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Characterization and biological activities of a novel polysaccharide isolated from raspberry (*Rubus idaeus* L.) fruits

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ABSTRACT

A water-soluble polysaccharide namely RCP-II from raspberry fruits was obtained by complex enzyme method followed by successive purification using macroporous resin D4020 and Sephadex G-100 columns. RCP-II was an acidic heteropolysaccharide and the characteristic structure of polysaccharide was determined. The carbohydrate of RCP-II was composed with galacturonic acid, rhamnose, arabinose, xylose, glucose and galactose in a molar ratio of 1.00:0.55:1.19:0.52:0.44:1.90 and the average molecular weight was estimated to be 4013 Da, based on dextran standards. RCP-II presented high scavenging activity toward DPPH•, HO•, $O_2^{\bullet-}$ in a concentration-dependent manner. The determination of the inhibitory activity on protein glycation showed that in 14 days of incubation the inhibitory ability of RCP-II was more effective on the development of non-enzymatic glycation reaction at early phase than that at the following two phases.

1. Introduction

In recent decades, it has been found that natural polysaccharides extracted from edible fruits possess various important biological activities, such as anti-cancer, antioxidant, immunological, hypolipidemic, anti-inflammatory and hypoglycaemic activities (Yang et al., 2006; Yang & Zhang, 2009; Cui et al., 2014; Dou et al., 2015). Therefore, increasing attention is being placed on extraction and characterization of new bioactive polysaccharides which may be applied to functional foods and medicine.

As a member of the 'berry family', raspberry (*Rubus idaeus* L.) is widely distributed in all temperate regions of Europe, North America and Asia (including the three provinces in Northeast China) (Chen, Xin, Zhang, & Yuan, 2013). The fruits of raspberry are subglobose and bright red when they are matured, and the majority of them are used in processed products such as juice, jam, wine and milk shake (Vladisavljević, Vukosavljević, & Veljović, 2013). Nowadays, raspberry gains most acceptance for its nutritive value, as they contain numerous bioactive compounds such as phenolics, ascorbic acids, minerals and anthocyanins (Kafkas, Özgen, Özoğul, & Türemis, 2008; Bobinaitė, Viškelis, & Venskutonis, 2012).

In recent years, many biological activities of raspberry have been found in many studies (Paredes-López, Cervantes-Ceja, Vigna-Pérez, & Hernández-Pérez, 2010; Maksimović, Milivojević, Poledica, Nikolić, & Maksimović, 2013), however, there are few reports on its polysaccharides, and the structure and function of these polysaccharides have never been well characterized. Our previous study demonstrated raspberry was also an excellent source of polysaccharides compared with other nutrients, and the yield of polysaccharides was 4.12% (counted with the weight of wet fruits) using complex enzyme method (Yu, Teng, Xu, & Li, 2014). In present study, a novel low-molecular-weight polysaccharide (RCP-II) from raspberry was isolated, and the structural characteristics were preliminary determined. The antioxidant activity and non-enzymatic

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glycation inhibition activity of RCP-II were also investigated to provide scientific evidence for further study.

2. Materials and methods

2.1. Materials

Raspberry (*R. idaeus* L.) fruits, with cultivar name Autumn bliss, were used in the experiment, which were obtained from the horticulture station of Northeast Agricultural University (Harbin, China) and stored at −18 °C until they were used. Before extraction, the frozen fruits were thawed at room temperature and then homogenised using a JJ-2 homogenizer (Changzhou Guohua Electric Appliance Co., Ltd, Jiangsu Province, China).

D4020 Macroporous Resin was purchased from NanKai University Chemical Plant (Tianjin, China). Sephadex G-100 and standard monosaccharides (D-glucose, D-galactose, D-rhamnose, D-mannose, D-arabinose and D-fructose) were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). T-series dextran standards (T-3, T-5, T-10, T-40 and T-70) were obtained from Beijing Baier Di Biotechnology Co., Ltd (Beijing, China). Ascorbic acid (Vc) and bovine serum albumin (BSA) were produced by Tianjin Regent Chemicals Ltd. (Tianjin, China) and Sino-American Biological Engineering Co., Ltd. (Henan Province, China), respectively. Pectinase (28.48 U/mg) and cellulase (13.83 U/mg) were obtained from Shanghai Lanji Biotechnology Co., Ltd (Shanghai, China). Papain (23.74 U/mg) was purchased from Beijing Obo Star Biotechnology Co., Ltd (Beijing, China). All other chemicals and reagents were of analytical grade.

