH-CDPs has a steeper weightlessness curve than other samples, indicating that EH-CDPs have poorer water retention and smaller molecular weight.

The DTG curves show mass loss rate of polysaccharides. As shown in Fig.5(B), in the first stage, the difference in DTG curves is due to water holding capacity. The CP-CDPs has a lower peak showing that there is a strong combination with water, while other samples have sharper peaks indicating that there is a higher water loss rate at around 60℃. In the second stage, it shows distinct differences, indicating that the polysaccharide chains, structures and molecular weight are different in polysaccharides. The DTA curves are used to measure enthalpy changes about the sample[56].The change trends of *C. deserticola* polysaccharides are shown in Fig.5(C). All curves have a weak endothermic peak with different peak value at approximately 270℃, indicating that the phase changed within the samples when

polysaccharide chains degrading.

3.10 Antioxidant activities

3.10.1. Scavenging activity of DPPH radical

The DPPH radical scavenging activity is widely used to evaluate antioxidant capacities[57]. Fig.6(A) shows DPPH radical scavenging rates of *C. deserticola* polysaccharides. According to the Fig.6(A), all polysaccharides have a scavenging activity for DPPH radical and show a strong dose-effect relationship. The isocratic VC-aqueous solution has higher clearance rate as positive control, while the scavenging abilities of *C. deserticola* polysaccharides are quite different. The DPPH radical scavenging activity generally increased in the order of CP-CDPs < EH-CDPs ≈ SBW-CDPs < HPS-CDPs < HAD-CDPs. The HAD-CDPs has the strongest scavenging ability, reaching 50% at approximately the concentration of 0.6 mg/mL. HPS-CDPs has a weaker scavenging ability than HAD-CDPs, reaching the maximal scavenging ability of 75.6% at a concentration of 1.5 mg/mL. The EH-CDPs and SBW-CDPs have similar scavenging ability according to the curves. The CP-CDPs shows a weak scavenging activity for the DPPH radicals compared with other samples. The scavenging ability increases from 3.56% to 54.27% when the concentration increasing from 0.3 to 1.8 mg/mL of CP- CDPs.

3.10.2. Scavenging activity of ABTS radical

ABTS radical scavenging activity is another method to evaluate antioxidant capacities. Fig.6(B) shows ABTS radical scavenging rates of *C. deserticola* polysaccharides. According to Fig.6(A) and Fig.6(B), the trends of DPPH and ABTS

radical scavenging rates are similar, showing strong dose-effect relationship. However, the distinctions among different polysaccharides are more marked. To be specific, the scavenging abilities of HAD-CDPs reaches 50% at around the concentration of 0.72 mg/mL and 96.83% at the concentration of 1.8 mg/mL. The ABTS radical scavenging activities of EH-CDPs and SBW-CDPs are extremely close at each concentration and the maximal radical scavenging rate is around 50% at the concentration of 1.8 mg/mL. Furthermore, it should be noted that the CP-CDPs shows a poor radical scavenging rate, and radical scavenging activity reaches 18.04% at around concentration of 1.8 mg/mL.

3.10.3. Scavenging activity of hydroxyl radical

Hydroxyl radical is a harmful radical which could lead to oxidative damage of nucleic acids, proteins, and lipids[58]. According to previous reports, the reason of biomolecules scavenging hydroxyl radicals was to inhibit the generation of hydroxyl radicals, but not directly scavenge[59]. Therefore, the hydroxyl radical scavenging activity of polysaccharides represent chelating ability with iron. The scavenging activities of *C. deserticola* polysaccharides on hydroxyl radicals generated through the Fenton reaction were evaluated. According to Fig.6(C), all samples show dose-effect relationship. When scavenging activity reaching to 50%, the concentrations of CP-CDPs, EH-CDPs, HAD-CDPs, SBW-CDPs, HPS-CDPs are 2.25 mg/mL, 0.25 mg/mL, 0.75 mg/mL, 1.8 mg/mL and 1.5 mg/mL, respectively. The scavenging activity of hydroxyl radicals on HAD-CDPs is the highest compared to other samples. It is noteworthy that the EH-CDPs has higher scavenging activity of

hydroxyl radical than DPPH and ABTS, indicating that the capability of scavenging hydroxyl radical of *C. deserticola* polysaccharides had greatly improved after being treated by enzymolysis.

