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Purification, antioxidant and moisture-preserving activities of polysaccharides from papaya

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ABSTRACT

Polysaccharides extracted from papaya are a group of heteropolysaccharides, and their antioxidant activities and moisture-preserving activities were investigated employing various established in vitro systems. Available data obtained with in vitro models suggested that among the three samples, the second fraction (named after P2) showed significant inhibitory effects on superoxide, hydroxyl and DPPH radical; its reducing power was also the strongest. Data also reveal that P2 has strong in vitro moisture absorption and retention capacities as compared to hyaluronic acid and glycerol. These results clearly establish the possibility that polysaccharides extracted from papaya could be effectively employed as a type of natural moisturizer. However, comprehensive studies need to be conducted in experimental animal models.

1. Introduction

It has been recognized that polysaccharides from microorganisms or higher plants possess bioactivities, and the antioxidant activities are most deeply studied. Antioxidant activity means that some antioxidants can protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. ROS have been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age (Patel, Cornwell, & Darley-Usmar, 1999). Thus, it is essential to develop and utilize effective antioxidants which can protect the human body from free radicals and retard the progress of many chronic diseases (Nandita & Rajini, 2004). In our previous reports, the polysaccharides extracted from seaweed and plants have been found to possess antioxidant activities (Zhang et al., 2009). In addition, polysaccharides are generally strongly hydrophilic, and will contribute to moisture absorption and retention. There have been several reports about polysaccharides with moisture-preserving activities (Li et al., 2011).

The papaya (from Carib via Spanish), papaw or pawpaw is the fruit of the plant *Carica papaya*, in the genus *Carica*. Papayas can be used as a food, a cooking aid, and in medicine. The stem and bark are also used in rope production. The juice has an in vitro antiproliferative effect on liver cancer cells, probably due to its component of lycopene or immune system stimulation (Rahmat, Rosli, Zain, Endrini, & Sani, 2002). Papaya seed could be used as an antibacterial agent for *Escherichia coli*, *Staphylococcus aureus* or *Salmonella typhi*, although further research is needed before advocating large-scale therapy (Yismaw, Tessema, Mulu, & Tiruneh, 2008). Papaya seed extract may be nephroprotective (protect the kidneys) in toxicity-induced kidney failure (Olagunju et al., 2009). However, few studies have dealt with papaya polysaccharides. The structure of acidic polysaccharides, present as contaminants in papain, has been investigated by methylation analysis (BeMiller & Dikko, 1986). The sugar composition and degree of esterification has been determined (Voragen, Timmers, Linssen, Schols, & Pilnik, 1983) for an aqueous ethanol-insoluble fraction of papaya fruit. Biswas, Mukherjee, and Rao (1969) reported that (1 → 3) and (1 → 4)-linked galacturonans as well as a galactan were present in a water-soluble fraction isolated from unripe papaya fruit. The aim of the present investigation was to fractionate and elucidate the chemical composition of water-soluble polysaccharides in the edible part of papaya fruit, and to evaluate the effect of polysaccharides from papaya by comparing their antioxidant and moisture-preserving activities and chemical characteristics.

2. Experimental

2.1. Materials

Papaya (*C. papaya* L. cv. Waimanalo) was collected in Huzhou city, China. The pulp of the fruit was sliced and extracted by

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sequential reflux with 80% aqueous (v/v) ethanol and chloroform to remove lipids. The residue was dried stored for use.

Butylated hydroxyanisole (BHA), Nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), nicotinamide adenine dinucleotide-reduced (NADH), 1-phenyl-3-methyl-5-pyrazolone (PMP), ethylene diamine tetraacetic acid (EDTA), ferrozine, trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. Ascorbic acid, sodium citrate and other reagents were of analytical grade. Dialysis membranes were produced by Spectrum Co., and molecular weight was cut off at 3600 Da.

2.2. Analytical methods

Total sugar content was determined by phenol–sulfuric acid method using D-glucose as standard (Dubois, Gillis, Hamilton, Rebers, & Smith, 1956). Uronic acid was estimated in a modified carbazole method using D-glucuronic acid as standard (Bitter & Muir, 1962). The protein was determined by Bradford assay as previously described (Pirie, 1987). Infrared spectra were measured by a Nicolet Magna-Avatar 360 with KBr disks.

Molecular weight of all samples was determined by HP-GPC on a Waters 515 GPC system at 35 °C, where 0.7% Na_2SO_4 solution was used as mobile phase with a flow rate of 0.5 mL/min. TSK G3000 column (300 mm × 7.8 mm) and 2140 refractive index detector was used. A series of different molecular weight dextrans purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China) were used as standard.