2.2. Extraction and purification of polysaccharides

Polysaccharides from raspberry were extracted according to our previous study (Yu et al., 2014). Homogenised raspberry fruit was mixed with complex enzymes (pectinase, cellulase and papain; 2.5:1.7:2.1) at 2.6% concentration, then citric acid–disodium hydrogen phosphate buffer (pH = 4.0) was added at certain liquid to solid ratios (10:1 mL/g). The enzymolysis reactions were carried out in a shaking bath (55 °C) for 2.6 h. After centrifugation at 5000 rpm for 15 min, the supernatants were combined, concentrated by rotary evaporator to a proper volume, precipitated by the addition of ethanol to a final concentration of 85% (*v*/*v*) and then kept overnight at 4 °C. The precipitates collected by vacuum filtration through a 0.45 μm microporous membrane (Shanghai Wanzi Shiye Co., Ltd, Shanghai, China) were dissolved in distilled water, concentrated and lyophilized, and then extracted polysaccharides were obtained.

The extracted polysaccharide solution (3 mg/mL) was injected to the macroporous resin (D4020) column (2.0 cm × 30 cm) and eluted with deionized water at a flow rate of 1.2 mL/min. The eluate (5 mL/tube) was collected automatically and monitored by phenol-sulfuric acid method at 490 nm (Masuko et al., 2005). One major polysaccharide peak namely RCP was obtained, and then lyophilized. The fraction was further purified by Sephadex G-100 column (2.6 cm × 60 cm) eluted with distilled water at a flow rate of 0.3 mL/min (1 mL/tube) to yield peaks RCP-I and RCP-II using the method mentioned above. The main fraction RCP-II was applied in the subsequent studies.

2.3. Physical and chemical properties analysis

The morphology and color were observed directly. Solubility of RCP-II in water and ordinary organic solvents (acetone, chloroform and ethanol) were investigated.

Chemical properties of RCP-II were determined using the following methods: Coomassie brilliant blue reaction (Bradford, 1976), ninhydrin reaction, iodine–potassium iodide reaction, Fehling reagent reaction (Li, Zhou, Cai, & Zhang, 1999), carbazole–sulphuric acid reaction (Bitter & Muir, 1962), $FeCl_3$ reaction. In addition, the content of esterified carboxyl was determined by the titrimetric method (FCC, 1981).

2.4. Characterization of RCP-II

2.4.1. Determination of molecular weight

The molecular weight of RCP-II was measured by high performance liquid chromatography (HPLC, LC-10AVP, Shimadzu Corporation, Japan) with a refractive index detector (RID-10A). The sample was dissolved in ultrapure water (1 mg/mL) and filtered through a 0.45 μm membrane. Then 10 μL RCP-II solution was applied to a gel-filtration chromatographic column of Waters Ultra-hydroge 2000 (7.8 mm × 30 cm) and eluted with 0.1 mol/mL $NaNO_3$ at a flow rate of 1.0 mL/min. Standard dextrans (T-3, T-5, T-10, T-40 and T-70) were separate passed through the column and their retention time were plotted against the logarithms of their respective molecular weights. The molecular weight of RCP-II at a given retention time was calculated from the calibration equation generated with the standard curve.

2.4.2. Analysis of monosaccharide composition

Gas chromatography (GC, GC-2010, Shimadzu Corporation, Japan) equipped with a RTX-1701 silica capillary column (30.0 m × 0.25 mm × 0.25 μm) and a flame ionization detector (FID) was used to analyze the monosaccharide components. Sample RCP-II (30.0 mg) was hydrolyzed with 2.0 mL of trifluoroacetic acid solution (TFA, 2.0 mol/mL) in a sealed glass tube at 100 °C for 2 h. The solution was evaporated to dryness at 30 °C and dissolved in 3 mL of methanol. This procedure was repeated until the TFA and methanol were removed completely. Then the sample and all standard sugars (D-galacturonic acid, D-glucuronic acid, D-xylose, D-galactose, D-mannose, D-glucose, D-arabinose and D-rhamnose) were acetylated following a published method (Ma et al., 2014) and analyzed by GC under the temperature condition as follows: the column temperature was initially set at 180 °C, increased to 220 °C at the rate of 5 °C/min and was held at 220 °C for 5 min, then elevated to 280 °C at 10 °C/min and finally maintained for 20 min. The heater temperatures of detector and injector were 280 °C. Nitrogen was used as the carrier gas and maintained at 1.0 mL/min. The content was calculated by peak area internal standard with inositol taken as the internal standard.

