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Characterization of antioxidant polysaccharides from *Auricularia auricular* using microwave-assisted extraction

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ABSTRACT

Influence of microwave-assisted extraction (MAE) to the characterization and corresponding antioxidant activity of polysaccharides from *Auricularia auricula* (AAP) was determined. According to gas chromatography–mass spectrometry, high performance size exclusion chromatography, Fourier transform infrared spectroscopy, partial acid hydrolysis, periodic acid oxidation, Smith degradation, methylation analysis and atomic force microscopy, AAP was a heteropolysaccharide (composed of glucose, galactose, mannose, arabinose and rhamnose at the molar ratio of 37.53:1:4.32:0.93:0.91) with the molecular weight of 2.77×10^4 Da, observed as a spherical lump, and the backbone of AAP was mainly composed by glucose with (1→3) linked. Moreover, AAP exhibited significant in vitro antioxidant activity in scavenging free radicals (ABTS, DPPH, superoxide and hydroxyl radicals), in lipid peroxidation and reducing power assays. The present result suggested that AAP by MAE had low molecular weight and remarkable antioxidant capability.

1. Introduction

Auricularia auricula, one of edible mushrooms, has been widely used as a healthy food in East Asian, especially in China and Korean (Luo et al., 2009). It is believed to be of high nutritional value since it has the high content of carbohydrates, amino acids, trace elements and vitamins, thus to be processed into a variety of foods (Fan, Zhang, Yu, & Ma, 2006). In previous studies, polysaccharides were isolated and identified to be the main active compound in *A. auricula* (Zhang, Yang, Ding, & Chen, 1995), with many biological functions, including hypoglycemic (Yuan, He, Cui, & Takeuchi, 1998), anticoagulant (Yoon et al., 2003), antitumor and immunobiological activities (Mizuno, Saito, Nishitoba, & Kawagishi, 1995). Recently, polysaccharides of *A. auricula* were found to have the potent antioxidant activity both *in vitro* (Fan et al., 2006; Kho, Vikineswary, Abdullah, Kuppusamy, & Oh, 2009) and *in vivo* (Chen, Luo, et al., 2008; Chen, Zhang, Qu, & Xie, 2008; Chen, Xie, Nie, Li, & Wang, 2008; Wu et al., 2010). A current research also reported that polysaccharides of *A. auricula* had the potential application as a new antioxidant agent in food industry (Fan et al., 2006).

Microwave-assisted extraction (MAE) is an alternative extraction technique that uses microwave energy and solvents to extract target compounds from various matrices (Eskilsson & Bjorklund, 2000). Recently, MAE has received a great attention as a potential alternative to conventional extraction methods, mainly due to considerable savings in processing time, solvent consumption and energy. Furthermore, it considerably enhances the efficiency of the extraction and is friendly to environment (Ballard, Mallikarjunan, Zhou, & O'Keefe, 2010). There are many applications of MAE which deal with the extraction of polysaccharides from mushrooms and herbs (Tao & Xu, 2008; Wang et al., 2010, 2009; Wei et al., 2010). Recently, MAE was also used to extract polyphenols from *A. auricular* (Chen, Ji, Huang, Wang, & Lin, 2010). In the current study, we had used response surface methodology to optimize the MAE process of polysaccharides from *A. auricula* and had gotten the optimum conditions (Zeng, Zhang, & Jia, 2012). However, the possibility influence of MAE to the characterization and biological activity of polysaccharides is limited. Therefore, it is necessary to do some research about the changes of polysaccharides in MAE process.

To the best of our knowledge, the investigation about the influence of MAE to the characterization and corresponding antioxidant activity of polysaccharides is rather limited. In the present study, high performance size exclusion chromatography (HPSEC), gas chromatography–mass spectrometry (GC–MS), Fourier transform infrared (FTIR) spectroscopy and atomic force microscopy (AFM) was employed to observe the characterization of polysaccharides from *A. auricula* using MAE. Furthermore, we attempted to study its *in vitro* antioxidant activities.

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2. Materials and methods

2.1. Chemicals and equipments

A. auricula was collected from Qingchuan of Sichuan, China, and was initially identified by the morphological features. The data and a voucher specimen were present in the Department of Biology, Sichuan University. The fruit bodies of *A. auricula* were collected, washed, and dried at 65 °C for 6 h, and then were crushed into powders with a mixer (JYL-350, Jiuyang Co., Ltd., China), sieved (through 60 mesh) and finally stored under vacuum.

1,1-Diphenyl-2-picrylhydrazyl (DPPH), inositol, pyridine, butyl hydroxy anisd (BHA), dihydronicotineamidadenine dinucleotide (NADH), phenazine methosulfate (PMS), 2,2′-azinobis-3-ethylbenzthiazoline-6-sulfonate (ABTS), nitro blue tetrazolium (NBT), ethylene diamine tetraacetic acid were (EDTA), trifluoroacetic acid (TFA), acetic anhydride and ascorbic acid (Vc) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The solvents for GC–MS were of chromatographic purity. All other reagents used were of analytical grade.