2.3. Neutral sugar analysis

2.3.1. Hydrolysis of polysaccharide

Polysaccharide sample (15.0–20.0 mg) was dissolved in 2 M trifluoroacetic acid (2.0 mL) in a 10 mL ampoule. The ampoule was sealed in a nitrogen atmosphere and incubated for 4 h at 110 °C. Following incubation, the ampoule was cooled to room temperature. The reaction mixture was then neutralized to pH 7 with 2 M sodium hydroxide, and we added 2.0 mL of the internal standard solution. The mixture was shaken well, diluted to 10 mL and filtered. The filtrate was retained for PMP derivatization.

2.3.2. Derivatization with PMP

0.5 M methanolic solution of PMP (100 μL) and 0.3 M aqueous sodium hydroxide (100 μL) were added to the monosaccharide reference solution or a reducing polysaccharide solution (100 μL each). The mixture was incubated at 70 °C for 30 min. The reaction mixture was then cooled at 8 °C, and neutralized with 0.3 M hydrochloric acid. We then added 1 mL of chloroform to the solution. The mixture was shaken well and centrifuged at 5000 r/min for 10 min at 6–8 °C. The chloroform layer was discarded and the aqueous layer was extracted twice with chloroform. The final aqueous layer was analyzed directly by HPLC.

2.3.3. Chromatography

Chromatographic conditions were generally as follows: column, YMC-Pack ODS-AQ (250 mm × 4.6 mm, 5 μm); temperature, 25 °C; solvent A, 0.4% triethylamine in 20 mM ammonium acetate buffer solution (pH 6.30 with acetic acid)–acetonitrile (9:1); solvent B, 0.4% triethylamine in 20 mM ammonium acetate buffer solution (pH 6.30 with acetic acid)–acetonitrile (4:6); gradient, 10–14% in 9 min, 14–64% from 9 min to 30 min, 64% during the next 5 min at 1 mL/min. The eluate was monitored at 245 nm.

2.4. Preparation of natural polysaccharide

2.4.1. Extraction of polysaccharides

The extracted residue was extracted at 90 °C for 3 h with 15 portions of water. The hot aqueous solution was separated from the residues by successive filtration through gauze and siliceous earth. The solution was dialyzed against running tap water for 48 h and then against distilled water for 24 h, and the solution was concentrated under reduced pressure. The concentrated extract was lyophilized to give crude polysaccharide (P) as a white powder.

2.4.2. Fractionation of polysaccharides

The crude polysaccharide was dissolved in water and fractionated by chromatography on a column (4.8 cm × 60 cm) of DEAE-Sepharose which was pretreated and equilibrated with distilled water for 24 h. The dissolved sample in was filtered through a 0.22 μm membrane filter then applied on to the column coupled to a peristaltic pump. The column was first eluted with distilled water at a flow rate of 1.0 mL/min followed by NaCl solution (0–2 mol/L). Fractions of 10 mL were collected using an automatic fraction collector and monitored for the presence of polysaccharides using the phenol–sulfuric acid assay. The elution profile was drawn according with the number of test-tube (*X*-axis) as well as the absorbance (*Y*-axis). Fractions containing polysaccharides from the elution step were collected; vacuum condensed, dialyzed and lyophilized.

2.5. Antioxidant activity

2.5.1. Superoxide radical assay

The superoxide radical-scavenging abilities of all the samples were assessed by the method of Nishimiki, Rao, and Yagi (1972). In this experiment, superoxide anion radicals were generated in 4.5 mL of Tris–HCl buffer (16 mM, pH 8.0) containing 0.5 mL of NBT (300 μM) solution, 0.5 mL of NADH (468 μM) solution and one sample (0.5–50.0 μg/mL). The reaction was started by adding 0.5 mL of PMS (60 μM) solution to the mixture. The reaction mixture was incubated at room temperature for 5 min and the absorbance was read at 560 nm by a spectrophotometer against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide radical-scavenging activity. The capability of scavenging the superoxide anion radicals was calculated using the following equation:

$$\text{scavenging effect}\,(\%) = \left(1 - \frac{\text{Asample 560}}{\text{Acontrol 560}}\right) \times 100$$

where Acontrol 560 is the absorbance of the control (Tris–HCl buffer, instead of sample).

