Ultrasonics Sonochemistry 70 (2021) 105355

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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

Ultrasound irradiation alters the spatial structure and improves the antioxidant activity of the yellow tea polysaccharide

Haisong Wang [a,b,*], Jinran Chen [b], Pengfei Ren [b], Yiwen Zhang [b], Stanley Omondi Onyango [c]

[a] School of Biology and Food Engineering, Changshu Institute of Technology, Jiangsu, PR China
[b] School of Tea and Food Science & Technology, Anhui Agricultural University, Anhui, PR China
[c] Department of Biotechnology, Faculty of Bioscience Engineering, Center for Microbial Ecology and Technology (CMET), Ghent University, Ghent, Belgium

ARTICLE INFO

Keywords:
Ultrasound
Yellow tea polysaccharide
Structure characteristic
Chain conformation
Antioxidant activity

ABSTRACT

In this study, the impact of ultrasound irradiation on the structural characteristics and antioxidant properties of yellow tea polysaccharides with different molecular weights (Mw) were investigated. Native yellow tea polysaccharide containing YTPS-3N, YTPS-5N and YTPS-7N were prepared through precipitation with ethanol at various concentrations of 30%, 50%, and 70%, respectively, and irradiated with high intensity ultrasound (20 kHz) for 55 min to yield yellow tea polysaccharide including YTPS-3U, YTPS-5U and YTPS-7U. The molecular weight (Mw) of YTPS-3N (from 37.7 to 15.1 kDa) and YTPS-5N (from 14.6 to 5.2 kDa) sharply decreased upon ultrasound irradiation, coincidentally particle size (Zavg) was also significantly reduced for YTPS-3N (40%), YTPS-5N (48%) and YTPS-7N (54%). The high-performance liquid chromatography and Fourier transform-infrared spectroscopy analysis revealed a partial degradation of native yellow tea polysaccharide treated with ultrasound, though the monosaccharide composition was not altered. Furthermore, changes in morphology and the breakdown of native yellow tea polysaccharide upon irradiation was confirmed with the circular dichroism spectrum, atomic force and scanning electron microscopy. As a consequence, irradiation of yellow tea polysaccharide increased free radical scavenging activity with YTPS-7U exhibiting the highest levels of 2, 2-diphenyl-1-picrylhydrazyl free radical, superoxide and hydroxyl radicals scavenging activity. These results suggest that the alteration of the spatial structure of yellow tea polysaccharide can enhance its antioxidant activity which is an important property for functional foods or medicines.

1. Introduction

Tea is derived from the fresh leaves of *Camellia sinensis* plant which is known to be rich in polyphenols, methylxanthines, polysaccharides, minerals, trace elements, amino acids and organic acids. The composition of tea are distinct to tea types considering the varying degree of processing techniques, geographical growing locations and conditions [1]. Yellow tea is one of the six main tea types in China and has gained popularity in recent years because of its pleasant mellow taste and perceived health benefits [2,3]. Recently, a new variety of yellow tea *Huangda cha* emerged. The yellow tea is specially produced using coarse blade of raw fresh tea leaves after fixation, rolling, sealed yellowing and intensive drying process. Among these processes the sealed yellowing and intensive drying are key to its successful production. This special processing not only generate unique burnt flavor, but also gives it the perceived health promoting properties [4]. Studies have suggested that the water extraction of this novel yellow tea have health benefits by the constituents attenuating macrophage-related chronic inflammation [5]; ameliorating metabolic syndrome [6] and may be anti-hyperglycemic [7].

Hot water extraction method has conventionally been used for preparing tea polysaccharides [8]. However, due to the long extraction time and low yields, better techniques are now emerging. The new techniques such as microwave-assisted extraction [9], supercritical fluid extraction [10] and ultrasound-assisted extraction [11] have been developed to speed up the extraction. Among these techniques, ultrasound-assisted extraction is most promising given its extraction efficiency and environmental friendliness. We have demonstrated that higher yields of yellow tea polysaccharide can be achieved with ultrasound-assisted extraction than the traditional hot water extraction. Although the ultrasound is a promising technique, it may lead to degradation of yellow tea polysaccharide. Particularly, ultrasound generates high-energy shear

* Corresponding author at: School of Biology and Food Engineering, Changshu Institute of Technology, Jiangsu, PR China.
E-mail address: whs@cslg.edu.cn (H. Wang).

https://doi.org/10.1016/j.ultsonch.2020.105355
Received 17 March 2020; Received in revised form 5 September 2020; Accepted 18 September 2020
Available online 22 September 2020

force through the process of acoustic cavitation which refers to the formation, growth, and collapse of small gas bubbles in liquids [12]. The violent collapse of these bubbles caused by cavitation can produce very intense localized heating and pressure spikes in very short lifetimes, which may reach up to thousands of degrees K and hundreds of atmospheres pressure, respectively [13,14]. These rather extreme conditions may promote pyrolysis of water and dissolved oxygen molecules into highly reactive species such as hydroxyl (•OH), hydrogen (•H), oxygen (•O) and hydroperoxyl (•OOH) radicals [15–17]. The diffusion of some of these reactive radicals out of the cavity to the surrounding liquid may react with the yellow tea polysaccharide. In addition, rapidly collapsing cavitation bubbles may cause mechanochemical action of ultrasound on polymers, which can induce high pressure gradients and high local velocities of liquid layers in their vicinity, this in turn may cause shear forces [18,19] capable of breaking down the chains of yellow tea polysaccharide. Besides the mechanochemical and radical attack mechanism, the yellow tea polysaccharide in aqueous solution may also undergo pyrolysis in the hot interfacial region between the bubble and the surrounding liquid [19].

Sonochemical degradation of polysaccharides such as chitosan, starch, and dextran has been extensively studied in the past decades [20–22], however, limited information on the sonolysis of tea polysaccharide. In addition, numerous physical characteristics such as viscosity and more complex properties like biological activities have been demonstrated in relation to polysaccharides with varying molecular weight [23–26]. As to how ultrasound irradiation impacts on the physicochemical properties and biological activities of tea polysaccharides with different molecular weight remains unclear. The present study therefore aimed at investigating the changes in physicochemical properties, structure, and chain conformation of yellow tea polysaccharide with different molecular weight under ultrasound irradiation. Furthermore, the potential antioxidant activity of ultrasound degrading yellow tea polysaccharide was explored by determining the scavenging of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical, superoxide and hydroxyl radicals. High performance liquid chromatography (HPLC), ultraviolet (UV), Fourier transform-infrared (FT-IR) spectroscopy, circular dichroism (CD), atomic force microscope (AFM), scanning electron and microscopy (SEM) were used to elucidate the structure of untreated control and ultrasound-treated yellow tea polysaccharide.

2. Materials and methods

2.1. Materials and chemicals

Yellow tea was purchased from BaoErZhongXiu Tea Co, Ltd. (Huoshan, Anhui, China), and the tea samples were stored in desiccator at room temperature for further study. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Dextran standards (2.7 kDa, 5.25 kDa, 9.75 kDa, 13.05 kDa, 36.8 kDa, 64.65 kDa, 135.35 kDa, and 300.6 kDa) were purchased from Beijing Zhongke quality inspection Biotechnology Co., Ltd (Beijing, China). Monosaccharide standards were bought from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were analytical grade or chromatographic grade.

2.2. Preparation and ultrasonic treatment of yellow tea polysaccharide

The dried yellow tea was shaken with 95% ethanol for 6 h at room temperature to remove the pigments, polyphenols, monosaccharides and small molecules. The residues were separated by filtration and dried in an oven at 37 °C to obtain pretreated yellow tea sample. The pretreated tea sample (100 g) was further extracted twice with hot deionized water (3.5 L) at 90℃ for 55 min. The aqueous extractions were combined and concentrated to approximately 10% of their original volume on a rotary evaporator under reduced pressure (60 °C, 100 rpm, 0.01 Mpa) and then precipitated with 80% (v/v) ethanol at 4 °C for 12 h. After centrifugation, the precipitate was collected, dissolved in deionized water, and subjected to the Sevag method in which the volume ratio of chloroform to n-butanol was 4:1 to remove free proteins [27]. As shown in Fig. 1, the deproteinized YTPS was sequentially precipitated with 30%, 50%, and 70% (v/v) ethanol at 4 °C for 12 h and freeze-dried to yield the native YTPS fractions (YTPS-3N, YTPS-5N and YTPS-7N) with different molecular weight. A 150 mg YTPS-N fractions were separately dissolved in 10 mL of deionized water and subjected to ultrasonic treatment using a sonicator (VCX800, Sonics & Materials Inc., Newton, CT, USA) equipped with a probe (tip diameter 3 mm). The probe was immersed 1 cm in the solution, before treating the solutions by ultrasound for 55 min at a frequency of 20 kHz and a power of 500 W. Finally, the ultrasound-treated samples were designated as YTPS-U including YTPS-3U, YTPS-5U and YTPS-7U with U representing ultrasound treated.