2.2. Extraction and isolation of AAP using MAE

The extraction of polysaccharides from *A. auricula* using MAE was carried out according to Zeng et al. (temperature 95 °C, time 25 min, microwave power of 860 W, pH 7.0) (Zeng et al., 2012). Being treated with Sevage reagent (Staob, 1965) and dialyzed (MWCO 5000, Sigma, USA), crude polysaccharides were precipitated with 5-fold volumes of absolute alcohol, and then were prepared by centrifugation and freeze-drying.

Crude polysaccharides (1 g) were applied to a DEAE-cellulose 52 column (3 cm × 30 cm) and Toyopearl HW-65F column (3 cm × 30 cm), and eluted using a linear gradient of 0–1 M NaCl solution at a flow rate of 0.2 mL/min to arrive at homogenous preparation. The polysaccharides obtained at this stage were designated as "AAP". The total saccharide content and quantification of uronic acid of AAP were detected by the phenol-sulfuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and the vitriol-carbazole method (Yang et al., 2008) with D-glucose and D-glucuronic acid as standard, respectively.

2.3. Homogeneity and molecular weight determination

The homogeneity and molecular weight of AAP were determined by high performance size exclusion chromatography (HPSEC) using a Waters 515 HPLC system equipped with a size exclusion chromatography (SEC) column (TSK gel G 5000PW$_{XL}$, 7.8 cm × 30 cm i.d. with a particle size of 10 μm, Tosoh, Tokyo, Japan). AAP (2 mg/mL; 20 μL) was injected into the column and eluted by ultrapure water at a flow rate of 0.8 mL/min. The molecular weight of AAP was estimated by the comparison to a calibration curve prepared with the T-series Dextran standards (Dextran T-10, T-40, T-70, T-90, T-100, T-200, Pharmacia, Uppsala, Sweden).

2.4. Monosaccharide composition analysis

AAP (5 mg) was dissolved in 2 M TFA (5 mL) and hydrolyzed at 120 °C for 2 h. The hydrolyzed products were evaporated to dryness and derivatized using the following method (Honda, Suzuki, Kakehi, Honda, & Takai, 1981). Hydroxylammonium chloride (10 mg), inositol (5 mg) and pyridine (0.6 mL) were added to the hydrolyzed sample. The mixture was placed in water bath at 90 °C incubated for 30 min. After cooling to room temperature, acetic anhydride (1 mL) was added, and then the reaction system was placed in water bath at 90 °C incubated for 30 min again. The reaction products were analyzed by GC–MS (Thermo GC-MSD, Thermo Fisher, USA) with a flame ionization detector and a TR-5 capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The operation conditions of GC–MS were as follows: flow rate of N_2, H_2 and air were 25 mL/min, 30 mL/min and 400 mL/min, respectively; the temperature of detector and inlet were 280 °C and 250 °C, respectively; the oven temperature program was set changing from 120 °C (standing for 3 min) up to 210 °C (standing for 4 min) at a rate of 3 °C/min. Standard monosaccharides (rhamnose, arabinose, fucose, xylose, mannose, glucose and galactose) were used as references.

2.5. Spectral analysis

AAP was resolved with pure water (0.5 mg/mL), and scanned on a Lambda 25 UV-visible spectrophotometer (Perkin Elmer, CA, USA) from 190 nm to 400 nm.

AAP was analyzed by FTIR for the vibrations of molecules and polar bonds. AAP and dry potassium bromide were mixed, ground and squashed. The spectroscopy was recorded on a Spectrum one FTIR spectrometer (Perkin Elmer, CA, USA) within the range of 4000–400 cm^{-1}.

2.6. Partial acid hydrolysis, periodic acid oxidation and Smith degradation

AAP (50 mg) was hydrolyzed using 0.05 M TFA (2 mL) at 95 °C for 16 h and residual TFA was removed by the repeated addition of ethanol. The hydrolyzed AAP was centrifuged to remove the precipitate (AAP-a), and the supernatant was dialyzed against pure water for 24 h. After dialysis, the fraction outside the dialysis sack was collected and named as AAP-d. Ethanol was added to the solution in dialysis sack, and the precipitation and supernatant were designated as AAP-b and AAP-c, respectively (Yuan et al., 2010). All fractions were dried for GC–MS analysis as mentioned above.