2.4.3. UV and IR spectroscopy

UV spectrum of RCP-II was recorded with a double beam UV spectrophotometer (TU-1901, Beijing Purkinje General Instrument Co., Ltd, Beijing, China) in the wavelength range of 600–190 nm^{-1}. IR spectrum of the RCP-II was determined by a FT-IR spectrophotometer (FTS135, BID-BAD Co., USA) over the wave number range from 4000 to 500 cm^{-1} with KBr pellets.

2.4.4. NMR spectroscopy

20.0 mg RCP-II was dissolved in 2.0 mL D_2O (99.9%) and transferred into a 5 mm NMR-tube. NMR spectra (^1H NMR, ^{13}C NMR) were recorded by AVANCEIII NMR spectrometer (Bruker Corporation, Switzerland).

2.5. Antioxidant activities of RCP-II

2.5.1. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by Fenton reaction described by Zhong et al. (2013). Different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) of RCP-II solution (2.0 mL) were incubated with a reaction mixture containing $FeSO_4$

(8.0 mmol/L, 2.0 mL), salicylic acid–ethanol solution (8.0 mmol/L, 2.0 mL) and H_2O_2 (8.8 mmol/L, 2.0 mL) at 37 °C for 30 min. The absorbance of the solution was read at 510 nm by TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd, Beijing, China). Vc was used as the positive control and deionized water was used as the blank. The antioxidant activity on hydroxyl radical was calculated with following formula:

$$\text{Scavenging activity}\ (\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where A_0 is the absorbance of deionized water and A_1 is the absorbance of sample.

2.5.2. Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity was assayed by the method of Li and Shah (2014) with some modifications. Briefly, 5.0 mL of Tris–HCl buffer (50.0 mmol/L, pH = 8.2) and 1.0 mL of RCP-II sample (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) were mixed and stored for 20 min at 25 °C, then 0.3 mL of pyrogallol solution (3.0 mmol/L) was injected into the mixture. The absorbance was measured at 320 nm every 60 s for 5 min. Vc was used as the positive control. The superoxide anion radical scavenging activity was calculated by the following equation:

$$\text{Scavenging activity}\ (\%) = \frac{\Delta A_0 - \Delta A_1}{\Delta A_0} \times 100 \quad (2)$$

where ΔA_0 is the difference of absorbance values per 60 s for different concentrations of samples and ΔA_1 is the difference of absorbance values per 60 s for water without samples.

2.5.3. DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the modified method of Ye and Huang (2012). 2.0 mL of DPPH solution (0.3 mmol/L DPPH in methanol) was mixed with RCP-II solution (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL). After the mixtures were shaken and incubated at 25 °C for 30 min in the dark, the sample absorbance values were measured at 517 nm. Vc was used as the positive control at the same concentration. The scavenging activity was calculated by the following equation:

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

where A_0 is the absorbance of samples and A_1 is the absorbance of the DPPH solution without samples.

2.6. Non-enzymatic glycation inhibition activity

2.6.1. Glycation of BSA

The BSA/glucose model was employed to evaluate the ability of polysaccharide antiglycation (Wu, Hsieh, Wang, & Chen, 2009). The total 20 mL of reaction mixture contained BSA (20 mg/mL, 10 mL), glucose (500 mmol/L, 5 mL), and samples with different concentrations dissolved in phosphate buffer (200 mmol/L, pH = 7.4, 5 mL). The negative control only contained BSA or glucose under the same conditions. All the mixtures were incubated in the dark at 37 °C and 2.0 mL of the glycated materials were taken from the whole system for the following experiments in 0, 2, 4, 6, 8, 10, 12, 14th days.

2.6.2. Analyses of Amadori products

The Amadori products were determined by the method of nitro blue tetrazolium (NBT) reductive assay (Zhang, Wang, & Dong, 2011). The glycated material (0.5 mL) and NBT reagent (0.3 mmol/L, 2.0 mL) were added to the sodium carbonate buffer (100 mmol/L, 2.5 mL, pH = 10.35). Then the mixture was incubated at room temperature for 20 min, and absorbance of sample was read at 530 nm against the blank of phosphate buffer. The inhibition rate (IR) was calculated as follows:

$$\text{IR}\,(\%) = \left(1 - \frac{A - A_c - A_d}{A_a - A_b}\right) \times 100 \quad (4)$$

where A is the absorbance of complete glycated system; A_α is the absorbance of glycated material without samples; A_b is the absorbance of glycated material without samples and glucose; A_c and A_d are the absorbance of reaction mixture without BSA and glucose, respectively.