2.3. Physicochemical properties analysis

2.3.1. Determination of monosaccharide composition

The monosaccharide composition of YTPS before and after ultrasonic treatment was analyzed by HPLC according to the procedure described by Nie and co-workers [28]. Briefly, 100 μL sample solution (5 mg/mL) and 100 μL trifluoroacetic acid (4 mol/L) were added to an ampoule bottle and hydrolyzed at 120℃ for 2 h, before 2 mL methanol were added to the hydrolysate with subsequent triple drying with a rotatory evaporator at 40℃ to ensure complete removal of excess trifluoroacetic acid. The dried hydrolysate was re-dissolved in 100 μL deionized water for subsequent derivatization. 100 μL NaOH solution (0.6 mol/L) was added to the hydrolysate solution, followed by the addition of 200 μL PMP methanol solution (0.5 mol/L) and mixed vigorously on a vortex mixer. The mixture was kept in water bath at 70℃ for 100 min. At the end of the reaction, the mixture was cooled to room temperature, followed by addition of 100 μL HCl solution (0.3 mol/L) to neutralize the residual alkali before drying with a rotatory evaporator. Then 1 mL of chloroform and deionized water were added and vigorously shaken before discarding the chloroform layer. The aqueous layer was then filtered through a 0.45 μm pore membrane filter for high performance liquid chromatography analysis (Agilent Technologies, Santa Clara, CA, USA) equipped with photodiode array detector was used for detecting PMP labeled monosaccharides. The mobile phase was a mixture of acetonitrile and 0.1 mol/mL phosphate buffer solution (PBS, pH 6.7) in a ratio of 83:17 (v/v). The samples with 20 μL injection volume were performed on a Zorbax Eclipse XDB-C18 column (4.6 × 250 mm, 5 μm, Agilent Technologies, USA) at 30℃ with 1 mL/min of flow rate and detected at 245 nm.

2.3.2. Component analysis

The carbohydrate contents of ultrasound treated and untreated yellow tea polysaccharide samples were determined according to the anthrone-sulfuric acid method using glucose as the standard [29]. The uronic acid contents of yellow tea polysaccharide samples were measured using sulfuric acid carbazole colorimetry method with galacturonic acid as a standard [30]. The content of protein was determined using the Bradford assay [31]. The total polyphenols content of yellow tea polysaccharide samples was estimated by the Folin-ciocalteu colorimetric method [32].

2.3.3. Determination of molecular weight and average particle size

The molecular weights of yellow tea polysaccharide before and after ultrasonic treatment were determined by high-performance gel permeation chromatography (HPGPC) on a Waters 600 HPLC apparatus equipped with 2410 differential refractive index (RI) detector and Empower workstation according to the procedure described by Chen and co-workers [33]. Briefly, a TSK-GEL guard column (PWXL 6.0 mm × 40 mm), a TSK-G5000 PWXL (300 × 7.8 mm, i.d., 10 μm) and a TSK-

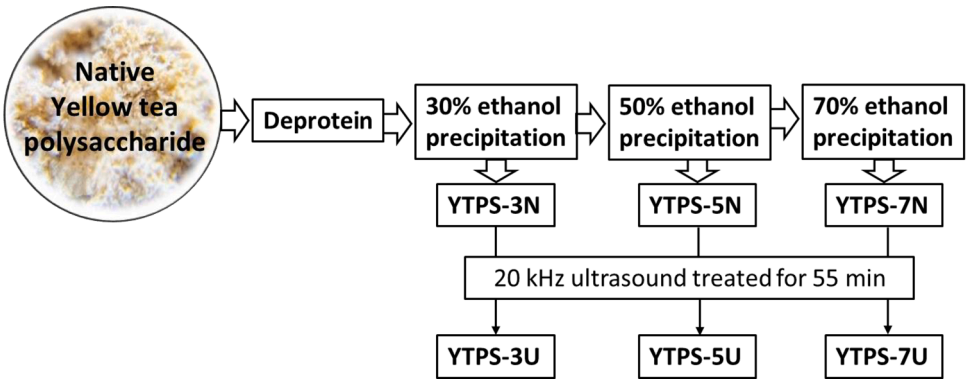

Fig. 1. Schematic diagram of yellow tea polysaccharide treatment process. The YTPS-3N, YTPS-5N, and YTPS-7N were the native yellow tea polysaccharide sequentially precipitated with 30%, 50%, and 70% (v/v) ethanol. The YTPS-3U, YTPS-5U, and YTPS-7U were ultrasound-treated YTPS-3N, YTPS-5N, and YTPS-7N samples, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

G3000 PWXL (300 × 7.8 mm, i.d., 7 μm: TOSOH Co., Ltd, Japan) were applied to the molecular weight determination, respectively. The sample was previously filtered through a membrane (0.22 μm, Millipore) before detection. The performance conditions were as follows: column temperature 35 °C, injection volume 20 μL (1 mg/mL, w) and flow rate 0.6 mL/min with 0.02 M KH_2PO_4 (pH 6.0). Dextran standards of various molecular weights were used to establish a standard curve.

Average particle size (Z_{avg}) of the YTPS was detected by particle size zeta potential analyzer (DelsaMax PRO Zeta, Beckman Coulter Commercial Enterprise Co., China) at 632.8 nm and a 90° scattering angle of 25 °C according to the method described by Xu et al. [34]. Briefly, different YTPS samples were diluted with deionized water to a concentration of 2.0 mg/mL, and three runs were performed for each sample.

2.4. Structure characterization

In order to determine the structure of yellow tea polysaccharide, the Ultraviolet–visible (UV–vis) spectrum, Fourier-transform infrared (FT-IR) spectrum, Circular dichroism (CD) spectrum, Atomic force microscopy (AFM), and Scanning electron microscopy (SEM) were used in this study. The UV absorbance of all yellow tea polysaccharide solutions (1 mg/mL) were performed on a UV–visible spectrophotometer (BioTek Instruments, Inc., Vermont, US) at the wavelength of 200–400 nm range. The IR spectroscopy was performed using the KBr pellet method [35]. Briefly, the sample was ground and compressed with KBr powder and subjected to infrared spectral scanning analysis in the range of 400–4000 cm^{-1} using a Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The CD spectra were recorded on a ChirascanV100 instrument (CD, Applied Photophysics Ltd., Surrey, UK). All solutions were filtered through 0.45 μm Millipore membrane before testing. Each CD spectrum was the accumulation of three scans at 100 nm/min, with a bandwidth of 1 nm and a time constant of 0.25 s. Data were collected from 190 nm to 250 nm. A correction for the solvent baseline was made digitally in each case. The morphology of ultrasound treated and untreated yellow tea polysaccharide was performed on a dimension Icon atomic force microscope (AFM, Bruker Optics, Inc., Ettlingen, Germany). Briefly, 5 μL yellow tea polysaccharide solution (10 μg/ml) was dropped on the surface of fresh mica (1 cm^2), completely dried, and then observed on an AMF. Finally, the image processing was performed using NanoScope Analysis software. A S-4800 scanning electron microscope (SEM, Hitachi, Ltd, Tokyo, Japan) with an acceleration voltage of 3 kV with an image magnification of 25.0 k × was also used for the morphological feature analysis of the yellow tea polysaccharide before and after ultrasound treatment. During the process, the dried samples were firmly fixed on the sample table with conductive adhesive and sputtered a 3 ~ 30 nm gold film using a vacuum sputter coater.

2.5. Antioxidant activity assay

2.5.1. DPPH radical scavenging activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of ultrasound treated and untreated yellow tea polysaccharide was measured as previously described by Shimada et al. [36] with slight modification. Briefly, 200 μL tea polysaccharides water solutions at various concentration from 0.05 mg/mL to 2.0 mg/mL were separately added to 600 μL DPPH ethanol solutions (0.1 mM), the mixture was shaken thoroughly and kept at room temperature in the dark for 30 min before measuring the absorbance at 517 nm. Each sample solution was replaced with an absolute ethanol solution as a blank control and vitamin C was used as a positive control. DPPH radical scavenging activity was calculated by the following equation:

Scavenging activity (%) = [1-(A1-A2) /A0] × 100

Where A0 is the absorbance of the blank control, A1 is the absorbance of the sample and A2 is the absorbance of the sample under identical conditions as A1 with ethanol instead of DPPH solution.

2.5.2. Superoxide radical scavenging activity

The superoxide radicals scavenging activity of ultrasound treated and untreated yellow tea polysaccharide at various concentration (0.05–2.0 mg/mL) was determined using pyrogallic acid method [37]. Briefly, 2.5 mL PBS buffer (0.1 mol/L, pH 8.2) and 4.0 mL tea polysaccharides solution were mixed thoroughly and kept at 25 °C for 15 min, followed by adding 2.5 mL of pyrogallic acid (6 mol/L) to the mixture. After 4 min reaction, 0.5 mL of thick hydrochloric acid was added to stop the reaction. The absorbance of the solution was read at 325 nm with vitamin C being used as a reference material. The scavenging activity was calculated as follows:

Scavenging activity (%) = [1-(Al-A2)/A3] × 100%

Where A1 is the absorbance of the sample and A2 is the absorbance of the sample solution and PBS mixture without pyrogallol solution. A3 is the absorbance of the control (ultra-pure water).

2.5.3. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined using the method described by Smirnoff et al. [38] with minor modification. 1 mL yellow tea polysaccharide solution (0.05–2.0 mg/mL), 1 mL of $FeSO_4$ (1 mM), 1.0 mL of H_2O_2 (0.3%) and 0.5 mL of salicylic acid–ethanol solution (9 mM) were mixed and kept at 37 °C for 30 min. The absorbance of the mixture was determined at 510 nm against a blank while vitamin C was used as a positive control. The hydroxyl radical scavenging activity was calculated by the following equation:

Scavenging activity (%) = [A0-(A1-A2)]/A0 × 100%

Where A0 is absorbance of the blank control group, A1 is the absorbance of the sample and A2 is the absorbance of the sample under identical conditions as A1 with salicylic acid–ethanol solution instead of $FeSO_4$ and H_2O_2 solution.