AAP (25 mg) was dissolved in $NaIO_4$ solution (25 mL, 15 mM) and kept in dark at 4 °C. The reaction mixture was monitored for the absorption at 223 nm, and the reaction was completed when absorbance stopped to decrease after 72 h and terminated by addition of glycol (1 mL). The periodate product (5 mL) was sampled to calculate the yield of HCOOH by titration with NaOH (5 mM).

The reaction mixture was reduced by adding $NaBH_4$ (50 mg) for about 20 h, and then was adjusted to pH 5.5–7.0 by using acetic acid (0.1 M). The reaction solution was dialyzed against running water and pure water each for 24 h. The dialyzed product was analyzed by using the method as described in Section 2.4. Standard monosaccharides, glycerol and erythritol were used as references (Niu et al., 2011).

2.7. Methylation analysis

NaOH powder (50 mg) was added to the AAP solution (2 mL, 5 mg/mL, dissolved in dimethyl sulfoxide) with interval vibration under the protection of N_2 at 25 °C for 1 h. Methyl iodide (1.0 mL) was added to react keeping 1 h. Then, pure water (0.5 mL) was used to stop the reaction. The reaction solution was dialyzed against running water for 48 h and distilled water for 24 h. This procedure was repeated to obtain completely methylated polysaccharide. Complete methylation was confirmed by the disappearance of O—H absorption band (3700–3100 cm^{-1}) in FTIR spectrum. The completely methylated polysaccharides was hydrolyzed with TFA, reduced with $NaBH_4$ and derivatized with pyridine-acetic anhydride by using the same method as described in Sections 2.4 and 2.6 (Qiao et al., 2010).

2.8. Observation with atomic force microscopy (AFM)

AAP solution (50 μg/mL) was dropped on the surface of a mica sample carrier, allowed to dry and then was imaged in air at room temperature. The AFM used in this study was a SPM-9600 Scanning Probe Microscope (Shimadzu Co., Kyoto, Japan) and was operated in the tapping-mode. The resulting imaging force was estimated to be 3–4 nN and the resonant frequency was about 2 kHz.

2.9. In vitro antioxidant activity of AAP

2.9.1. ABTS radical scavenging assay

ABTS radical scavenging assay was conducted according to the procedure of Re et al. (1999) with a modification. ABTS radical cation solution was prepared through 16 h of reaction of ABTS (7 mM) with potassium persulfate (2.45 mM) at 23 °C in dark. The solution was diluted with pure water to obtain an absorbance of 0.700 ± 0.005 at 734 nm, and the diluted solution (3.9 mL) was then mixed with 0.1 mL AAP solution. The mixture was allowed to react at 23 °C for 6 min and the absorbance at 734 nm was recorded by using Lambda 25 UV-visible spectrophotometer. The ability to scavenge ABTS radical was calculated as a percentage according to the equation: $(1 - A_{\text{sample } 734}/A_{\text{control } 734}) \times 100\%$.

2.9.2. DPPH radical scavenging assay

DPPH radical scavenging assay was performed according to the previous method with a modification (Mendes, Freitas, Baptista, & Carvalho, 2011). DPPH solution (0.1 mM, in 95% ethanol) was prepared and used fresh on the day of each test. AAP solution (2 mL) was mixed with 2 mL DPPH solution. The mixture was shaken vigorously and kept in dark for 30 min, prior to measuring the absorbance at 517 nm. The ability to scavenge DPPH was calculated as a percentage according to the equation: $(1 - A_{\text{sample } 517}/A_{\text{control } 517}) \times 100\%$.

2.9.3. Superoxide radical scavenging assay

Superoxide radical scavenging assay was carried out according to a modified method (Zhang et al., 2003). NADH, PMS and NBT were prepared at 557 μM, 45 μM and 108 μM in Tris–HCl (16 mM, pH 8.0), respectively. AAP solution (0.1 mL) was mixed with 1 mL NADH, 1 mL PMS and 1 mL NBT. The mixture was incubated at 25 °C for 5 min and the absorbance was measured at 560 nm. The superoxide radical scavenging activity was calculated according to the equation: $(1 - A_{\text{sample } 560}/A_{\text{control } 560}) \times 100\%$.

2.9.4. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging assay was conducted according to the previous method with a modification (Zhang et al., 2003). Deoxyribose (2.67 mM) and EDTA (0.13 mM) were dissolved in phosphate buffered saline (PBS, 0.2 M, pH 7.4). The PBS solution (0.6 mL) was mixed with 0.1 mL AAP solution, 0.2 mL ferrous ammonium sulfate (0.4 mM), 0.05 mL Vc (2.0 mM) and 0.05 mL H_2O_2 (20 mM). The solution was incubated at 37 °C for 15 min, and then 1 mL thiobarbituric acid (1%, w/v) and 1 mL trichloroacetic acid (2%, w/v) were added. The mixture was boiled for 15 min and cooled in ice, and its absorbance was measured at 532 nm. The scavenging activity was calculated according to the equation: $(1 - A_{\text{sample } 532}/A_{\text{control } 532}) \times 100\%$.