2.6.3. Analyses of dicarbonyl compounds

Dicarbonyl compounds were measured by Girard-T assay using spectrophotometric analyses (Zhang et al., 2011). Briefly, the glycated material (0.4 mL) and Girard-T stock solution (500 mmol/L, 0.2 mL) in sodium formate (500 mmol/L, 3.4 mL, pH = 2.9) were incubated at room temperature for 1 h. The absorbance was tested at 294 nm against a blank of sodium formate. Glyoxal was used as a standard in the drawing process of the calibration curve which was treated in a similar manner. IR was calculated as formula (4).

2.6.4. Analyses of AGEs

The glycated solution (0.5 mL) was diluted to 10 mL with sodium buffer (200 mmol/L, pH = 7.4). The fluorescence was determined at 370 nm of excitation wavelength and 440 nm of emission wavelength with LS45 spectrofluorometer (PerkinElmer Co., Ltd, Waltham, MA, USA). IR was calculated according to the following formula:

$$\text{IR}\,(\%) = \left(1 - \frac{F - F_c - F_d}{F_\alpha - F_b}\right) \times 100 \quad (5)$$

where F is the fluorescence of complete glycated material. F_α, F_b, F_c and F_d are the fluorescence of reaction mixture without samples, samples and glucose, BSA, glucose, respectively.

2.7. Statistical analysis

All presented data were expressed as mean ± standard deviation of three determinations, followed by analysis of statistical significance using SPSS version 12.0 software. Results for $p < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Extraction and purification

The extracted polysaccharides were obtained from raspberry by complex enzyme extraction, ethanol precipitation, and lyophilization. The yield of 4.09% (counted with the weight of wet fruits) was higher than the yield of 3.44% by hot water extraction (Wang & Xu, 2007). After purification by macroporous resin D4020, the polysaccharide of RCP was obtained and further purified by Sephadex G-100 column to yield RCP-I and RCP-II. The yield of RCP, RCP-I and RCP-II were 2.26%, 0.08% and 0.41%, respectively. Because of the low yield of RCP-I, only RCP-II was collected and used for further study. The total sugar content of RCP-II was determined to be 92.76% by phenol–sulfuric acid method and the content of uronic acid was 23.6% determined by carbazole–sulphuric acid method. According to the result of chemical titration, the content of esterified carboxyl was determined as 37.5%. In addition, RCP-II had no absorption peak at 260 nm, 280 nm and 520 nm in UV spectrum, which indicated that nucleic acid, protein and pigment were removed basically.

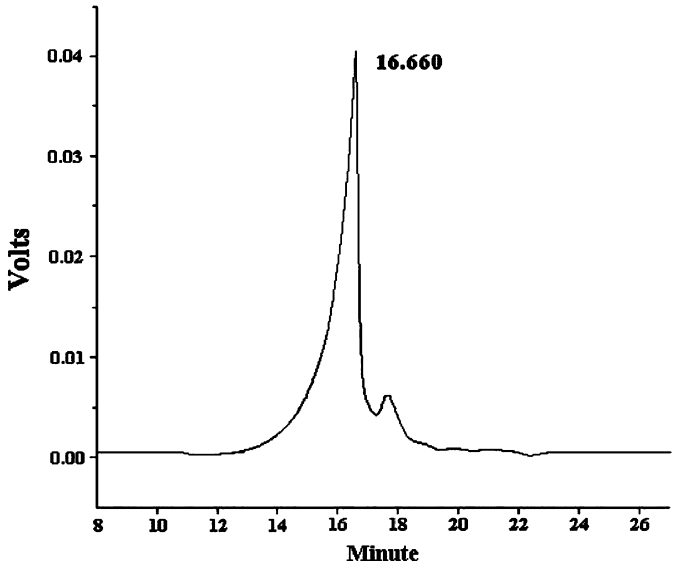

Fig. 1. HPLC elution profile of RCP-II.

3.2. Physicochemical property analysis

Freeze-dried RCP-II appeared as odorless white powder. RCP-II was soluble in water and practically insoluble in acetone, chloroform, and ethanol. It had negative response to ninhydrin and coomassie brilliant blue reactions, indicating no amino acids or protein remained in the powder. The absence of starch, phenols and reducing sugar in RCP-II was confirmed by iodine–potassium iodide, $FeCl_3$ and Fehling reagent reactions, respectively.

3.3. Molecular weight and monosaccharide composition of RCP-II

The elution peak of RCP-II was single, narrow and sharp in HPLC, with the feature of homogeneity (Fig. 1). According to the regression equation of the standard curve made by different dextran standards ($y = -0.475x + 11.517$, $R^2 = 0.9924$), the molecular weight of the purified RCP-II was calculated to be 4013 Da with retention time of 16.660 min.