3. Results and discussion

3.1. Effects of ultrasound on physicochemical properties

3.1.1. Monosaccharide composition

In this study, monosaccharide composition of yellow tea polysaccharide before and after ultrasound treatment were determined. As shown in Table 1, both ultrasound treated and untreated yellow tea polysaccharide were predominantly composed of rhamnose but also contained mannose, ribose, glucuronic acid, galacturonic acid, glucose, galactose, and arabinose. The results indicated that yellow tea polysaccharide was a heterogeneous polysaccharide and upon ultrasonic treatment did not alter the monosaccharide composition, though their molar ratios were slightly modified. This finding indicated that ultrasonic treatment had no major impact on the monosaccharide composition of yellow tea polysaccharide and confirmed the previous findings by Dou et al. [39].

3.1.2. Molecular weight, particle size and component analysis

The molecular weight, particle size and the component analysis of the yellow tea polysaccharide before and after ultrasound treatment were summarized in Table 2. Increasing the concentration of ethanol from 30 to 70% decreased the molecular weight and particle size (Zavg) of the native yellow tea polysaccharide. While there was no decrease in molecular weight of the YTPS-7N (3.3 to 3.1 kDa), the ultrasound irradiation sharply decreased the molecular weight of YTPS-3N and YTPS-5N from 37.7 to 15.1 and 14.6 to 5.2 kDa, respectively, confirming that ultrasound irradiation reduce the molecular weight of polysaccharides [22], the degradation of the high molecular weight polysaccharides was more pronounced than the low molecular weight polysaccharides which are consistent with previous findings [40].

The changes of particle size of the native yellow tea polysaccharide after ultrasonic treatment showed that ultrasound irradiation significantly ($P < 0.05$) decreased the particle size of yellow tea polysaccharide precipitated with different ethanol concentration. Zavg results showed 40%, 48% and 54% reductions in ultrasonic-treated YTPS-3N, YTPS-5N, and YTPS-7N, respectively, which indicated that the small particle size native yellow tea polysaccharide was easier to break down than the large size particles when subjected to the same ultrasonic-treated conditions.

Component analysis revealed that the neutral sugar content was the highest in all samples, followed by the uronic acid and protein content, with polyphenol content being the lowest. The yellow tea polysaccharide with high molecular weight had low content of neutral sugar and protein yet more uronic acid than that in low molecular weight. Upon ultrasound treatment, a significant ($P < 0.05$) lowering of the neutral sugar content and uronic acid content in YTPS-3N, as well as the neutral sugar content in and YTPS-7N was observed. The protein content analysis revealed that yellow tea polysaccharide might be a type of glycoprotein and ultrasound treatment caused glycosidic bond hydrolysis thereby increasing the protein content. This further demonstrates the bond between native yellow tea polysaccharide and protein.

Table 1
Effect of ultrasonic treatment on monosaccharide composition of yellow tea polysaccharide.

Monosaccharide composition (Molar ratio)								
	Man	Rib	Rha	GlcUA	GalUA	Glc	Gal	Ara
YTPS-N	1.65	1	10.95	1.06	2.03	5.49	3.50	4.02
YTPS-U	1.72	1	11.05	1.09	2.13	5.36	3.62	4.17

YTPS-N: native yellow tea polysaccharide, YTPS-U: ultrasound-treated yellow tea polysaccharide.
Man: mannose Rib: ribose, Rha: rhamnose, GalUA: galacturonic acid, GlcUA: glucuronic acid, Glc: glucose, Gal: galactose, Ara: arabinose.

3.1.3. Ultraviolet–visible (UV–vis) spectra

Not only protein with aromatic amino acids or polyphenol show strong absorption at the band of 250–290 nm in the UV–vis spectrum due to the benzene ring, compounds with carbonyl groups also show weak absorption at the vicinity of 280 nm [41,42]. The UV–vis spectra of native yellow tea polysaccharide with different molecular weight (YTPS-3N, YTPS-5N, and YTPS-7N) and ultrasound treated yellow tea polysaccharide (YTPS-3U, YTPS-5U, and YTPS-7U) are shown in Fig. 2. All the yellow tea polysaccharides except YTPS-3N showed absorption at the vicinity of 280 nm indicating that protein with aromatic amino acids, polyphenol, and carbonyl groups exist in the yellow tea polysaccharides as revealed by the content of protein, polyphenol, and uronic acid (Table 2). Ultrasound irradiation slightly increased the absorption strength of YTPS-3N and YTPS-5N at the vicinity of 280 nm in the UV–vis spectra. Whereas the polyphenol content was not affected in YTPS-3N but slightly decreased in YTPS-5N, the uronic acid content decreased in both yellow tea polysaccharides after ultrasound irradiation (Table 2), these results indicated that ultrasonic treatment assisted exposure of proteins containing aromatic amino acids in YTPS-3N and YTPS-5N. It is intriguing that ultrasound irradiation has no observable effect on the absorption of YTPS-7N at the vicinity of 280 nm in the UV–vis spectra and could be ascribed to the low amounts of aromatic amino acids in the protein. However, detailed studies are required to determine how varying ethanol concentrations influence the protein types of natural yellow tea polysaccharides.

3.1.4. Fourier-transform infrared (FT-IR) spectra

The FT-IR spectra of yellow tea polysaccharide treated and untreated by ultrasound are shown in Fig. 3. The broad band at 3425 cm^{-1} was ascribed to the stretching vibration of O–H [43]. The weak absorption peak at around 2920 cm^{-1} and 1430 cm^{-1} was attributed to the stretching vibration and bending vibration of C–H [39]. The absorption peaks observed around 1636 cm^{-1} was assigned to the stretching of C=O indicating the presence of carbonyl groups in native and ultrasound treated yellow tea polysaccharides. Additionally, the protein amino band may also present absorption at 1636 cm^{-1} [44] suggesting the existence of protein in YTPS. The strong absorption peak at 1324 cm^{-1} was depicted the presence of carboxyl groups which indicated the characteristic absorption of uronic acid [45]. Moreover, the absorption at 1115 cm^{-1}, 1052 cm^{-1}, 939 cm^{-1}, and 877 cm^{-1} were ascribed to the existence of arabinose, rhamnose, galactose, and mannose in YTPS, respectively [46,47]. The strong peak around 722 cm^{-1} was ascribed to the four adjacent hydrogen atoms in the benzene ring which indicated the existence of aromatic amino acids [48]. The absorption peak at 788 cm^{-1} was the C–H angle vibration of the furan ring [49], and the absorption peak at 617 cm^{-1} was the out-of-plane vibration of O–H, which revealed the existence of phenolic compounds in YTPS-5N, YTPS-7N, YTPS-5U and YTPS-7U [50]. Ultrasonic treatment did not alter the main absorption peaks of yellow tea polysaccharide at 3425, 2920 and 1636 cm^{-1}. The changes only occurred in the characteristic peaks between 1500 and 500 cm^{-1} after ultrasonic treatment. Our results confirm a well-established knowledge on sonochemistry of polysaccharides that ultrasonic treatment mainly causes the breaking of glycosidic linkages with no change on the primary functional groups in yellow tea polysaccharide [51].

3.1.5. Circular dichroism (CD) spectra

The tertiary structure is very important to diverse biological activities of yellow tea polysaccharide. The conformational transition of native yellow tea polysaccharide induced by ethanol concentration and ultrasound treatment was detected on the CD spectra (see Fig. 4). All

Table 2
Molecular weight, particle size and component analysis of native yellow tea polysaccharide and ultrasound-treated yellow tea polysaccharide by LSD. n = 3.

Samples	Neutral sugar (%)	Uronic acid (%)	Protein (%)	Polyphenol (%)	Zavg (nm)	Molecular weight (kDa)
YTPS-3N	58.46 ± 0.14^{c}	24.11 ± 0.57^{a}	3.44 ± 0.24^{c}	0.13 ± 0.01^{b}	1280 ± 5 ^{a}	37.7
YTPS-3U	52.71 ± 0.48^{d}	20.27 ± 0.66^{b}	3.49 ± 0.19^{c}	0.13 ± 0.01^{b}	761 ± 4^{b}	15.1
YTPS-5N	58.77 ± 0.55^{c}	12.35 ± 0.13^{b}	4.38 ± 0.14^{b}	1.04 ± 0.58^{a}	687 ± 3^{c}	14.6
YTPS-5U	57.06 ± 0.75^{c}	11.28 ± 0.22^{b}	4.41 ± 0.65^{b}	1.00 ± 0.30^{a}	352 ± 2 ^{d}	5.2
YTPS-7N	75.82 ± 1.70^{a}	7.63 ± 0.27^{c}	6.00 ± 0.36^{a}	1.21 ± 0.30^{a}	11 ± 1^{e}	3.3
YTPS-7U	71.15 ± 1.40^{b}	6.84 ± 0.25^{c}	6.36 ± 0.29^{a}	1.17 ± 0.64^{a}	5 ± 0^{f}	3.1

Note: Each value represents the mean ± standard deviation (n = 3). Means with different letters (a–d) for each column (neutral sugar, uronic acid, protein, polyphenols, and Zavg) showed significant difference ($p < 0.05$). The YTPS-3N, YTPS-5N, and YTPS-7N were the native yellow tea polysaccharide sequentially precipitated with 30%, 50%, and 70% (v/v) ethanol. The YTPS-3U, YTPS-5U, and YTPS-7U were ultrasound-treated YTPS-3N, YTPS-5N, and YTPS-7N samples, respectively.