2.5.2. Hydroxyl radical assay

The reaction mixture, containing all the samples (0.6–7.0 mg/mL), was incubated with EDTA-Fe^{2+} (2 mM), saffron (360 μg/mL), and H_2O_2 (3%) in potassium phosphate buffer (150 mM, pH 7.4), was incubated for 30 min at 37 °C (Wang et al., 1994). The absorbance was read at 520 nm against a blank. Hydroxyl radical bleached the saffron, so decreased absorbance of the reaction mixture indicated a decrease in hydroxyl radical-scavenging ability. The capability of scavenging hydroxyl radical was calculated using the following equation,

$$\text{scavenging effect}\,(\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

where A_0 is the absorbance of the control (without samples) and A_1 is the absorbance of the mixture containing samples.

2.5.3. DPPH radical-scavenging assay

The effect of porphyrans on scavenging DPPH-radical was studied employing the modified method described earlier by Yamaguchi, Takamura, Matoba, and Terao (1998). Briefly, 1 mL of DPPH solution (0.1 mM, in 50% ethanol solution) was incubated with varying concentrations of the sample. The reaction mixture was shaken well and incubated for 20 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{Scavenging effect}\,(\%) = \left(\frac{1 - \text{Asample}\,517}{\text{Acontrol}\,517}\right) \times 100$$

2.5.4. Reducing power assay

The reducing power was determined as described previously by Yen and Chen (1995). Briefly, 1.0 mL different concentrations of samples (0.47–6.0 mg/mL) in phosphate buffer (0.2 M, pH 6.6) was mixed with 1.0 mL potassium ferricyanide (1%, w/v), and was incubated at 50 °C for 20 min. Afterwards, 2.0 mL trichloroacetic acid (10%, w/v) was added to the mixture to terminate the reaction. Then the solution was mixed with 1.2 mL ferric chloride (0.1%, w/v) and the absorbance was measured at 700 nm. Increased absorbance of reaction mixture indicated increased reducing power.

2.6. Evaluation of moisture-preserving activities

2.6.1. Moisture absorption activity

All the samples (polysaccharides, glycerol and hyaluronic acid) were ground into fine powder and oven-dried at 100 °C for 4 h. Then they were placed in a sealed humidity chamber maintained by saturated K_2CO_3 (43% relative humidity; RH) and saturated $(NH_4)_2SO_4$ (81% RH) at 25 °C for indicated times. The weights of all the samples were obtained after 0 h, 4 h, 24 h, 48 h, 72 h and 96 h. The moisture absorption rate (Ra) of a sample was evaluated by the gain of weight:

$$\text{Ra}\,(\%) = \frac{W_t - W_0}{W_0} \times 100$$

where W_0 was the weight of an oven-dried sample and W_t was the weight of the sample after moisture absorption for a specific time in the humidity chamber.

2.6.2. Moisture retention activity

The oven-dried samples (100 mg each) were added in distilled water and then placed in a humidification chamber dried silica gel containing saturated K_2CO_3 (43% RH) to dehydrate at 25 °C for the indicated times. The moisture retention rate (Rr) was evaluated by weight loss of the water after 0 h, 4 h, 24 h, 48 h, 72 h and 96 h:

$$\text{Rr}\,(\%) = \frac{W_t}{W_0} \times 100$$

where W_0 was the weight of added distilled water and W_t was the weight of the water after a specific time.

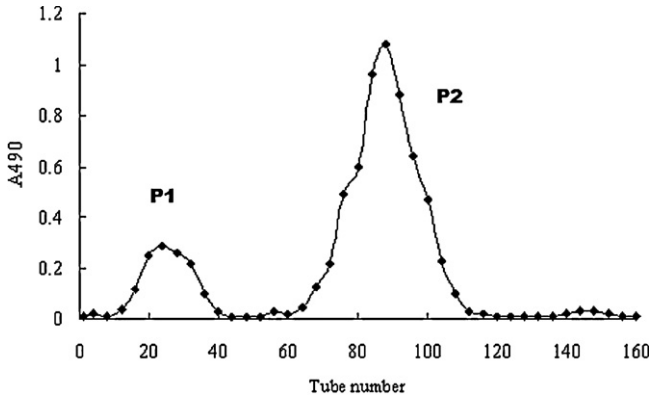

Fig. 1. Purification of P by DEAE-Sepharose column chromatography.

2.7. Statistical analysis

Results were expressed as the mean ± standard deviation of triplicate analysis. Statistical comparisons were performed using Student's *t*-test. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Chemical analysis

Crude polysaccharides (P) were further fractionated by a DEAE-Sepharose column chromatography eluted with water and NaCl solution (0–2 mol/L) to yield two peaks, P1 and P2 (Fig. 1). From the figure, P2 was the predominant peak.

The chemical compositions of three natural polysaccharides were shown in Table 1. The result showed that the main chemical components of these samples are total sugar and protein, along with uronic acid.