2.9.5. Lipid peroxidation assay

The lipid peroxidation assay used in this study was based on the method of Dasgupta and De (2004). Egg yolk homogenate (10%, v/v) was prepared as a lipid-rich media. AAP solution (0.1 mL) was mixed with 0.5 mL egg yolk homogenate and 0.4 mL pure water. Ferrous sulfate (50 μL, 70 mM) was then added to induce lipid peroxidation and the mixture was incubated at 37.5 °C for 30 min. Subsequently, 1.5 mL acetic acid (20%, v/v, pH 3.5) and 1.5 mL thiobarbituric acid (0.8%, w/v, in 1.1% sodium dodecyl sulfate) were added and the mixture was shaken and heated at 95 °C for 60 min. After the reaction solution was cooled, 5 mL of 1-butanol was added and the mixture was centrifuged at 5000 rpm for 15 min. The upper layer was collected and its absorbance at 532 nm was measured. The inhibition of lipid peroxidation was calculated according to the equation: $(1 - A_{\text{sample } 532}/A_{\text{control } 532}) \times 100\%$.

2.9.6. Reducing power assay

The reducing power of AAP was determined according to the method reported by Qi et al. (2005) with minor modification. AAP solution (1 mL) was mixed with 2.5 mL PBS (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%, w/v). The mixture was incubated at 50 °C for 20 min, followed by addition of trichloroacetic acid (2 mL, 10%, w/v) and centrifugation at 2000 rpm for 15 min. A 2.5 mL aliquot of the supernatant was mixed with 2.5 mL pure water and 0.5 mL ferric chloride (0.1%, w/v), and the absorbance was measured at 700 nm.

2.10. Statistical analysis

The data of all experiments were recorded as means ± standard deviations and were analyzed with SPSS (version 12.0 for Windows, SPSS Inc., CO, USA). Differences were considered significant at $P < 0.05$.

3. Results

3.1. Purification and characterization of AAP

Fig. 1A represents the chromatogram of crude polysaccharides subjected to a DEAE-cellulose 52 column. Two distinct peaks were observed. The second peak (Peak-II) showed prominent biological activity and was further subjected to a Toyopearl HW-65F column (Fig. 1B) to obtain AAP with the color of grey. The total saccharide and uronic acid contents of AAP were 95.8% and 10.7%, respectively.

HPSEC analysis (Fig. 1C) indicated that AAP was a homogeneous polysaccharide with a high purity, and its equivalent dextran molecular weight was estimated to 2.77×10^4 Da based on the equation of the standard curve made with a group of dextran standards.

According to the analysis of monosaccharide using GC–MS, AAP was a heteropolysaccharide and consisted of glucose, galactose, mannose, arabinose and rhamnose, with the molar ratio of 37.53:1:4.32:0.93:0.91.

3.2. Spectra analysis of AAP

The ultraviolet spectra revealed that there was no absorption at 280 or 260 nm, indicating that AAP contained no protein or nucleic acid. Fig. 1D showed the FTIR spectrum of AAP. Two characteristic absorptions of polysaccharides, at about 3600–3200 cm^{-1} and 3000–2800 cm^{-1}, were due to the stretching vibration of —OH and C—H. The absorption at 1700–1750 cm^{-1} was the special absorption of uronic acid. No absorption at this region may be due to the limited content of uronic acid in AAP or be concealed by the strong absorption at 1592 cm^{-1}. The absorption at 903 cm^{-1} indicated that β-glycosidic linkages were present between the sugar units. Each particular polysaccharide has a specific band in the 1200–1000 cm^{-1} region. This region is dominated by ring vibrations overlapped with stretching vibrations of (C—OH) side groups and the (C—O—C) glycosidic band vibration. The absorptions at 1145 cm^{-1}, 1075 cm^{-1} and 1042 cm^{-1} indicated a pyranose form of sugar.