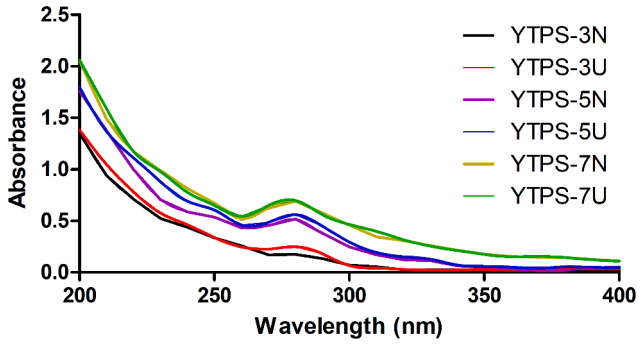

Fig. 2. UV–vis spectra of native yellow tea polysaccharide and ultrasonic treated yellow tea polysaccharide in the range of 200–400 nm. The YTPS-3N, YTPS-5N, and YTPS-7N were the native yellow tea polysaccharide sequentially precipitated with 30%, 50%, and 70% (v/v) ethanol. The YTPS-3U, YTPS-5U, and YTPS-7U were ultrasound-treated YTPS-3N, YTPS-5N, and YTPS-7N samples, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

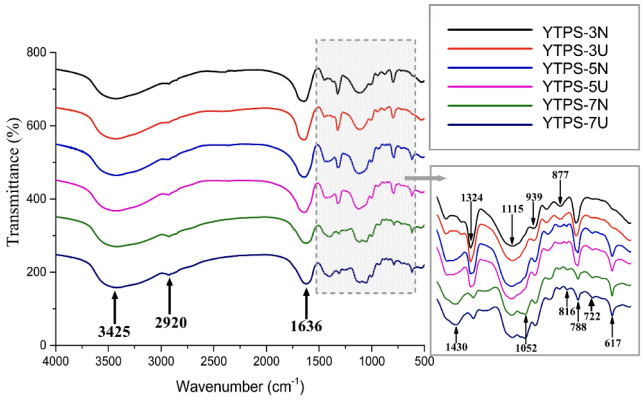

Fig. 3. FT-IR spectra of native YTPS and ultrasonic treated YTPS in the range of 4000–500 cm^{-1}. The YTPS-3N, YTPS-5N, and YTPS-7N were the native yellow tea polysaccharide sequentially precipitated with 30%, 50%, and 70% (v/v) ethanol. The YTPS-3U, YTPS-5U, and YTPS-7U were ultrasound-treated YTPS-3N, YTPS-5N, and YTPS-7N samples, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

three native yellow tea polysaccharide including YTPS-3N, YTPS-5N, and YTPS-7N have positive and negative cotton effect, which suggested that native yellow tea polysaccharide was in highly ordered structures. However, the ellipticity and maximum/minimum peak position of the native yellow tea polysaccharide precipitated with different ethanol concentration were significantly different. The CD spectrum of YTPS-3N showed positive Cotton effect with maximum absorption at 199 nm and negative Cotton effect with minimum absorption at 215 nm in the scanned range. As the ethanol concentration increased from 30% to 70%, the maximum absorption gradually increased and shifted to 205 nm, while the minimum absorption peak appeared at 226 nm, indicating remarkable transformation of native yellow tea polysaccharide in conformation after the ultrasound treatment. Meanwhile, the increased ellipticity of YTPS-3N, YTPS-5N and YTPS-7N revealed the changes of yellow tea polysaccharide are heterogenous [52]. Ultrasound irradiation altered the ellipticity and the appearance of maximum/minimum absorption peaks of yellow tea polysaccharide, as shown in Fig. 4b, ultrasonic treatment changed the maximum and minimum band of YTPS-3N, YTPS-5N, and YTPS-7N. As can be seen in Fig. 4c, the CD spectrum of YTPS-3N had a red shift after ultrasonic treatment in which the minimum peaks shifted from 215 nm to 222 nm with the increasing of negative ellipticity which might attribute to the n → π* transition of carboxyl group, and the optical activity of the carboxyl chromophore could be affected by intra- and intermolecular interactions [53,54]. It was in accordance with the result of component analysis of yellow tea polysaccharide in which uronic acid was observed (Table 2). A minor blue shift of n → π* transition with increasing positive ellipticity at around 200 nm in YTPS-5U and decreasing negative ellipticity at around 220 nm in YTPS-7U were observed in Fig. 4d and Fig. 4e, respectively, it was proposed that such changes in the ellipticity and minor red/blue shift of the n → π* transition reflect the degrading of yellow tea polysaccharide to expose more carboxyl group inducing the alteration of the microenvironment around the carboxyl site [55]. Collectively, the changes of the CD spectrum of yellow tea polysaccharide indicated the conformation transformation of yellow tea polysaccharide in aqueous solutions after ultrasonic treatment.

3.1.6. Morphology observation

To provide direct evidence of the chain conformation of the yellow tea polysaccharide, Atomic Force Microscope (AFM) was used to observe morphology of the polysaccharide in aqueous solution. As can be seen from the AFM images all the yellow tea polysaccharides were irregularly spherical lumps and uneven in size (Fig. 5). The height of the lumps ranged from 0.1 to 4.8 nm while the height of single polysaccharide particle was generally in the range of 0.1–1.0 nm which suggested inter- and/or intra-molecular aggregation was involved during the drying process of yellow tea polysaccharide solution on the surface of fresh mica [56]. On the other hand, the large diameter of over 1.0 nm may also suggest the aggregates of chains and branched structure entangling with each other for all the polysaccharide fractions. The average height of the polysaccharide particles in YTPS-3N, YTPS-5N, and YTPS-7N were 5, 3, and 2 nm with an average diameter approximately being 129, 128, and 101 nm, respectively. Ultrasound treatment significantly decreased the particle size of the yellow tea polysaccharide in which YTPS-3N, YTPS-5N and YTPS-7N showed reductions in average height of 41%, 17% and 7%, and widths to 93, 83 and 69 nm, respectively. These results are in agreement with previous studies which demonstrated that ultrasound can break the original structure and morphology of yellow tea polysaccharide [57].