The GC profile of RCP-II with acid hydrolysis and acetylation (Fig. 2) was obtained based on the retention time. According to the monosaccharide standards, RCP-II was composed of D-galacturonic acid, D-rhamnose, D-arabinose, D-xylose, D-glucose and D-galactose in a molar ratio of 1.00:0.55:1.19:0.52:0.44:1.90. These results indicated that RCP-II was an acidic heteropolysaccharide. By comparing with the reported data, monosaccharide composition of RCP-II was different from the polysaccharides in other berries, such as bilberry, black currant and wild raspberry (Hilz, Bakx, Schols, & Voragen, 2005; Fu et al., 2015).

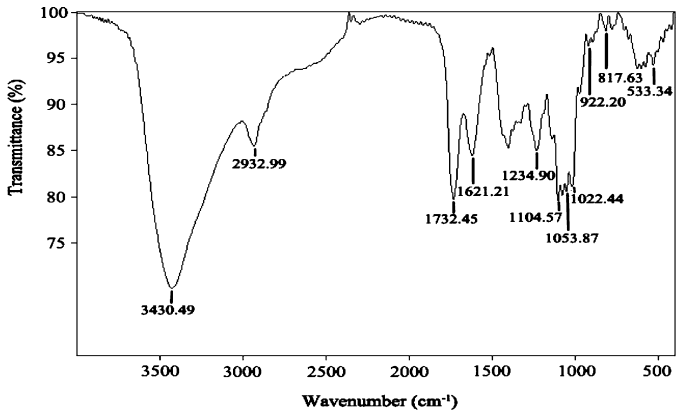

Fig. 3. IR spectrum of RCP-II.

3.4. IR spectra analysis of RCP-II

The FT-IR spectra of RCP-II ranging from 4000 to 400 cm^{-1} were presented in Fig. 3. The intense broad band around 3430 cm^{-1} and the weak absorption band at about 2932 cm^{-1} were due to O—H and C—H stretching vibration, respectively (Jiang et al., 2013). Furthermore, the absorption peaks of 1732 and 1621 cm^{-1} were attributed to the absorption of ester carbonyl (—COOR) groups and carboxylate ion stretching band (—COO—), respectively (Zha et al., 2014), which was consistent with the result of carbazole–sulphuric acid reaction. The peak at 1234 cm^{-1} was related to non-symmetrical C—O—C stretching vibration. Three absorption peaks within the range of 1100–1010 cm^{-1} suggested the possible presence of pyranoid ring in RCP-II (Li & Shah, 2014). In addition, the characteristic absorption peaks around 818 cm^{-1} and 922 cm^{-1} were ascribed to α-glycoside bond and β-glycoside bond, respectively (Li et al., 2014).

3.5. NMR spectroscopy analysis

The ^1H and ^{13}C NMR spectra of RCP-II were shown in Fig. 4a and b. The ^1H NMR spectrum was crowded in a narrow region ranging from 3 to 5 ppm which is typical signal of polysaccharides (Nep & Conway, 2010). As could be seen in Fig. 4a, the chemical shifts of C1 protons were higher or lower than 5.0 ppm, indicating the existence of both α- and β-configuration in RCP-II. The signals in δ95–101 ppm corresponded to α-anomeric carbons and the signals in δ101–105 ppm corresponded to β-anomeric carbons in ^{13}C NMR spectrum, which was consistent with the ^1H NMR spectrum (Huang, Li, Li, Wang, 2011). The signal at δ5.20 ppm in ^1H NMR spectrum was due to H_1 resonance of α-galactose residues and the signal at δ98.02 ppm in ^{13}C NMR spectra supported this deduction. The signal at δ4.11 ppm in ^1H NMR spectrum and δ101.43 ppm in ^{13}C NMR spectrum suggested the polysaccharide contained β-arabinose residues (Yu & Yang, 1999). The

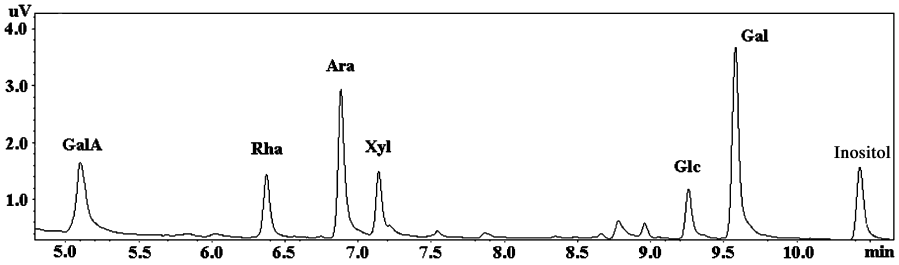

Fig. 2. GC profile of RCP-II with acid hydrolysis and acetylation.