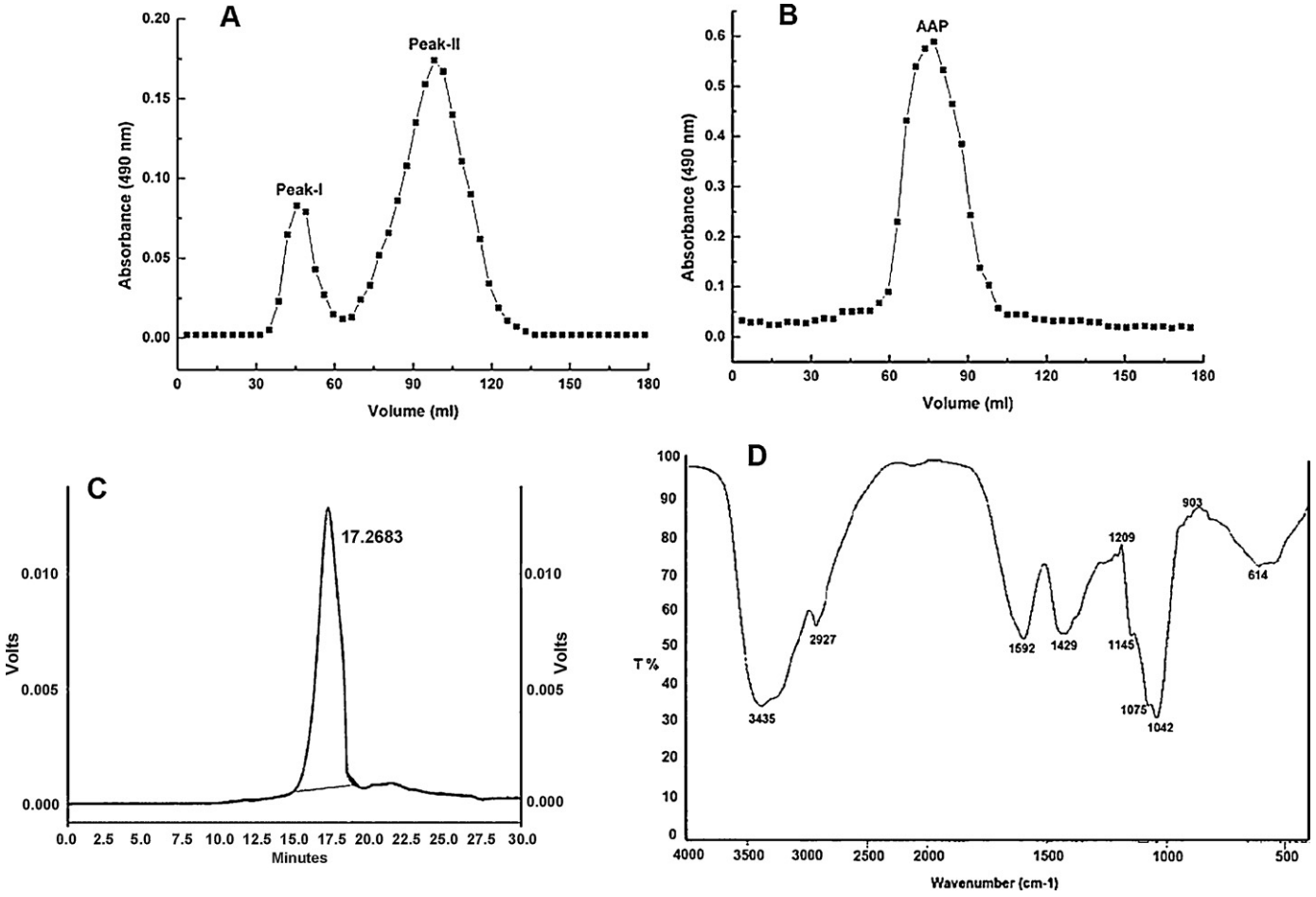

Fig. 1. Purification and characterization of AAP. (A) Chromatogram of crude polysaccharides by DEAE-cellulose 52 chromatography; (B) chromatogram of the second peak (peak-II) by Toyopearl HW-65F chromatography; (C) HPSEC chromatogram of AAP; (D) FTIR spectra of AAP.

Table 1
Analysis result of partial acid hydrolysis.

Fractions	Molar ratios				
	Glucose	Galactose	Mannose	Arabinose	Rhamnose
AAP-a[a]	4.62	1	2.34	0.86	0.82
AAP-b[b]	25.11	1	2.42	0.45	1.03
AAP-c[c]	24.65	1	2.03	2.97	0.98
AAP-d[d]	22.46	1	9.93	0.79	1.37

[a] Precipitation after hydrolysis.
[b] Precipitate in the sack.
[c] Supernatant in the sack.
[d] Fraction out of sack.

3.3. Structural elucidation of AAP

3.3.1. Partial acid hydrolysis

Four fractions (AAP-a, AAP-b, AAP-c and AAP-d) were obtained through partial acid hydrolysis. Each fraction was subjected to GC–MS analysis and the results were shown in Table 1. Results indicated that glucose may be the backbone of the structure of AAP, and galactose, arabinose and rhamnose may be the branched structure of AAP. Glucose and mannose may be the terminal residues of backbone and branched.

3.3.2. Periodate oxidation and Smith degradation

Results of periodate oxidation showed that 1 mol of sugar residue consumed 0.56 mol and produced 0.18 mol of formic acid, indicating the presence of a few of monosaccharide that are (1→) linked or (1→6) linked. The fact that the amount of periodate consumption was more than the amount of formic acid (0.18 mol × 2) demonstrated that there were other linkage types that cannot produce formic acid, such as (1→3) or (1→3, 6).