Neutral monosaccharide constitutions of the polysaccharides were analyzed by HPLC. Results showed that galactose and arabinose were the main sugar form in all the samples. In addition, the more content of monosaccharides was rhamnose. The other common monosaccharides were also seen in these samples, which show that chemical property may have great influence on antioxidant activities.

The FT-IR spectra of the products are shown in Fig. 2. Infrared spectroscopy analysis indicated that all the three samples showed typical peaks of polysaccharide. The peaks at 1640–1650 cm^{-1} are caused by the bending vibration C–O of uronic acids. Signals at 3420–3450 and 1050–1070 cm^{-1} correspond to stretching vibration of O–H and C–O, respectively.

3.2. Superoxide radical assay

The superoxide radical is a highly toxic species that is generated by numerous biological and photochemical reactions (Banerjee, Dasgupta, & De, 2005). Fig. 3(a) showed that the inhibitory effect of all samples on superoxide radicals was marked and concentration

Table 1
Yield and chemical composition of the samples (% (w/w) of dry weight).

Samples	Yield (%)	Total sugar (%)	Uronic acids (%)	MW (×10^6 Da)	Neutral sugar (mole ratio)[a]					
					Gal	Glc	Ara	Rha	Xyl	Man
P	–	83.4	8.20	2.65	42.5	<0.1	41.2	15.4	<0.1	<0.1
P1	4.70	86.7	5.45	2.51	45.3	9.34	30.9	11.7	3.28	1.51
P2	23.14	76.9	10.92	2.54	52.0	11.0	21.6	10.2	<0.1	3.04

[a] Gal, galactose; Glc, glucose; Ara, arabinose; Rha, rhamnose; Xyl, xylose; Man, mannose.

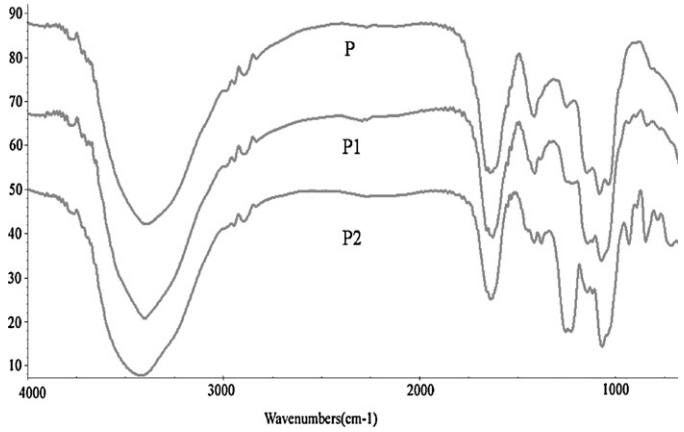

Fig. 2. IR spectra of the crude polysaccharide and the isolated P1 and P2 fractions.

related. Significant scavenging of superoxide radical was evident at all the tested concentrations of all products. Results show that EC_{50} of P, P1 and P2 were 1.1, 3.8 and 2.4 μg/mL. When the concentration was over 5 μg/mL, the increase of scavenging abilities of all the samples was slower. At the higher concentration (40 μg/mL), the fraction P2 showed less superoxide radical scavenging activity than BHA (97%), but were much better than P and P1 (69% and 63%).

It was reported that addition of electron-withdrawing groups to the pyrrole enhanced antioxidant activity (Yanagimoto, Lee, Ochi, & Shibamoto, 2002). Although superoxide has been a relatively weak oxidant, it decomposed to form stronger, reactive oxidative species, such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids. Furthermore, superoxides were also known to indirectly initiate lipid peroxidation as a result of H_2O_2 formation, creating precursors of hydroxyl radicals (Duh, Du, & Yen, 1999). Based on this, the antioxidant activities of all the samples were also related to their ability to scavenge superoxide radical.

3.3. Hydroxyl radical assay

The hydroxyl radical, known to be generated through the Fenton reaction in this system, was scavenged by polysaccharide samples. The scavenging effect of all samples was shown in Fig. 3(b). For all the samples, the effects of scavenging hydroxyl radicals were in a concentration-dependent manner. With regard to the EC_{50} values, the fraction P (6.41 ± 0.43 mg/mL) was a considerably less effective ($p < 0.05$) hydroxyl radical scavenger compared to P1(2.38 ± 0.16 mg/mL) and P2 (0.98 ± 0.41 mg/mL).