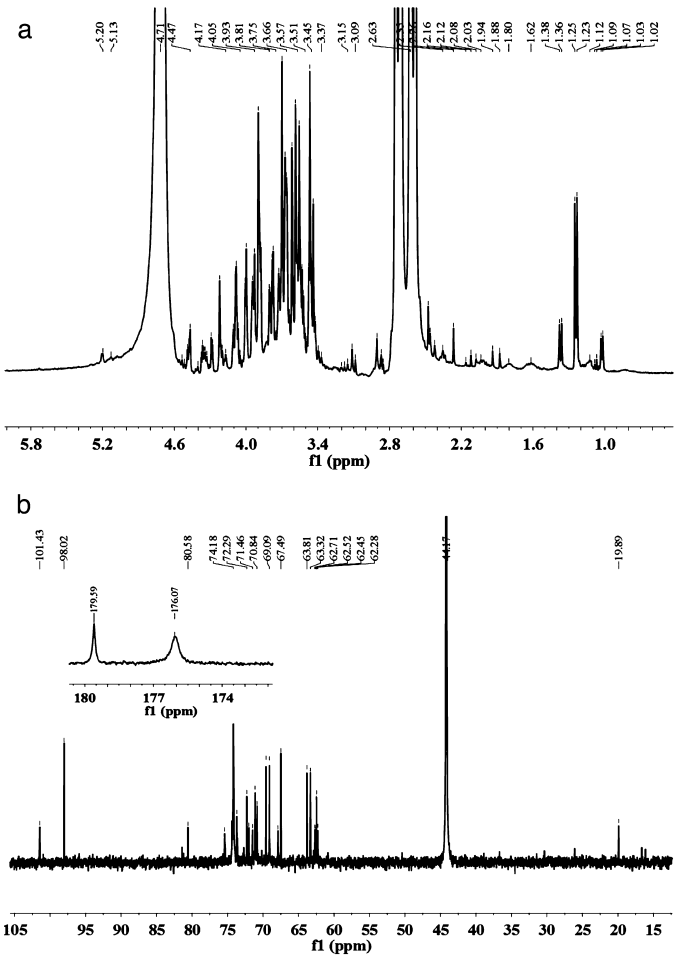

Fig. 4. (a) ^{1}H NMR spectrum of RCP-II, (b) ^{13}C NMR spectrum of RCP-II.

signals at 16–18 ppm were assigned to the C-6 of the rhamnose residues (Wang, Liu, & Fang, 2005). These results were also confirmed by GC analysis. Signals at 2.08 ppm (^{1}H NMR), correlating with carbon atoms at 19.89 ppm (^{13}C NMR) were due to the *O*-acetyl groups of galacturonic acid residues (Tamaki, Konishi, Fukuta, & Tako, 2008). Signals at δ179.59 and δ176.07 ppm (^{13}C NMR) corresponded to the C-6 of unesterified and esterified galacturonic acid units (Li, Ai, & Yang, 2013), which was consistent with the results of carbazole–sulphuric acid reaction and IR data.

3.6. Biological activities of RCP-II

3.6.1. Assay of antioxidant activity

Hydroxyl radical scavenging activities of RCP-II and Vc were determined and shown in Fig. 5a. The scavenging activity of RCP-II directly increased with the promoting concentration and had a significant difference ($p < 0.01$) within the range (0.2–1.0 mg/mL). The scavenging ability of RCP-II was lower than that of Vc at every concentration point. The IC_{50} values of RCP-II and Vc were 0.96 mg/mL and 0.63 mg/mL, respectively.

As could be seen in Fig. 5b, both RCP-II and Vc showed obvious scavenging activity on superoxide anion radicals. The scavenging activity of RCP-II was concentration-dependent, but Vc exhibited high scavenging activity (87.1 ± 1.13%) at low concentration (0.2 mg/mL). The maximum scavenging rate of RCP-II was 77.59 ± 1.30% at 1.0 mg/mL, whereas that of Vc was 97.52 ± 0.92% at the same concentration. The IC_{50} value of RCP-II was 0.49 mg/mL after the calculation.