In a word, the antioxidant activities of *C. deserticola* polysaccharides had significant changes after different processing. HAD-CDPs had the highest antioxidant activities than other samples. EH-CDPs and SBW-CDPs had similar antioxidant activities on DPPH and ABTS radicals. According to the Congo red analysis, CP-CDPs and EH-CDPs have a triple-helix conformation, indicating the native conformation of polysaccharides is easier to be preserved from fresh *C. deserticola* and CP-CDPs exhibits the weakest antioxidant activity. Meanwhile, the triple-helix conformation of *C. deserticola* polysaccharides disappeared after processing, accompanying by an increase in antioxidant activity. It shows that conformational structure is an important feature related to antioxidant activity in *C. deserticola* polysaccharides. It shows that processing could change the structures and improve the biological activities of *C. deserticola* polysaccharides.

4. Conclusion

In our study, we obtained a pure white polysaccharide for the first time from fresh *C. deserticola*, and conducted a systematic discussion. Besides, we also systematically explored the changes of structural and antioxidant activity of *C. deserticola* polysaccharides from different processing approaches. We got five different polysaccharides from processed *C. deserticola*. Then, the chemical composition, monosaccharide composition, molecular weight and color were measured. The results

showed that the processing could affect the physical properties of *C. deserticola* polysaccharides. Besides, UV–Vis and FT-IR spectroscopic, SEM, AFM and thermal stabilities were also used to analyze the structures of *C. deserticola* polysaccharides. Results showed that the processing could change the microstructures, molecular morphology and polysaccharide chain structure. According to our findings, the traditional processing in *C. deserticola* could improve the antioxidant activities of *C. deserticola* polysaccharides, and all polyssacharides of *C. deserticola* showed dose-dependent connection. However, this study provides a basic study on different *C. deserticola* polysaccharides, and the other efficacies can be further validated in the future, such as antidiabetic, anti-obesity, hepatoprotective, anti-inflammatory, neuroprotective and regulating gut microbiota activities. This study provided a theoretical basis and laboratory evidence for the application of *C. deserticola* polysaccharides as functional antioxidant foods or medicines.

CRediT authorship contribution statement

Shoubu Hou: Investigation, Writing - original draft, Data curation, Writing - review & editing. **Minghui Tan**: Methodology. **Senlin Chang**: Investigation. **Yuan Zhu**: Data curation. **Guang Rong**: Writing – review. **Gaojie Wei**: Investigation. **Jinyu Zhang**: Project administration, Funding acquisition. **Bing Zhao**: Supervision. **Qing-Sheng Zhao:** Investigation, Methodology, Project administration, Writing - review & editing.

Declaration of competing interests

The authors declare no potential conflicts of interest.

Acknowledgments

This work was conducted with financial support from Special fund for transformation of scientific and technological achievements in Inner Mongolia Province (AMCG201910). The authors would like to thank Inner Mongolia Alashan Cistanche Co. ltd for their generous supply of the experimental material for this study. We would like to thank Sanshu Bio-tech (Shanghai, China) for assistance with monosaccharide composition analysis.

Journal Pre-proof

References

[1] Y. Jiang, P.F. Tu, Analysis of chemical constituents in Cistanche species, J Chromatogr A 1216(11) (2009) 1970-9.

[2] T. Wang, X. Zhang, W. Xie, Cistanche deserticola Y. C. Ma, "Desert ginseng": a review, Am J Chin Med 40(6) (2012) 1123-41.

[3] C.P. Commission, Pharmacopoeia of the People's Republic of China (part I, 2020 edition), Chinese Medical Science and Technology Press, Beijing, 2020.

[4] N. Wang, S. Ji, H. Zhang, S. Mei, L. Qiao, X. Jin, Herba Cistanches: anti-aging, aging Dis 8(6) (2017) 740-759.