3.1.7. Scanning electron microscopy (SEM) analysis

The surface topography of the yellow tea polysaccharide particles

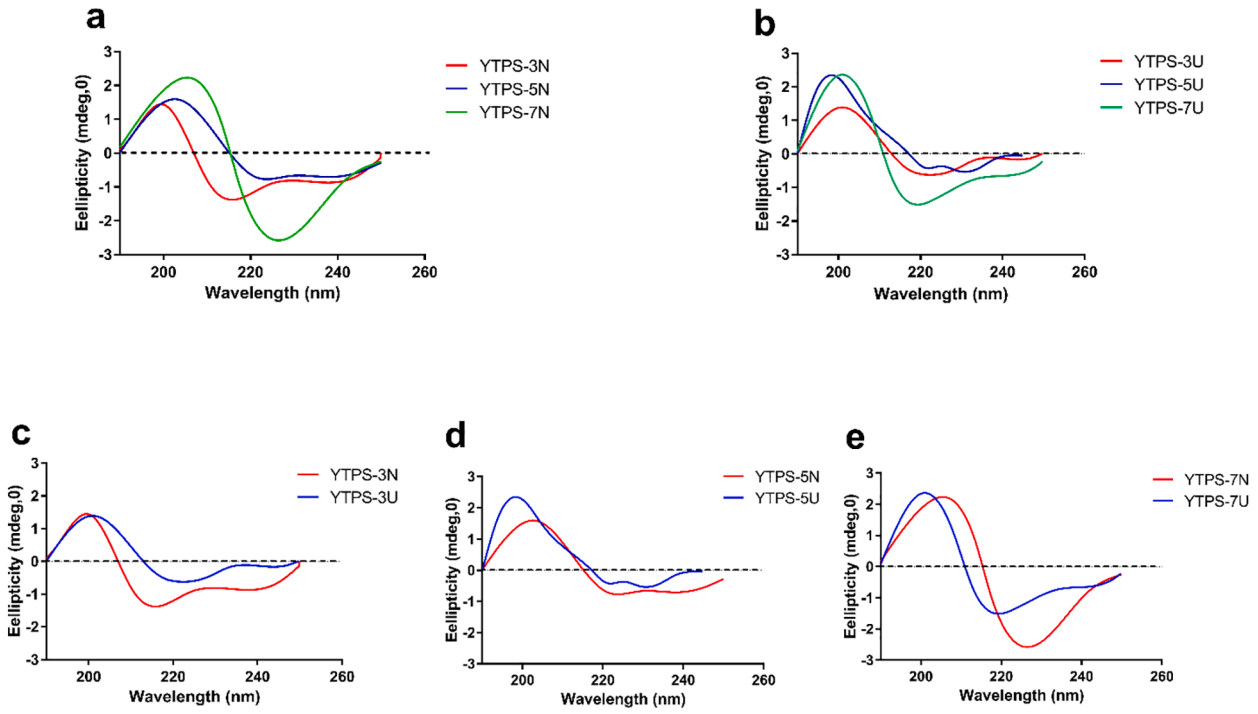

Fig. 4. Circular dichroism (CD) spectra of native yellow tea polysaccharide and ultrasonic treated yellow tea polysaccharide in the wavelength region from 190 to 250 nm. (a) CD spectra of native yellow tea polysaccharide including YTPS-3N, YTPS-5N and YTPS-7N, (b) CD spectra of ultrasonic-treated yellow tea polysaccharide including YTPS-3U, YTPS-5U and YTPS-7U, (c) CD spectra of YTPS-3N and YTPS-3U, (d) CD spectra of YTPS-5N and YTPS-5U, (e) CD spectra of YTPS-7N and YTPS-7U. The YTPS-3N, YTPS-5N, and YTPS-7N were the native yellow tea polysaccharide sequentially precipitated with 30%, 50%, and 70% (v/v) ethanol. The YTPS-3U, YTPS-5U, and YTPS-7U were ultrasound-treated YTPS-3N, YTPS-5N, and YTPS-7N samples, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was analyzed using Scanning electron microscope (SEM). As shown in Fig. 6, the YTPS-3N showed an irregular thin slice shape with sharp corner and a rough surface (Fig. 6a). After ultrasonic treatment, the particles of native yellow tea polysaccharide were degraded, the slices in YTPS-3U were much thinner than that in YTPS-3N, and the laminar corner was blunt with porous on the surface (indicated by the red arrow in Fig. 6b). When the native yellow tea polysaccharide was precipitated with 50% ethanol, the YTPS-5N showed a block structure with smooth surface and edge (Fig. 6c), ultrasonication altered the YTPS-5N structure to smaller fragments with porous on surface and cavities on fracture surface (as shown in YTPS-5U image) (Fig. 6d), which may be caused by the substantial cavitation activity, turbulence shear, and instantaneous high pressure [58]. However, the YTPS-7N had a smooth surface with characteristic small wrinkles which was very distinct from YTPS-3N and YTPS-5N (Fig. 6e). After ultrasound treatment, the yellow tea polysaccharide developed a flat and rough surface with plenty of tiny pits and pores (as shown in YTPS-7U image) (Fig. 6f). The results were consistent with those of Chen et al. [9], in which the *Moringa oleifera* Lam polysaccharides precipitated with various concentrations of ethanol with varying surface structures. The differences in the surfaces between YTPS-3N, YTPS-5N, and YTPS-7N could be attributed to the changes in interconnection and intermolecular distance caused by different ethanol concentration. Collectively, our results confirmed that ultrasonic energy is sufficient to break the covalent bonds, such as glycosidic linkages that connects the monosaccharides and promotes the segregation of polysaccharide molecules [59].

3.2. Effects of ultrasound on antioxidant activities

The *in vitro* antioxidant activities of yellow tea polysaccharide treated and untreated by ultrasound were investigated by detection of radical scavenging activities of 2, 2-diphenyl-1-picrylhydrazyl (DPPH), superoxide, and hydroxyl. The DPPH radical is one of the few stable radical species widely used to evaluate the free radical scavenging ability of antioxidants. As shown in Fig. 7a and b, the DPPH radical scavenging activity was improved with the increasing of yellow tea polysaccharide concentration. However, the antioxidant activity of the ultrasound treated and untreated yellow tea polysaccharides were much lower than the positive control (vitamin C, 2.035×10^{-6} mg/mL), in which the IC_{50} values were 0.63 ± 0.09, 0.36 ± 0.15, 0.25 ± 0.03, 0.52 ± 0.1, 0.34 ± 0.15 and 0.06 ± 0.02 mg/mL. respectively. As compared to the native yellow tea polysaccharide, ultrasonication lowered the IC_{50} values of yellow tea polysaccharide, but only that of YTPS-7U were significantly decreased ($P < 0.05$). This could be attributed to the native hydrogen bond of the polysaccharide being broken during the ultrasonic treatment process; DPPH free radicals generated from ultrasonic treatment can receive more hydrogen atoms from degraded polysaccharide [60] and contributed to the higher DPPH free radical scavenging capability of yellow tea polysaccharide.

The superoxide radical is one of the highly toxic reactive oxygen species which can generate other reactive oxygen species with a potential to cause tissue damage [61]. Thus, it is very important to test superoxide radical scavenging activity of antioxidants to characterize their safety. Despite yellow tea polysaccharide showing a radical scavenging ability in a concentration dependent manner (Fig. 7c), its antioxidant ability was found to be lower than vitamin C at the same concentration. Upon ultrasonication, the superoxide radical scavenging capacity of YTPS-3U and YTPS-5U significantly improved ($P < 0.05$) and exhibited a much lower IC_{50} compared to YTPS-3N and YTPS-5N. Of all the six yellow tea polysaccharide fractions, the YTPS-7U showed the best superoxide radical scavenging ability (Fig. 7d). These results suggests the degradation effect of ultrasound on the yellow tea polysaccharide can expose more hydroxyl groups. The hydroxyl group may subsequently donate electrons to reduce the radicals to more stable forms and/or by directly reacting with the free radicals to terminate the radical chain reaction thereby causing the antioxidation of tea polysaccharides

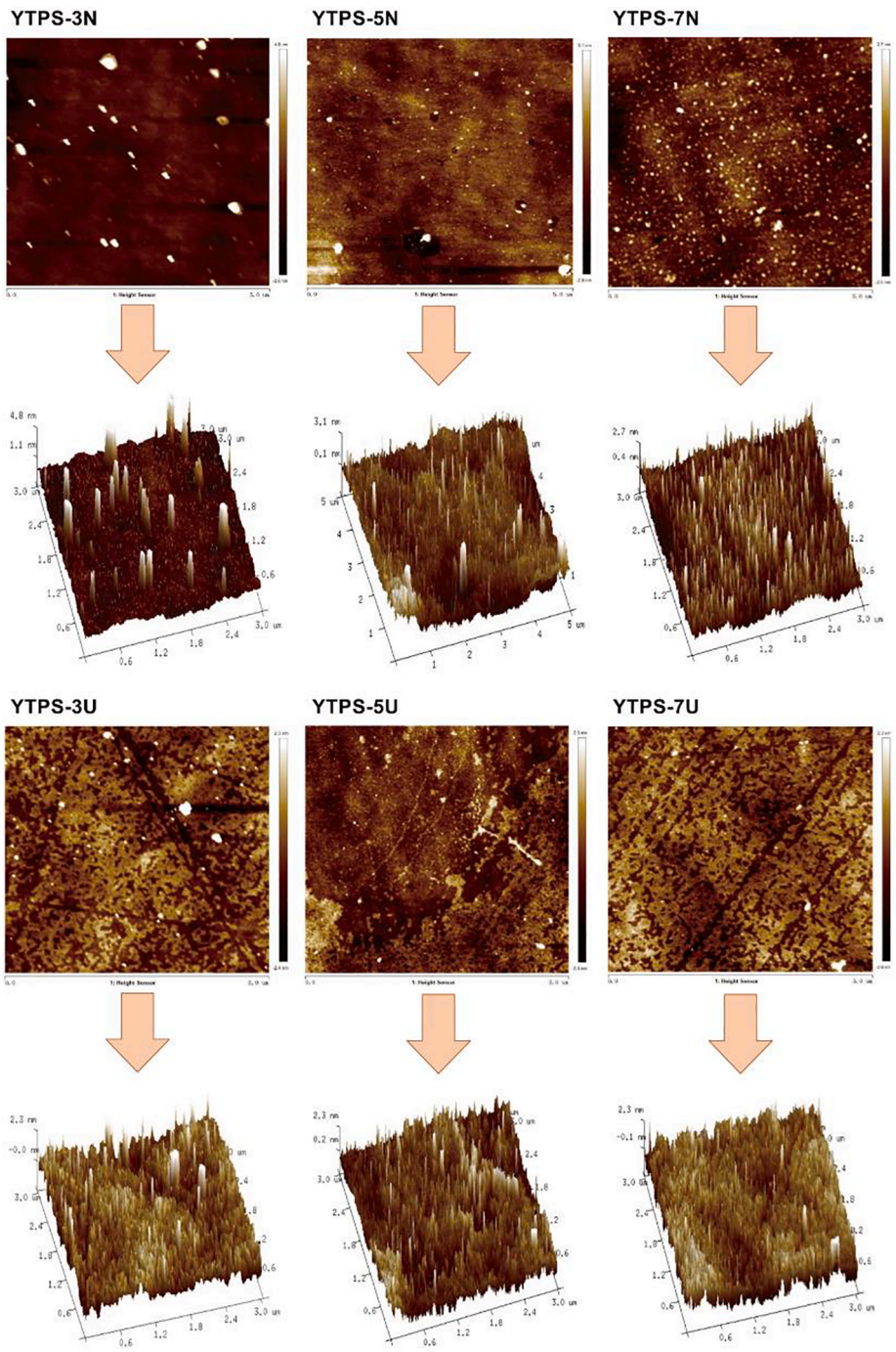

Fig. 5. Morphology of native yellow tea polysaccharide and ultrasonic treated yellow tea polysaccharide by atomic force microscope. The YTPS-3N, YTPS-5N, and YTPS-7N were the native yellow tea polysaccharide sequentially precipitated with 30%, 50%, and 70% (v/v) ethanol. The YTPS-3U, YTPS-5U, and YTPS-7U were ultrasound-treated YTPS-3N, YTPS-5N, and YTPS-7N samples, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[62,63]. In addition, the uronic acid in yellow tea polysaccharide (Table 2) can also play a role in scavenging of superoxide radicals as has been reported [64].