As shown in Fig. 5c, the DPPH scavenging activity of RCP-II was increased rapidly within the test dosage range. At the concentration of 1.0 mg/mL, RCP-II showed scavenging rate of 40.01 ± 1.09%, which is close to that of Vc (42.14 ± 1.27%). This ability was lower than that of hydroxyl radicals (52.77 ± 0.53%) and superoxide anion radicals (77.59 ± 1.30%). On the basis of the results above, RCP-II showed the strongest scavenging activity against the superoxide anion radicals.

Published data indicate that some fruit polysaccharides also have strong antioxidant activities and could be developed into

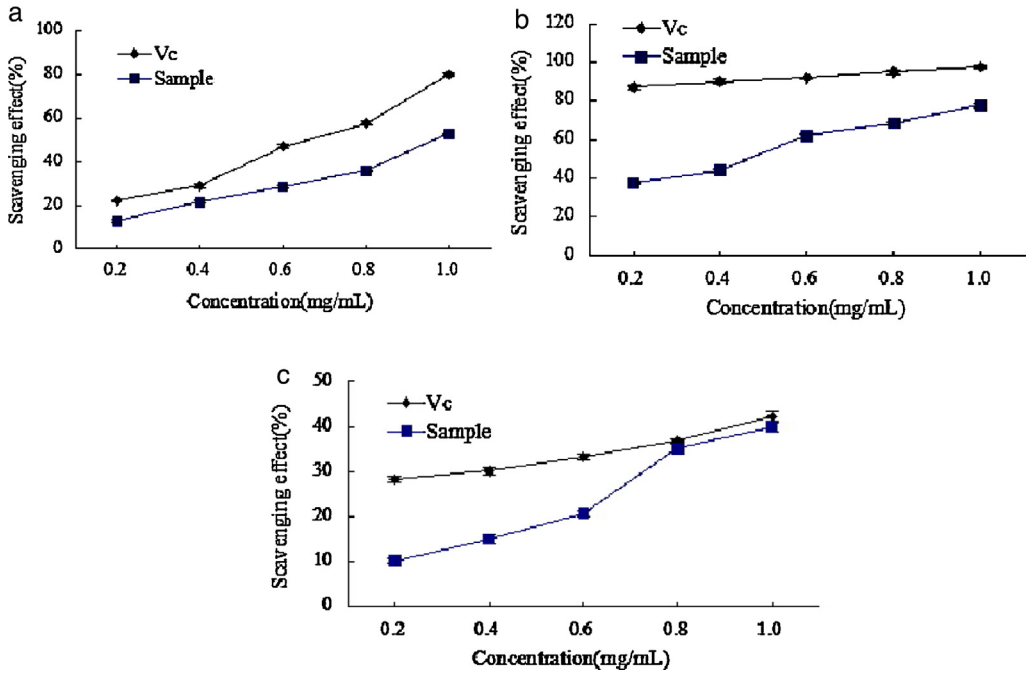

Fig. 5. Antioxidant effects of RCP-II: (a) hydroxyl radical scavenging activity, (b) superoxide anion radical scavenging activity, (c) DPPH radical scavenging activity.