[5] D. Wang, H. Wang, L. Gu, The antidepressant and cognitive improvement activities of the traditional chinese herb Cistanche, Evid Based Complement Alternat Med 2017 (2017) 3925903.

[6] W.T. Xiong, L. Gu, C. Wang, H.X. Sun, X. Liu, Anti-hyperglycemic and hypolipidemic effects of Cistanche tubulosa in type 2 diabetic db/db mice, J Ethnopharmacol 150(3) (2013) 935-45.

[7] Y. Guo, L. Cao, Q. Zhao, L. Zhang, J. Chen, B. Liu, B. Zhao, Preliminary characterizations, antioxidant and hepatoprotective activity of polysaccharide from Cistanche deserticola, Int J Biol Macromol 93(Pt A) (2016) 678-685.

[8] R.L. Cai, M.H. Yang, Y. Shi, J. Chen, Y.C. Li, Y. Qi, Antifatigue activity of phenylethanoid-rich extract from Cistanche deserticola, Phytother Res 24(2) (2010) 313-5.

[9] H. Zhang, Z. Xiang, X. Duan, J.L. Jiang, Y.M. Xing, C. Zhu, Q. Song, Q.R. Yu,

Antitumor and anti-inflammatory effects of oligosaccharides from Cistanche deserticola extract on spinal cord injury, Int J Biol Macromol 124 (2019) 360-367.

[10] L. Zhang, X.X. Yue, L. Zhang, J.F. Zhao, Y.M. Chen, Z.J. Cao, Y.L. Liu, Anti-osteoporosis effect of Cistanche deserticola Ma extract in ovariectomized rats, Tropical Journal of Pharmaceutical Research 15(9) (2016) 1929-1933.

[11] X.Y. Cheng, H.Y. Zhou, X. Cui, W. Ni, C.Z. Liu, Improvement of phenylethanoid glycosides biosynthesis in Cistanche deserticola cell suspension cultures by chitosan elicitor, J Biotechnol 121(2) (2006) 253-60.

[12] X.M. Liu, J. Li, Y. Jiang, M.B. Zhao, P.F. Tu, Chemical constituents from Cistanche sinensis (Orobanchaceae), Biochemical Systematics and Ecology 47 (2013) 21-24.

[13] A. Bougandoura, B. D'Abrosca, S. Ameddah, M. Scognamiglio, R. Mekkiou, A. Fiorentino, S. Benayache, F. Benayache, Chemical constituents and in vitro anti-inflammatory activity of Cistanche violacea Desf. (Orobanchaceae) extract, Fitoterapia 109 (2016) 248-53.

[14] Z. Fu, X. Fan, X. Wang, X. Gao, Cistanches Herba: An overview of its chemistry, pharmacology, and pharmacokinetics property, J Ethnopharmacol 219 (2018) 233-247.

[15] T. Morikawa, Y. Pan, K. Ninomiya, K. Imura, H. Matsuda, M. Yoshikawa, D. Yuan, O. Muraoka, Acylated phenylethanoid oligoglycosides with hepatoprotective activity from the desert plant Cistanche tubulosa, Bioorg Med Chem 18(5) (2010) 1882-90.

[16] M.U. Kakar, M. Naveed, M. Saeed, S. Zhao, M. Rasheed, S. Firdoos, R. Manzoor, Y. Deng, R. Dai, A review on structure, extraction, and biological activities of polysaccharides isolated from Cyclocarya paliurus (Batalin) Iljinskaja, Int J Biol Macromol 156 (2020) 420-429.

[17] H. Huang, G. Huang, Extraction, separation, modification, structural characterization, and antioxidant activity of plant polysaccharides, Chem Biol Drug Des 96(5) (2020) 1209-1222.

[18] H.L. Zeng, S. Miao, Y. Zhang, S. Lin, Y.Y. Jian, Y.T. Jian, B.D. Zheng, Isolation, preliminary structural characterization and hypolipidemic effect of polysaccharide fractions from Fortunella margarita (Lour.) Swingle, Food Hydrocolloids 52 (2016) 126-136.