The periodate-oxidized products were fully hydrolyzed and analyzed by GC–MS after periodate oxidation (Table 2). The predominant presence of glycerol demonstrated that the linkages of backbone are (1→) linked, (1→6) linked, (1→2) linked, (1→2, 6) linked that can be oxidized to produce glycerol. The presence of erythritol indicated that the (1→4) linked and (1→4, 6)linked were present in the backbone of AAP. Combining with the results of partial acid hydrolysis, we can conclude that glucose was present in the backbone and some residues of glucose in backbone were (1→) linked, (1→4) linked, and (1→6) linked that could be oxidized completely.

3.3.3. Methylation analysis

The fully methylated AAP was hydrolyzed with acid and analyzed by GC–MS. The results (Table 3) showed the presence of two components, namely 2,3,4,6-tetra-O-methyl-d-hexitol and 2,4,6-tri-O-methyl-d-hexitol with a molar ration of 1:19. This results from analysis of GC–MS, which were consistent with the results from partial acid hydrolysis, periodate oxidation and Smith degradation, indicated that a few of branched structure was present in AAP; the backbone of AAP was mainly composed by glucose in the form of (1→3) linked; a few of (1→) linked, (1→4) linked, (1→6) linked and (1→4, 6) linked were also present in AAP; glucose and

Table 2
Analysis result of Smith degradation.

Fractions	Molar ratios						
	Glucose	Galactose	Mannose	Arabinose	Rhamnose	Glycerol	Erythritol
Full acid hydrolysis	37.53	1	4.32	0.93	0.91	–	–
Out of sack	8.32	2.43	1.42	1.23	0.61	15.32	1.65
Supernatant in sack	1.44	–	–	1.63	–	+	1.25
Precipitation in sack	1.83	–	–	1.26	–	1.31	+

Table 3
Results of methylation analysis.

Methylation product	Molar ratio	Mass fragments (m/z)	Type of linkage
2,3,4,6-tetra-O-methyl-d-hexitol	1	43, 101, 117, 129, 145, 161, 205	1→
2,4,6-tri-O-methyl-d-hexitol	19	43, 71, 87, 101, 117, 129, 161, 233	1→3

mannose were the major components of AAP; part of galactose, arabinose and rhamnose were distributed in branches.

3.4. Atomic force micrograph

The atomic force micrograph of AAP dissolved in pure water with a concentration of 50 μg/mL was shown in Fig. 2. In Fig. 2A, many spherical lumps can be seen and the diameter of them ranged from 7 to 56 nm (average diameter: 30 nm). The heights of the spherical structures shown in Fig. 2B indicated that the height of lumps ranged from 1.1 to 1.5 nm; the average height was 1.33 nm; the volume of lumps ranged from 25.97 to 2552 nm^3; the average volume was 665.2 nm^3.

3.5. Antioxidant activity

AAP showed the obvious antioxidant properties which are concentration-dependent, as presented in Fig. 3. The ABTS-scavenging ratio was in the range of 43.5–81.7% and the half-effective concentration (EC_{50}) was estimated at 1.23 ± 0.32 mg/mL (Fig. 3A), while EC_{50} of BHA was 5.79 ± 0.58 mg/mL. As shown in Fig. 3B, AAP scavenged 33.5–75.4% of DPPH radicals and its EC_{50} (3.29 ± 0.41 mg/mL) was lower than that (4.45 ± 0.53 mg/mL) of BHA. AAP also showed potent superoxide and hydroxyl radical scavenging activity (Fig. 2C and D), with estimated EC_{50} values of 0.73 ± 0.25 mg/mL and 9.01 ± 0.46 mg/mL, respectively.

AAP was observed to significantly inhibit peroxidation of egg yolk homogenate (Fig. 2E). The inhibition ratio was 47.6% at 0.0625 mg/mL of AAP, and reached 80.4% when the concentration increased to 1 mg/mL. The estimated EC_{50} was 0.067 ± 0.015 mg/mL. The reducing property of AAP was expressed as an A_{700} value, with higher A_{700} meaning stronger reducing activity. As shown in Fig. 2F, AAP showed a strong reducing power and its reducing activity increased with increasing concentration.