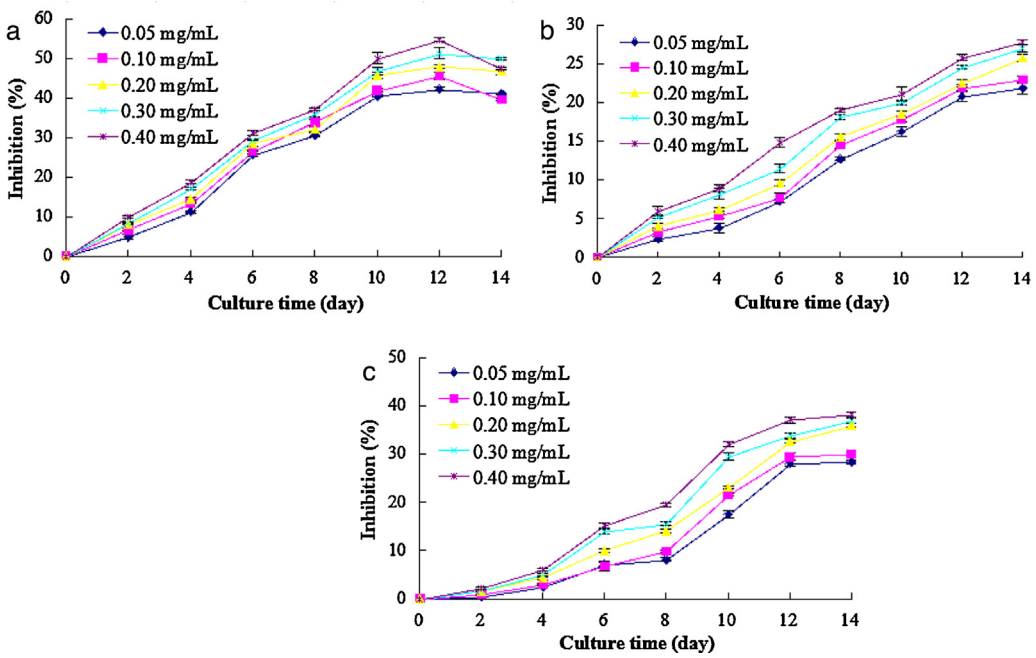

Fig. 6. Inhibitory effects of RCP-II on protein nonenzymatic glycation: (a) Inhibit Amadori product formation, (b) inhibit dicarbonyl compound formation, (c) inhibit AGEs formation.

health food (Yang & Zhang, 2009). Compared to the antioxidant activities of polysaccharides (TYAP-1, TYAP-2 and TYAP-3) from thinned-young apple, RCP-II had more prominent scavenging activity on DPPH•, HO•, $O_2^{\bullet-}$ (Dou et al., 2015). But at the same tested concentrations, the scavenging effects of WFPs from wolfberry fruits against these three radicals were slightly higher than RCP-II (He, Yang, Jiao, Tian, & Zhao, 2012). The activity differential between the polysaccharides may be due to their different structure, such as monosaccharide composition, molecular weight and the content of uronic acid. In the future, RCP-II might be expected to play a promising role as dietary free radical scavengers for oxidative damage prevention.

3.6.2. Non-enzymatic glycation inhibition activity

Schiff base and Amadori products are formed during the early stages of non-enzymatic glycation of proteins. Amadori products can reduce NBT in alkaline solution to yield a coloured reactant with a maximum absorption at 530 nm (Baker, Zyzak, Thorpe, & Baynes, 1994). As shown in Fig. 6a, the inhibitory activities of different dosages of RCP-II increased with the culture proceeding from 0 to 12 days and then decreased slowly. RCP-II showed the highest inhibitory ability of 54.68 ± 0.49% under the concentration of 0.40 mg/mL at the culture time of 12 day. Moreover, the inhibitory rate had a significant difference ($p < 0.01$) in the tested concentrations (0.05–0.40 mg/mL) of RCP-II.

Many studies had showed that dicarbonyl compounds can yield stable AGEs by inducing cross-linking of protein (Chen, Liu, Liu, & Zhao, 2012). According to our research (Fig. 6b), the inhibitory rate of different dosage increased gradually from 0 day to 14 days of incubation. The peak inhibition of 27.84 ± 1.26% was observed in the group treated with 0.4 mg/mL RCP-II at the culture time of 14 day.

Fluorescence intensity can also be used to measure the degree of non-enzymatic glycation system and the inhibition activity of samples (Zhang et al., 2011). The result was showed in Fig. 6c, the inhibition increased slowly during the first 4 days and then increased faster significantly and became flat after 12 days. The inhibition increased significantly ($p < 0.01$) from 0.05 to 0.40 mg/mL at the culture time of 14 day and the maximal inhibitory rate was 38.04 ± 0.54%.

Contrasting the inhibition of non-enzymatic glycation of proteins in these three phases, the IC_{50} values of RCP-II were 0.26, 1.63 and 0.77 mg/mL, respectively. These results indicated that early phase was more effective than the following two phase. It was reported that NBT reduction could also be caused by superoxide radical anion that formed by glucose autoxidation (Wolff & Dean, 1987). Based on antioxidant experimental results, RCP-II could be used as effective superoxide anion radical scavengers according to its strong scavenging ability. Thus, these results showed that the inhibitory effects of RCP-II on non-enzymatic protein glycation may associate with its antioxidative activity.

4. Conclusion

A novel water-soluble polysaccharide named RCP-II was isolated from raspberry fruits, which was an acidic heteropolysaccharide mainly composed of galacturonic acid, rhamnose, arabinose, xylose, glucose and galactose in a molar ratio of 1.00:0.55:1.19:0.52:0.44:1.90. RCP-II was a low-molecular-weight polysaccharide and the average molecular weight was 4013 Da. The characteristic absorptive peaks of polysaccharide structure were determined by IR and the presence of α-galactose and β-arabinose residues were confirmed by NMR analysis. RCP-II was proved to have certain antioxidant capability and non-enzymatic glycation inhibition activity. All of these findings provide a scientific basis for the further use of polysaccharides from raspberry fruits.

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