[19] J. Zhu, W. Liu, J. Yu, S. Zou, J. Wang, W. Yao, X. Gao, Characterization and hypoglycemic effect of a polysaccharide extracted from the fruit of Lycium barbarum L, Carbohydr Polym 98(1) (2013) 8-16.

[20] L. Xu, J. Cao, W. Chen, Structural characterization of a broccoli polysaccharide and evaluation of anti-cancer cell proliferation effects, Carbohydr Polym 126 (2015) 179-84.

[21] H. Zhu, L. Tian, L. Zhang, J. Bi, Q. Song, H. Yang, J. Qiao, Preparation, characterization and antioxidant activity of polysaccharide from spent Lentinus edodes substrate, Int J Biol Macromol 112 (2018) 976-984.

[22] L. Ren, J. Zhang, T. Zhang, Immunomodulatory activities of polysaccharides from Ganoderma on immune effector cells, Food Chem 340 (2021) 127933.

[23] Y. Xu, H. Jiang, C. Sun, M. Adu-Frimpong, W. Deng, J. Yu, X. Xu, Antioxidant and hepatoprotective effects of purified Rhodiola rosea polysaccharides, Int J Biol Macromol 117 (2018) 167-178.

[24] C. Li, Q. Huang, X. Fu, X.J. Yue, R.H. Liu, L.J. You, Characterization, antioxidant and immunomodulatory activities of polysaccharides from Prunella vulgaris Linn, Int J Biol Macromol 75 (2015) 298-305.

[25] Y. Liu, M. Luo, F. Liu, X. Feng, S.A. Ibrahim, L. Cheng, W. Huang, Effects of freeze drying and hot-air drying on the physicochemical properties and bioactivities of polysaccharides from Lentinula edodes, Int J Biol Macromol 145 (2020) 476-483.

[26] D.T. Wu, Y.X. Zhao, H. Guo, R.Y. Gan, L.X. Peng, G. Zhao, L. Zou, Physicochemical and biological properties of polysaccharides from Dictyophora indusiata prepared by different extraction techniques, Polymers (Basel) 13(14) (2021).

[27] J.H. Zhou, P. Zou, C.L. Jing, Z.C. Xu, S. Zhou, Y.Q. Li, C.S. Zhang, Y. Yuan, Chemical characterization and bioactivities of polysaccharides from Apocynum venetum leaves extracted by different solvents, Journal of Food Measurement and Characterization 14(1) (2020) 244-253.

[28] S. Song, R. Qiu, X. Jin, Z. Zhou, J. Yan, Q. Ou, X. Liu, W. Li, Y. Mao, W. Yao, T. Lu, Mechanism exploration of ancient pharmaceutic processing (Paozhi) improving the gastroprotective efficacy of Aucklandiae Radix, J Ethnopharmacol 287 (2022) 114911.

[29] Z. Wu, R. Gao, H. Li, X. Liao, X. Tang, X. Wang, Z. Su, How steaming and drying processes affect the active compounds and antioxidant types of Gastrodia elata

Bl. f. glauca S. chow, Food Res Int 157 (2022) 111277.

[30] J. Hu, Y. Liu, L. Cheng, R. Shi, A. Qayum, A. Bilawal, M.A. Gantumur, M.A. Hussain, Z. Jiang, B. Tian, Comparison in bioactivity and characteristics of Ginkgo biloba seed polysaccharides from four extract pathways, Int J Biol Macromol 159 (2020) 1156-1164.

[31] P. Rozi, A. Abuduwaili, S. Ma, X. Bao, H. Xu, J. Zhu, N. Yadikar, J. Wang, X. Yang, A. Yili, Isolations, characterizations and bioactivities of polysaccharides from the seeds of three species Glycyrrhiza, Int J Biol Macromol 145 (2020) 364-371.

[32] M.G. Sevag, D.B. Lackman, J. Smolens, The isolation of the components of streptococcal nucleoproteins in serologically active form, Journal of Biological Chemistry 124(2) (1938) 0425-0436.