Hydroxyl radicals is similarly regarded as one of the most harmful free radicals among reactive oxygen species. It can attack almost all the biomacromolecules, such as carbohydrates, proteins, lipids, and DNA in living cells and induce severe damage [65]. Fig. 7e and f showed the hydroxyl radical scavenging activity of yellow tea polysaccharide and their IC_{50}, respectively. As shown in Fig. 7e, all the yellow tea polysaccharide fractions exhibited higher hydroxyl radicals scavenging ability than vitamin C in the concentration range from 0.05 mg/ml to 2 mg/ml. The IC_{50} of YTPS-3N, YTPS-5N, and YTPS-7 were 0.14 ± 0.002, 0.13 ± 0.004, 0.09 ± 0.003, respectively. Ultrasonic treatment significantly increased ($P < 0.05$) the hydroxyl radical scavenging activity of YTPS-5N and YTPS-7N, in which the IC_{50} were decreased to 0.10 ± 0.006 and 0.07 ± 0.004 mg/mL, respectively (Fig. 7f). The higher hydroxyl radical scavenging activity in YTPS-3U, YTPS-5U, and YTPS-7U may be due to the induction of the hydroxyl groups by the ultrasonication in yellow tea polysaccharide which further enhanced the radical scavenging capacity. Our findings are in accordance with those of Yan et al. [66]. Additionally, the monosaccharide composition, chemical structure, and spatial conformation of yellow tea polysaccharide may also affect hydroxyl radical scavenging activity.

The results clearly demonstrated that ultrasonication had improved the free radical scavenging activity of yellow tea polysaccharide based on molecular weight and particle size. Previous studies have also reported notable radical scavenging ability of polysaccharides from different sources [11,47,67]. This phenomenon could be ascribed to the

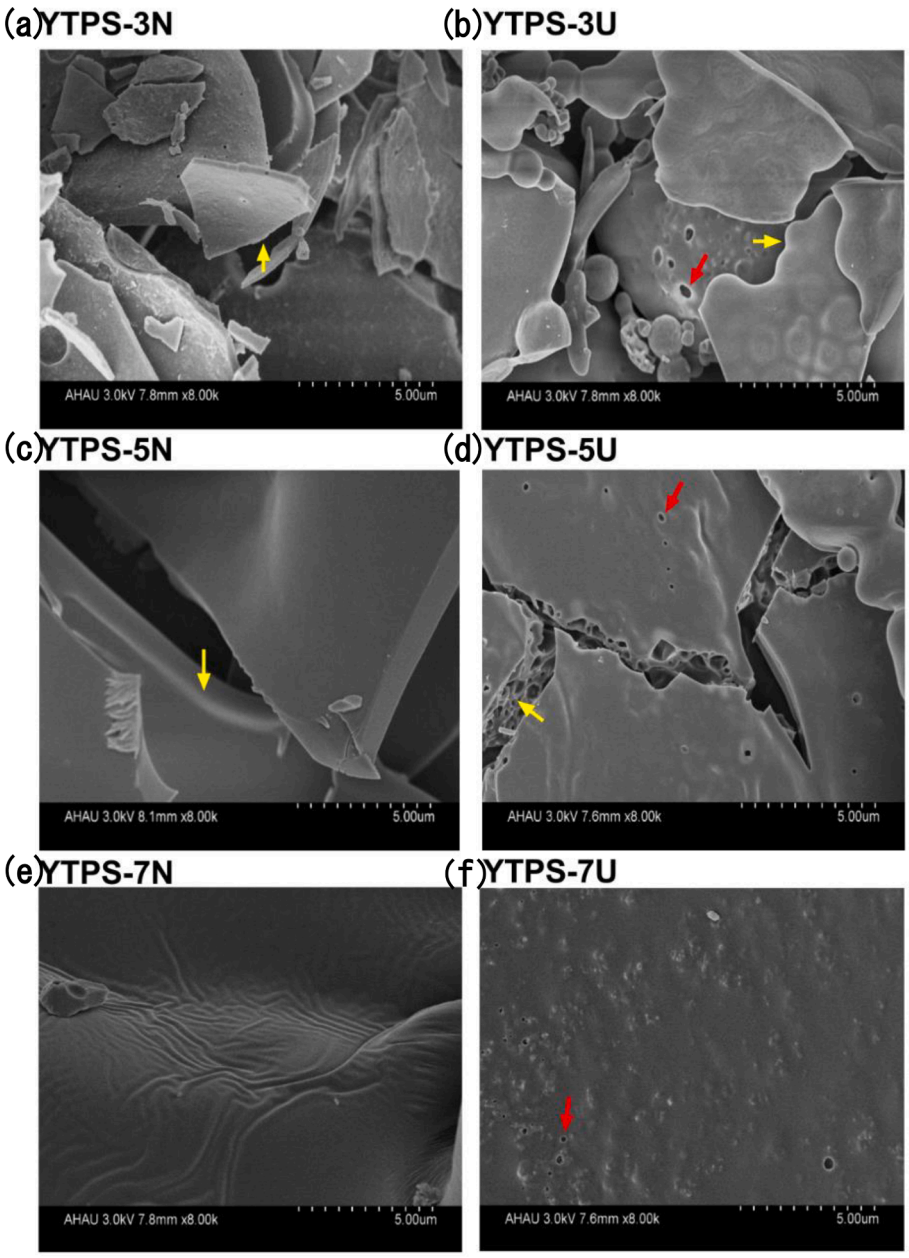

Fig. 6. Scanning electron microscope (SEM) images of yellow tea polysaccharide fractions before and after ultrasonic treatment. The YTPS-3N, YTPS-5N, and YTPS-7N were the native yellow tea polysaccharide sequentially precipitated with 30%, 50%, and 70% (v/v) ethanol. The YTPS-3U, YTPS-5U, and YTPS-7U were ultrasound-treated YTPS-3N, YTPS-5N, and YTPS-7N samples, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ultrasonic cavitation effect which break up the aggregates and reduce their particle size [68]. Generally, the degraded yellow tea polysaccharide particles provided a larger surface area and more reactive sites with free radicals and/or suppress the reactions between the free radicals. Consequently, the ultrasonic cavitation contributed to the improving of scavenging abilities of yellow tea polysaccharide against different types of free radicals.

4. Conclusion

The structural characteristic and antioxidant activity of yellow tea polysaccharide with different molecular weight were influenced by ultrasound irradiation. Compared to the native yellow tea polysaccharide, the ultrasound treatment caused a degradation of polysaccharides without altering the primary chemical composition of the monosaccharides. Ultrasound treatment nonetheless significantly altered the tertiary structure and surface morphology based on FT-IR, AFM, CD, and SEM analysis and subsequently the yellow tea polysaccharide free radical scavenging activity. The smaller particle size yellow tea polysaccharide exhibited stronger free radical scavenging activity than the larger counterparts. Similarly, the degraded yellow tea polysaccharide by ultrasound also showed strong antioxidant capacity compared to the native yellow tea polysaccharide. Thus, ultrasonic treatment can be an effective way to improve the free radical scavenging activity of yellow tea polysaccharide with different molecular wights. In addition, there is a complex relationship between structural changes and antioxidant properties of polysaccharides before and after ultrasound. In the future structure–function relationship of yellow tea polysaccharide and its action mechanism will also be studied.

CRediT authorship contribution statement

Haisong Wang: Conceptualization, Methodology, Supervision, Project administration, Writing - review & editing. **Jinran Chen:** Investigation, Formal analysis, Writing - original draft. **Pengfei Ren:** Formal analysis. **Yiwen Zhang:** Investigation. **Stanley Omondi**