4. Discussions

Over the past decade, MAE has drawn significant research attention in various fields, such as chemical engineering, textile industry and medicine. In particular in food industry, MAE has been widely applied to prepare the secondary metabolites from plants for food production, due to its rapid heating rate, moderate capital cost and good performance under atmospheric conditions (Zhang, Yang, & Wang, 2011). In our current study (Zeng et al., 2012), MAE was employed to extract polysaccharides from *A. auricula*, and was optimized by response surface methodology. Results indicated that the yield of polysaccharides was highly increased. However, it has been reported that microwave is an electromagnetic radiation with a wavelength from 0.001 m to 1 m (frequency from 3×10^{11} Hz to 3×10^{8} Hz), and may have the possibility influence to the characterization and biological activity of biopolymers (Mahesar et al., 2008). In present study, the characterization of AAP by MAE was

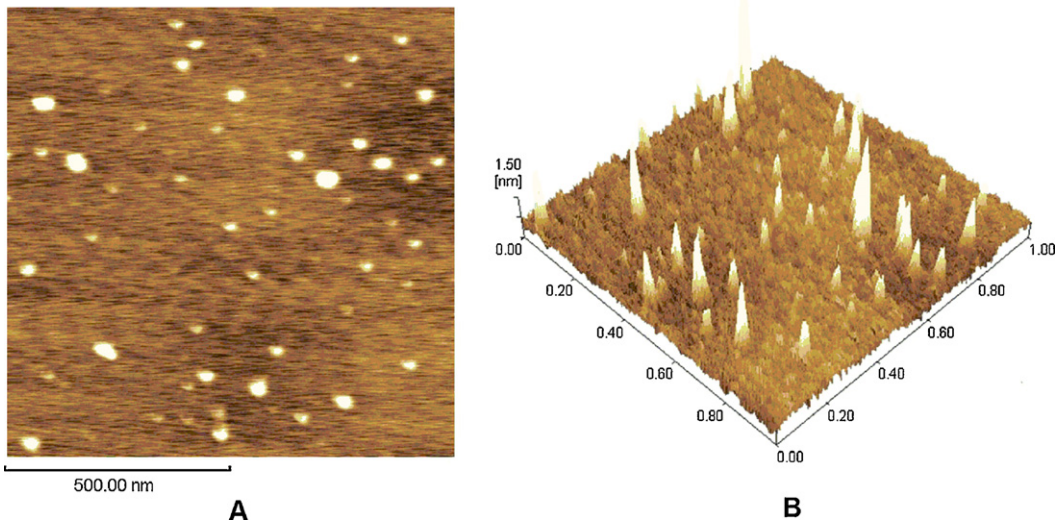

Fig. 2. Molecular structure of AAP observed by atomic force microscopy. (A) Planar image; (B) cubic image.