[33] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Analytical Chemistry 28(3) (1956) 350-356.

[34] M.M. Bradford, Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding, Analytical Biochemistry 72(1-2) (1976) 248-254.

[35] N. Blumenkrantz, G. Asboe-Hansen, New method for quantitative determination of uronic acids, Anal Biochem 54(2) (1973) 484-9.

[36] M. Tan, Q. Zhao, B. Zhao, Physicochemical properties, structural characterization and biological activities of polysaccharides from quinoa (Chenopodium quinoa Willd.) seeds, Int J Biol Macromol 193(Pt B) (2021)

1635-1644.

[37] B. Zhao, J. Zhao, M. Lv, X. Li, J. Wang, Z. Yue, J. Shi, G. Zhang, G. Sui, Comparative study of structural properties and biological activities of polysaccharides extracted from Chroogomphus rutilus by four different approaches, Int J Biol Macromol 188 (2021) 215-225.

[38] D.F. Yin, X.J. Sun, N. Li, Y.Y. Guo, Y. Tian, L.Z. Wang, Structural properties and antioxidant activity of polysaccharides extracted from Laminaria japonica using various methods, Process Biochemistry 111 (2021) 201-209.

[39] A. Wadouachi, J. Kovensky, Synthesis of glycosides of glucuronic, galacturonic and mannuronic acids: an overview, Molecules 16(5) (2011) 3933-3968.

[40] Z. Meng, T.T. Dong, A.U. Malik, S. Zhang, Q.G. Wang, Harvest maturity affects the browning of fresh-cut potatoes by influencing contents of amino acids, Postharvest Biology and Technology 173 (2021).

[41] S. Lin, H. Guo, M. Lu, M.Y. Lu, J.D.B. Gong, L. Wang, Q. Zhang, W. Qin, D.T. Wu, Correlations of molecular weights of beta-glucans from Qingke (Tibetan Hulless Barley) to their multiple bioactivities, Molecules 23(7) (2018).

[42] N.X. Yang, D.P. Wang, Y.Y. Geng, J.M. Man, Y.Y. Gao, Y. Hang, H.J. Zheng, M.S. Zhang, Structure, physicochemical characterisation and properties of pectic polysaccharide from Premma puberula pamp., Food Hydrocolloids 128 (2022).

[43] X.X. Zhang, Z.J. Ni, F. Zhang, K. Thakur, J.G. Zhang, M.R. Khan, R. Busquets, Z.J. Wei, Physicochemical and antioxidant properties of Lycium barbarum seed dreg polysaccharides prepared by continuous extraction, Food Chem X 14 (2022) 100282.

[44] Z. Chen, C. Yin, X. Fan, K. Ma, F. Yao, R. Zhou, D. Shi, W. Cheng, H. Gao, Characterization of physicochemical and biological properties of Schizophyllum commune polysaccharide extracted with different methods, Int J Biol Macromol 156 (2020) 1425-1434.

[45] D. Yuan, C. Li, Q. Huang, X. Fu, Ultrasonic degradation effects on the physicochemical, rheological and antioxidant properties of polysaccharide from Sargassum pallidum, Carbohydr Polym 239 (2020) 116230.

[46] Y. Wang, X. Chen, P. Zhao, L. Ren, X. Li, W. Gao, Physicochemical characteristics and immunoregulatory activities of polysaccharides from five cultivars of Chrysanthemi Flos, Food Sci Nutr 10(5) (2022) 1391-1400.

[47] G.D. Manrique, F.M. Lajolo, FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit, Postharvest Biology and Technology 25(1) (2002) 99-107.

[48] J. Gu, H. Zhang, J. Zhang, C. Wen, H. Ma, Y. Duan, Y. He, Preparation, characterization and bioactivity of polysaccharide fractions from Sagittaria sagittifolia L, Carbohydr Polym 229 (2020) 115355.

[49] B. Wang, J. Niu, B. Mai, F. Shi, M. Li, L. Chen, P. Wang, Q. Liu, Effects of extraction methods on antioxidant and immunomodulatory activities of polysaccharides from superfine powder Gynostemma pentaphyllum Makino, Glycoconj J 37(6) (2020) 777-789.