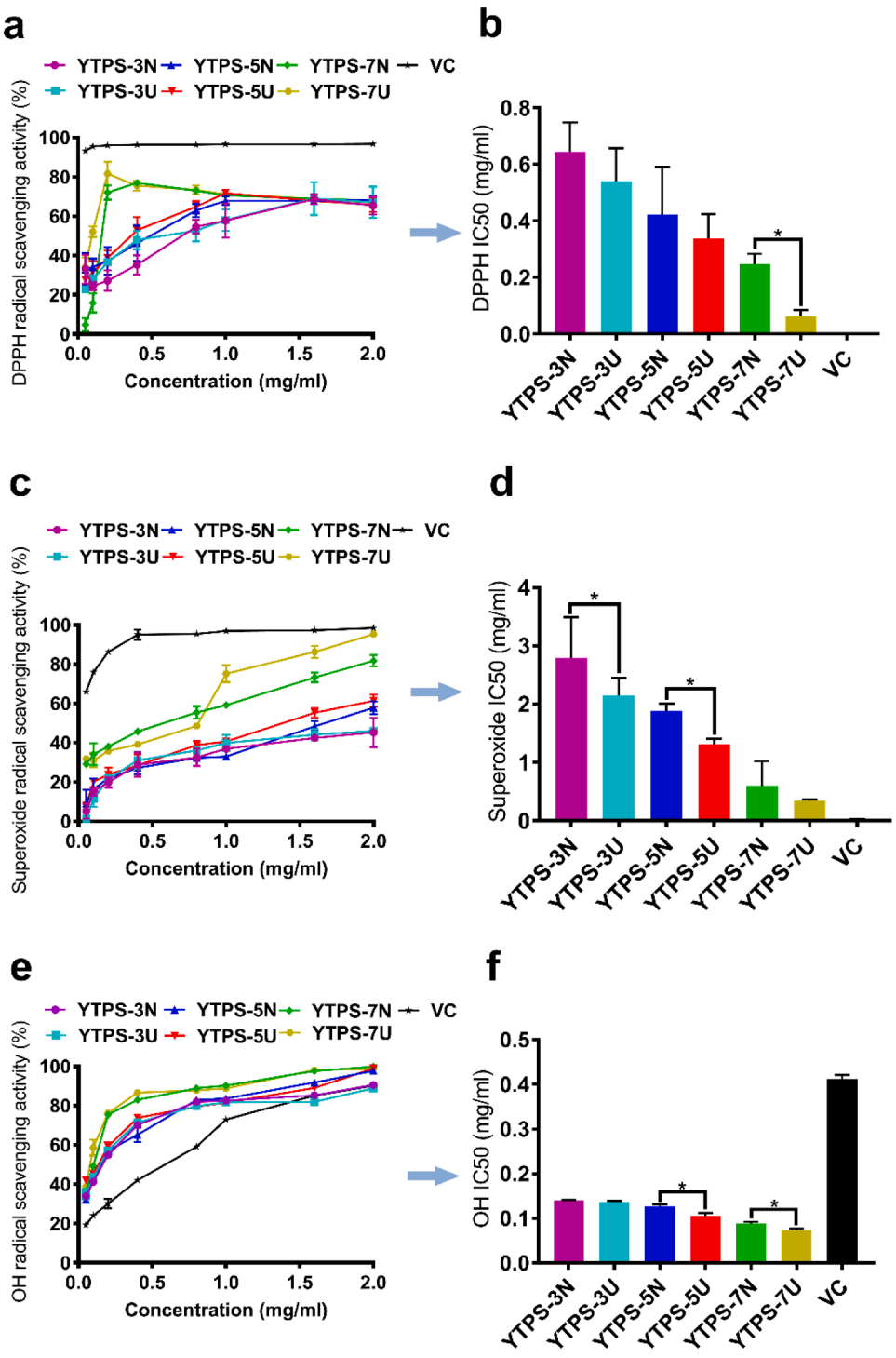

Fig. 7. (a) DPPH radical scavenging activity (b) IC_{50} of DPPH free radical scavenging rate; (c) superoxide radical scavenging activity; (d) IC_{50} of superoxide anion radical scavenging rate; (e) hydroxyl radical scavenging activity; (f) IC_{50} of hydroxyl radical scavenging rate. Values were presented as mean ± SD (n = 3). *, $P < 0.05$, indicated that was significantly different between two groups. The YTPS-3N, YTPS-5N, and YTPS-7N were the native yellow tea polysaccharide sequentially precipitated with 30%, 50%, and 70% (v/v) ethanol. The YTPS-3U, YTPS-5U, and YTPS-7U were ultrasound-treated YTPS-3N, YTPS-5N, and YTPS-7N samples, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Onyango: Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors are grateful for the financial support by the National Natural Science Foundation for Young Scientists of China (31601424) for this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2020.105355.

References

[1] J. Xu, M. Wang, J. Zhao, Y.H. Wang, Q. Tang, I.A. Khan, Yellow tea (Camellia sinensis L.), a promising Chinese tea: processing, chemical constituents and health benefits, Food Res. Int. 107 (2018) 567–577.

[2] A. Gramza-Michałowska, J. Kobus-Cisowska, D. Kmiecik, J. Korczak, B. Helak, K. Dziedzic, D. Górecka, Antioxidative potential, nutritional value and sensory profiles of confectionery fortified with green and yellow tea leaves (Camellia sinensis), Food Chem. 211 (2016) 448–454.

[3] X. Guo, C.-T. Ho, W. Schwab, C. Song, X. Wan, Aroma compositions of large-leaf yellow tea and potential effect of theanine on volatile formation in tea, Food Chem. 280 (2019) 73–82.

[4] J. Zhou, L. Zhang, Q. Meng, Y. Wang, P. Long, C.-T. Ho, C. Cui, L. Cao, D. Li, X. Wan, Roasting improves the hypoglycemic effects of a large-leaf yellow tea infusion by enhancing the levels of epimerized catechins that inhibit α-glucosidase, Food Funct. 9 (10) (2018) 5162–5168.

[5] N.a. Xu, J. Chu, M. Wang, L. Chen, L. Zhang, Z. Xie, J. Zhang, C.-T. Ho, D. Li, X. Wan, Large yellow tea attenuates macrophage-related chronic inflammation and metabolic syndrome in high-fat diet treated mice, J. Agric. Food Chem. 66 (15) (2018) 3823–3832.

[6] Y. Teng, D. Li, P. Guruvaiah, N. Xu, Z. Xie, Dietary supplement of large yellow tea ameliorates metabolic syndrome and attenuates hepatic steatosis in db/db mice, Nutrients 10 (2018) 75.

[7] M. Han, G. Zhao, Y. Wang, D. Wang, F. Sun, J. Ning, X. Wan, J. Zhang, Safety and anti-hyperglycemic efficacy of various tea types in mice, Sci. Rep. 6 (2016) 31703.

[8] G. Chen, Q. Yuan, M. Saeeduddin, S. Ou, X. Zeng, H. Ye, Recent advances in tea polysaccharides: extraction, purification, physicochemical characterization and bioactivities, Carbohydr. Polym. 153 (20) (2016) 663–678.

[9] C. Chen, B. Zhang, Q. Huang, X. Fu, R.H. Liu, Microwave-assisted extraction of polysaccharides from Moringa oleifera Lam. leaves: characterization and hypoglycemic activity, Ind. Crops Prod. 100 (2017) 1–11.

[10] J. Chen, J. Li, A.-D. Sun, B.-L. Zhang, S.-G. Qin, Y.-Q. Zhang, Supercritical CO2 extraction and pre-column derivatization of polysaccharides from Artemisia sphaerocephala Krasch. seeds via gas chromatography, Ind. Crops Prod. 60 (2014) 138–143.

[11] X. Guo, X. Shang, X. Zhou, B. Zhao, J. Zhang, Ultrasound-assisted extraction of polysaccharides from Rhododendron agganiphum: antioxidant activity and rheological properties, Ultrason. Sonochem. 38 (2017) 246–255.

[12] P. RIESZ, T. KONDO, Free radical formation induced by ultrasound and its biological implications, Free Radical Biol. Med. 13 (3) (1992) 247–270.

[13] E.B. Flint, K.S. Suslick, The temperature of cavitation, Science 253 (1991) 1397.

[14] K.S. Suslick, Sonochemistry, Science 247 (4949) (1990) 1439–1445.

[15] K.S. Suslick, The chemical effects of ultrasound, Sci. Am. 260 (2) (1989) 80–86.

[16] K. Makino, M.M. Mossoba, P. Riesz, Chemical effects of ultrasound on aqueous solutions. Formation of hydroxyl radicals and hydrogen atoms, J. Phys. Chem. 87 (1983) 1369.

[17] N. Serpone, P. Colarusso, I. Sonochemistry, Effects of ultrasounds on heterogeneous chemical reactions - a useful tool to generate radicals and to examine reaction mechanisms, Res. Chem. Inter. med. 20 (1994) 635.

[18] R. Czechowska-Biskup, B. Rokita, S. Lotfy, P. Ulanski, J.M. Rosiak, Degradation of chitosan and starch by 360-kHz ultrasound, Carbohydr. Polym. 60 (2) (2005) 175–184.

[19] F. Chemat, N. Rombaut, A.-G. Sicaire, A. Meullemiestre, A.-S. Fabiano-Tixier, M. Abert-Vian, Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review, Ultrason. Sonochem. 34 (2017) 540–560.

[20] E.S.K. Tang, M. Huang, L.Y. Lim, Ultrasonication of chitosan and chitosan nanoparticles, Int. J. Pharm. 265 (2003) 103–114.

[21] G. Portenlaenger, H. Heusinger, The influence of frequency on the mechanical and radical effects for the ultrasonic degradation of dextranes, Ultrason. Sonochem. 4 (1997) 127–130.

[22] N. Kardos, J.-L. Luche, Sonochemistry of carbohydrate compounds, Carbohydr. Res. 332 (2) (2001) 115–131.

[23] C. Chen, Q. Huang, L.-J. You, X. Fu, Chemical property and impacts of different polysaccharide fractions from Fructus Mori. on lipolysis with digestion model in vitro, Carbohydr. Polym. 178 (2017) 360–367.

[24] C.-H. Chou, T.-J. Sung, Y.-N. Hu, H.-Y. Lu, L.-C. Yang, K.-C. Cheng, P.-S. Lai, C.-W. Hsieh, Chemical analysis, moisture-preserving, and antioxidant activities of polysaccharides from Pholiota nameko by fractional precipitation, Int. J. Biol. Macromol. 131 (2019) 1021–1031.

[25] J. Xu, R.-Q. Yue, J. Liu, H.-M. Ho, T. Yi, H.-B. Chen, Q.-B. Han, Structural diversity requires individual optimization of ethanol concentration in polysaccharide precipitation, Int. J. Biol. Macromol. 67 (2014) 205–209.

[26] T.A. Gorshkova, L.V. Kozlova, P.V. Mikshina, Spatial structure of plant cell wall polysaccharides and its functional significance, Biochemistry 78 (2013) 836–853.

[27] M.G. Sevag, D.B. Lackman, J. Smolens, The isolation of the components of streptococcal nucleoproteins in serologically active form, J. Biol. Chem. 124 (1938) 425–436.

[28] C. Nie, P. Zhu, M. Wang, S. Ma, Z. Wei, Optimization of water-soluble polysaccharides from stem lettuce by response surface methodology and study on its characterization and bioactivities, Int. J. Biol. Macromol. 105 (2017) 912–923.

[29] A.F. Gaudy, Colorimetric determination of protein and carbohydrate, Water Wastes 7 (1962) 17–22.

[30] J. Li, K. Kisara, S. Danielsson, M.E. Lindström, G. Gellerstedt, An improved methodology for the quantification of uronic acid units in xylans and other polysaccharides, Carbohydr. Res. 342 (11) (2007) 1442–1449.