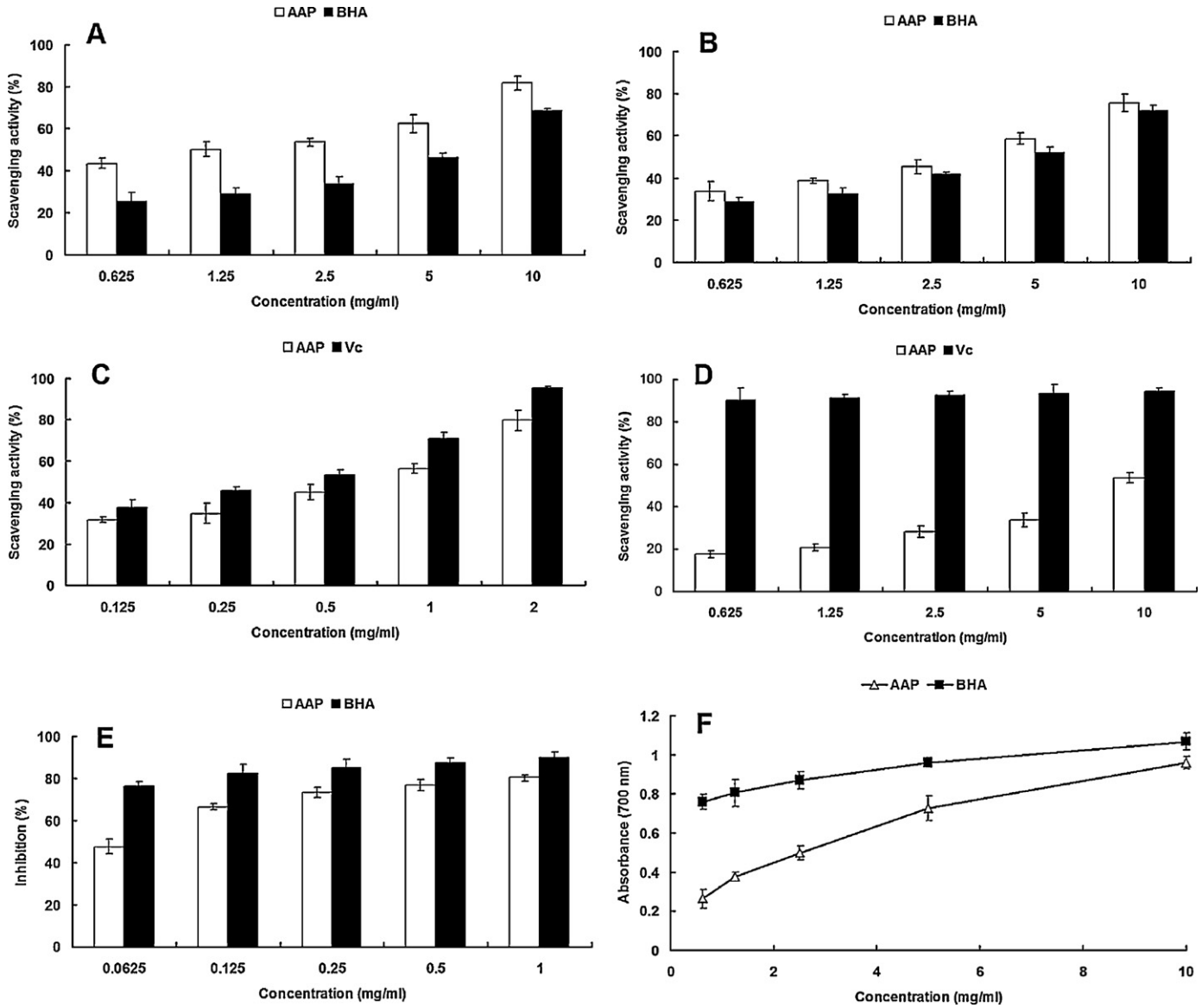

Fig. 3. Radical-scavenging activity (A, ABTS; B, DPPH; C, superoxide radical; D, hydroxyl radical), lipid peroxidation inhibition (E) and reducing property (F) of AAP.

determined using chromatogram technology and AFM. According to the results of GC–MS and HPSEC, AAP was a heteropolysaccharide (composed of glucose, galactose, mannose, arabinose and rhamnose at the molar ratio of 37.53:1:4.32:0.93:0.91) with the molecular weight of 2.77×10^4 Da. Comparing with the previous report (Zhang et al., 1995), AAP had the lower molecular weight and higher content of glucose. These changes may be attributed to the heating mechanism of MAE. The heating process of MAE is based on two principles, which are ionic conduction and dipole rotation. Ionic conduction refers to the electrophoretic migration of the charge carriers under the influence of the electric field produced by microwave; dipole rotation happens when the dipolar molecules attempts to follow the electric field in the same alignment (Mahesar et al., 2008). Microwave energy acts as an electromagnetic radiation that causes the highly localized temperature and leads to the degradation of polysaccharides during the microwave heating process. Moreover, recent studies also observed the possibility disassembly of polysaccharides during MAE (Fishman, Chau, Cooke, Yadav, & Hotchkiss, 2009; Tao & Xu, 2008). In addition, structure analysis of AAP indicated that the backbone of AAP was mainly composed by glucose in the form of (1→3) linked with a few of (1→) linked, (1→4) linked, (1→6) linked and (1→4, 6) linked; the diameter and volume of AAP were small (average diameter: 30 nm, average volume: 665.2 nm^3). These observations revealed that the molecular structure of AAP was similar to the observations in previous research (Wu et al., 2010; Zhang et al., 1995).

Further, our results demonstrated that AAP had the significant antioxidant activity. As shown in Fig. 3, AAP was observed to have obvious scavenging activity against ABTS, DPPH, superoxide, and hydroxyl radicals. In addition, AAP effectively inhibited the lipid oxidation of egg yolk homogenate and exhibited strong reducing power. Compared with the previous study (Fan et al., 2006), AAP had better antioxidant ability. Previous studies had showed that structures of polysaccharides had closely relationship with their biological activity, especially for antioxidant activity (Chen, Luo, et al., 2008; Chen, Zhang, et al., 2008; Chen, Xie, et al., 2008; Yang, Du, Huang, Wan, & Li, 2002). It also has been reported that the molecular weight of polysaccharides was an important parameter influencing antioxidant activity and the lowest molecular weight showed the highest antioxidant activities (Chen, Luo, et al., 2008; Chen, Zhang, et al., 2008; Chen, Xie, et al., 2008; Wang et al., 2009). In present study, the remarkable antioxidant activity of AAP may

be attributed to its lower molecular weight. However, the exact mechanism of antioxidant activity of polysaccharides is still not fully understood.

In conclusion, results of the present study suggested that MAE had the possibility influence to the molecular weight of AAP, but no obvious impact to its structure. AAP has the remarkable in vitro antioxidant capacities and can be useful as a potential antioxidant agent applied in food systems. Further studies are undergoing for the possible mechanism of antioxidant activity of AAP and the toxicological evaluation for the safety usage of AAP used in food products.

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