[50] J. Wu, R. Chen, L. Tan, H. Bai, L. Tian, J. Lu, M. Gao, C. Bai, H. Sun, Y. Chi, Ultrasonic disruption effects on the extraction efficiency, characterization, and

bioactivities of polysaccharides from Panax notoginseng flower, Carbohydr Polym 291 (2022) 119535.

[51] W. Wang, Y. Zou, Q. Li, R.W. Mao, X.J. Shao, D. Jin, D.H. Zheng, T. Zhao, H.F. Zhu, L. Zhang, L.Q. Yang, X.Y. Wu, Immunomodulatory effects of a polysaccharide purified from Lepidium meyenii Walp. on macrophages, Process Biochemistry 51(4) (2016) 542-553.

[52] L. Wang, H.M. Liu, G.Y. Qin, Structure characterization and antioxidant activity of polysaccharides from Chinese quince seed meal, Food Chem 234 (2017) 314-322.

[53] A. Abuduwaili, P. Rozi, P. Mutailifu, Y.H. Gao, R. Nuerxiati, H.A. Aisa, A. Yili, Effects of different extraction techniques on physicochemical properties and biological activities of polysaccharides from Fritillaria pallidiflora Schrenk, Process Biochemistry 83 (2019) 189-197.

[54] F.S. Kittur, K.V.H. Prashanth, K.U. Sankar, R.N. Tharanathan, Characterization of chitin, chitosan and their carboxymethyl derivatives by differential scanning calorimetry, Carbohydrate Polymers 49(2) (2002) 185-193.

[55] Q. Li, J. Li, H. Li, R. Xu, Y. Yuan, J. Cao, Physicochemical properties and functional bioactivities of different bonding state polysaccharides extracted from tomato fruit, Carbohydr Polym 219 (2019) 181-190.

[56] U.V. Deore, H.S. Mahajan, Isolation and structural characterization of mucilaginous polysaccharides obtained from the seeds of Cassia uniflora for industrial application, Food Chem 351 (2021) 129262.

[57] M.S. Blois, Antioxidant determinations by the use of a stable free radical, Nature

181(4617) (1958) 1199-1200.

[58] H.M. Liu, X.Y. Liu, Y.Y. Yan, J.H. Gao, Z. Qin, X.D. Wang, Structural properties and antioxidant activities of polysaccharides isolated from sunflower meal after oil extraction, Arabian Journal of Chemistry 14(12) (2021).

[59] A.T. Diplock, Will the 'Good fairies' please prove to us that vitamin E lessens human degenerative disease? (vol 26, pg 565, 1996), Free Radical Research 27(5) (1997) 511-532.

Journal Pre-proof

Figure 1, UV–Vis and FT-IR spectroscopic analysis of *C. deserticola* polysaccharides. (A) UV–vis spectra analysis; (B) FT-IR spectra analysis.

Figure. 2, Congo red analysis of *C. deserticola* polysaccharides.

Figure. 3, The images of *C. deserticola* polysaccharides. (A) Appearance image; (B) Scanning electron micrographs; (C and D) atomic force microscopy images (C-2D image; D-3D image).

Figure. 4, The X-ray diffraction curves of *C. deserticola* polysaccharides.

Figure. 5, Thermodynamic analysis of *C. deserticola* polysaccharides. (A) TG curve; (B) DTG curve; (C) DTA curve.

Figure. 6, Antioxidant activity of *C. deserticola* polysaccharides. (A) DPPH radical scavenging ability; (B) ABTS radical scavenging ability; (C) Hydroxyl radical scavenging ability.

Journal Pre-proof

Highlights

- The polysaccharides from different processed *C. deserticola* were discussed.
- Processing could affect the structure of *C. deserticola* polysaccharide.
- The polysaccharides from hot-air dried *C. deserticola* had the highest antioxidant activities.
- The polysaccharide from fresh *C. deserticola* had the best exterior.

Journal Pre-proof