[31] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1-2) (1976) 248–254.

[32] V.L. Singleton, R. Orthofer, R.M. Lamuela Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, Methods Enzymol. 464 (299) (1999) 152–178.

[33] C. Chen, B. Zhang, X. Fu, L.-J. You, A.M. Abbasi, R.H. Liu, The digestibility of mulberry fruit polysaccharides and its impact on lipolysis under simulated saliva, gastric and intestinal conditions, Food Hydrocolloids 58 (2016) 171–178.

[34] Y. Xu, Y. Guo, S. Duan, H. Wei, Y. Liu, L. Wang, X. Huo, Y.u. Yang, Effects of ultrasound irradiation on the characterization and bioactivities of the polysaccharide from blackcurrant fruits, Ultrason. Sonochem. 49 (2018) 206–214.

[35] H. Yang, Y. Wu, C. Gan, T. Yue, Y. Yuan, Characterization and antioxidant activity of a novel polysaccharide from Pholidota chinensis Lindl. Carbohydr. Polym. 138 (2016) 327–334.

[36] K. Shimada, K. Fujikawa, K. Yahara, T. Nakamura, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, J. Agric. Food Chem. 40 (6) (1992) 945–948.

[37] C.-L. Ye, W.-L. Hu, D.-H. Dai, Extraction of polysaccharides and the antioxidant activity from the seeds of Plantago asiatica L. Int. J. Biol. Macromol. 49 (4) (2011) 466–470.

[38] N. Smirnoff, Q.J. Cumbes, Hydroxyl radical scavenging activity of compatible solutes, Phytochemistry 28 (4) (1989) 1057–1060.

[39] Z. Dou, C. Chen, X. Fu, The effect of ultrasound irradiation on the physicochemical properties and α-glucosidase inhibitory effect of blackberry fruit polysaccharide, Food Hydrocolloids 96 (2019) 568–576.

[40] R.H. Chen, J.R. Chang, J.S. Shyur, Effects of ultrasonic conditions and storage in acidic solutions on changes in molecular weight and polydispersity of treated chitosan, Carbohydr. Res. 299 (1997) 287–294.

[41] E. Layne, Spectrophotometric and turbidimetric methods for measuring proteins, Methods Enzymol. 3 (1957) 447–455.

[42] J.D. Koch, J. Gronki, R.K. Hanson, Measurements of near-UV absorption spectra of acetone and 3-pentanose at high temperatures, J. Quant. Spectrosc. Ra. 109 (11) (2008) 2037–2044.

[43] C.G. Kumar, H.-S. Joo, J.-W. Choi, Y.-M. Koo, C.-S. Chang, Purification and characterization of an extracellular polysaccharide from haloalkalophilic Bacillus sp. I-450, Enzyme Microb. Technol. 34 (7) (2004) 673–681.

[44] Y. Wang, Y. Wang, Q. Luo, H. Zhang, J. Cao, Molecular characterization of the effects of Ganoderma Lucidum polysaccharides on the structure and activity of bovine serum albumin, Spectrochim. Acta, Part A 206 (2019) 538–546.

[45] S. NIE, M. XIE, Z. FU, Y. WAN, A. YAN, Study on the purification and chemical compositions of tea glycoprotein, Carbohydr. Polym. 71 (4) (2008) 626–633.

[46] M. Kacuráková, P. Capek, V. Sasinková, N. Wellner, A. Ebringerová, FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses, Carbohydr. Polym 43 (2000) 195–203.

[47] N. Liao, J. Zhong, X. Ye, S. Lu, W. Wang, R. Zhang, J. Xu, S. Chen, D. Liu, Ultrasonic-assisted enzymatic extraction of polysaccharide from Corbicula fluminea: characterization and antioxidant activity, LWT-Food Sci. Technol. 60 (2015) 1113–1121.

[48] B. Raja, V. Balachandran, B. Revathi, Structural study, NCA, FT-IR, FT-Raman spectral investigations, NBO analysis, thermodynamic functions of N-acetyl-l-phenylalanine, Spectrochim. Acta, Part A 138 (2015) 283–295.

[49] A. Chiovitti, M.L. Liao, G. Kraft, S. Munro, D. Craik, A. Bacic, Cell wall polysaccharides from Australian red algae of the family Solieriaceae (Gigartinales, Rhodophyta): highly methylated carrageenans from the genus Rhabdonia, Bot. Mar. 39 (1996) 47–60.

[50] M.A. Coimbra, F. Gonçalves, A.S. Barros, I. Delgadillo, Fourier transform infrared spectroscopy and chemometric analysis of white wine polysaccharide extracts, J. Agric. Food Chem. 50 (12) (2002) 3405–3411.

[51] X. Guo, X. Ye, Y. Sun, D. Wu, N. Wu, Y. Hu, S. Chen, Ultrasound effects on the degradation kinetics, structure, and antioxidant activity of sea cucumber Fucoidan, J. Agric. Food Chem. 62 (5) (2014) 1088–1095.

[52] D.G. Cziner, E.S. Stevens, E.R. Morris, D.A. Rees, Vacuum ultraviolet circular dichroism of dermatan sulfate: Iduronate ring geometry in solution and solid state, J. Am. Chem. Soc. 108 (1986) 3790–3795.

[53] K.J. Sreeram, H.Y. Shrivastava, B.U. Nair, Studies on the nature of interaction of iron (III) with alginates, Biochim. Biophys. Acta, Gen. Subj. 1670 (2004) 121–125.

[54] I.G. Plaschina, E.E. Braudo, V.B. Tolstoguzov, Circular-dichroism studies of pectin solutions, Carbohydr. Res. 60 (1) (1978) 1–8.

[55] J. Wang, W. Yang, Y. Tang, Q. Xu, S. Huang, J. Yao, J. Zhang, Z. Lei, Regioselective sulfation of Artemisia sphaerocephala polysaccharide: solution conformation and antioxidant activities in vitro, Carbohydr. Polym. 136 (2016) 527–536.

[56] T. Zhao, G. Mao, M. Zhang, W. Feng, R. Mao, Y. Zhu, X. Gu, Q. Li, L. Yang, X. Wu, Structure analysis of a bioactive heteropolysaccharide from Schisandra chinensis (Turcz.) Baill, Carbohydr. Polym. 103 (2014) 488–495.

[57] N. Raoufi, R. Kadkhodaee, Y. Fang, G.O. Phillips, Ultrasonic degradation of Persian gum and gum tragacanth: effect on chain conformation and molecular properties, Ultrason. Sonochem. 52 (2019) 311–317.

[58] X. Chen, D. Fang, R. Zhao, J. Gao, B.M. Kimatu, Q. Hu, G. Chen, L. Zhao, Effects of ultrasound-assisted extraction on antioxidant activity and bidirectional immunomodulatory activity of Flammulina velutipes polysaccharide, Int. J. Biol. Macromol. 140 (2019) 505–514.

[59] Z.-M. Wang, Y.-C. Cheung, P.-H. Leung, J.-Y. Wu, Ultrasonic treatment for improved solution properties of a high-molecular weight exopolysaccharide produced by a medicinal fungus, Bioresour. Technol. 101 (14) (2010) 5517–5522.

[60] O.P. Sharma, T.K. Bhat, DPPH antioxidant assay revisited, Food Chem. 113 (4) (2009) 1202–1205.
[61] P. Shao, M. Chen, Y. Pei, P. Sun, In intro antioxidant activities of different sulfated polysaccharides from chlorophytan seaweeds Ulva fasciata, Int. J. Biol. Macromol. 59 (2013) 295–300.
[62] R.C. Lindsay, Food additives, in: O.R. Fennema (Ed.), Food Chemistry, Marcel Dekker Inc, New York, 1996, pp. 778–780.
[63] J.K. Yan, L. Li, Z.M. Wang, P.H. Leung, W.Q. Wang, J.Y. Wu, Acidic degradation and enhanced antioxidant activities of exopolysaccharides from Cordyceps sinensis mycelial culture, Food Chem. 117 (4) (2009) 641–646.
[64] Z. Zhang, X. Wang, J. Li, G. Wang, G. Mao, Extraction and free radical scavenging activity of polysaccharide from 'Anji Baicha' (Camellia sinensis (L.) O. Kuntze), Int. J. Biol. Macromol. 84 (2016) 161–165.
[65] E. Rollet-Labelle, M.-J. Grange, C. Elbim, C. Marquetty, M.-A. Gougerot-Pocidalo, C. Pasquier, Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis, Free Radical Biol. Med. 24 (4) (1998) 563–572.
[66] J.-K. Yan, Y.-Y. Wang, H.-L. Ma, Z.-B. Wang, Ultrasonic effects on the degradation kinetics, preliminary characterization and antioxidant activities of polysaccharides from Phellinus linteus mycelia, Ultrason. Sonochem. 29 (2016) 251–257.
[67] T. Lin, Y. Liu, C. Lai, T. Yang, J. Xie, Y. Zhang, The effect of ultrasound assisted extraction on structural composition, antioxidant activity and immunoregulation of polysaccharides from Ziziphus jujuba Mill var. spinosa seeds, Ind. Crops Prod. 125 (2018) 150–159.
[68] W. Cai, T. Hu, A.M. Bakry, Z. Zheng, Y. Xiao, Q. Huang, Effect of ultrasound on size, morphology, stability and antioxidant activity of selenium nanoparticles dispersed by a hyperbranched polysaccharide from Lignosus rhinocerotis, Ultrason. Sonochem. 42 (2018) 823–831.