For hydroxyl radical, there were two types of antioxidation mechanism; one suppresses the generation of the hydroxyl radical, and the other scavenges the hydroxyl radicals generated. In the former, the antioxidant activity may ligate to the metal ions which react with H_2O_2 to give the metal complexes. The metal complexes thus formed cannot further react with H_2O_2 to give hydroxyl radicals (Ueda, Saito, Shimazu, & Ozawa, 1996). Iron, a transition metal, is capable of generating free radicals from peroxides by the Fenton reaction and is implicated in many diseases. Fe^{2+} has also been shown to produce oxyradicals and lipid peroxidation, and reduction of Fe^{2+} concentrations in the Fenton reaction would protect against oxidative damage. In the present study, all the samples contain the high content of total sugar, which may be the main factor on the scavenging activities on the hydroxyl radical. The mechanism on the hydroxyl radicals needs to be further investigation.

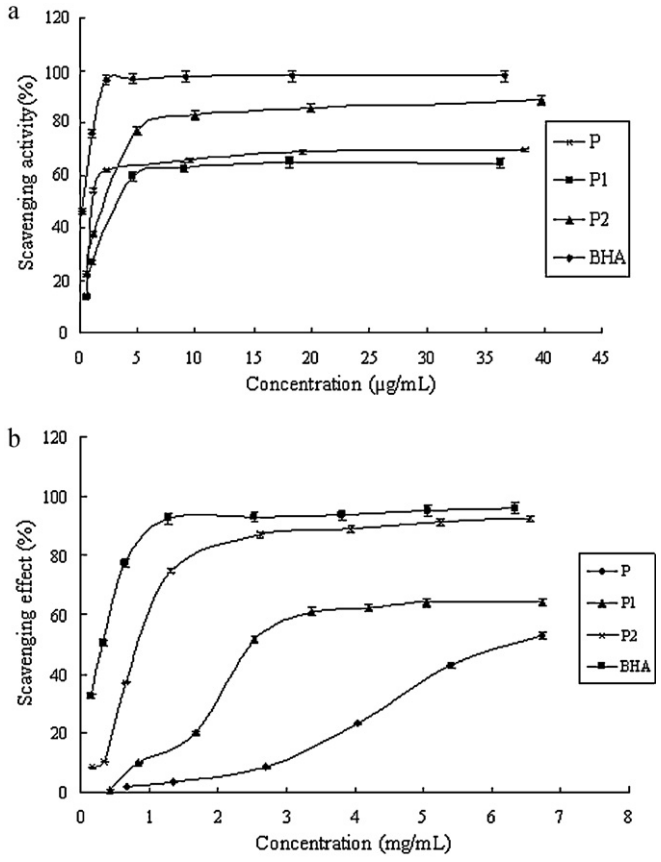

Fig. 3. Scavenging effects of the samples on superoxide radical (a) and hydroxyl radical (b). Values are means ±S.D. ($n = 3$).

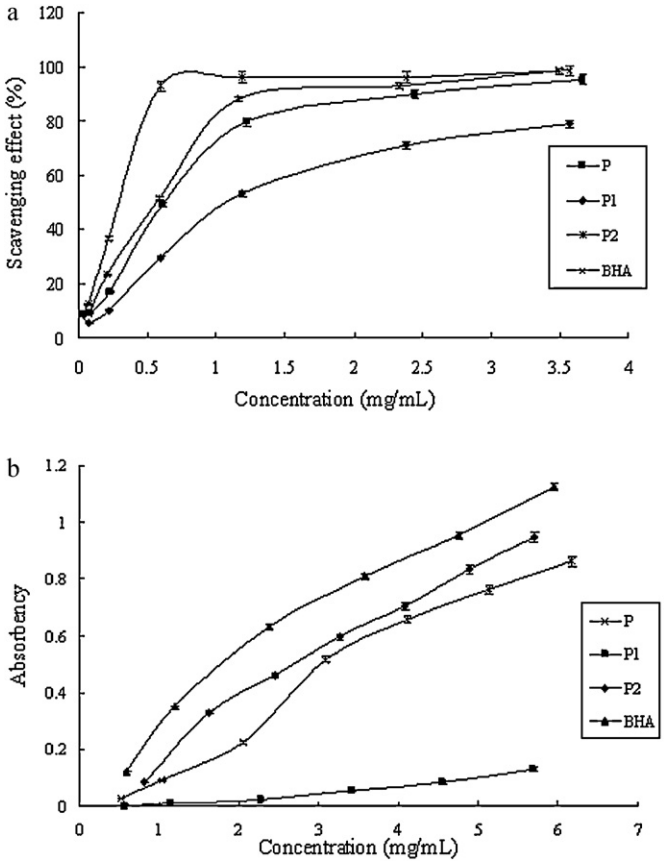

Fig. 4. Scavenging effects of the samples on DPPH radical (a) and reducing power (b). Values are means ±S.D. ($n = 3$).

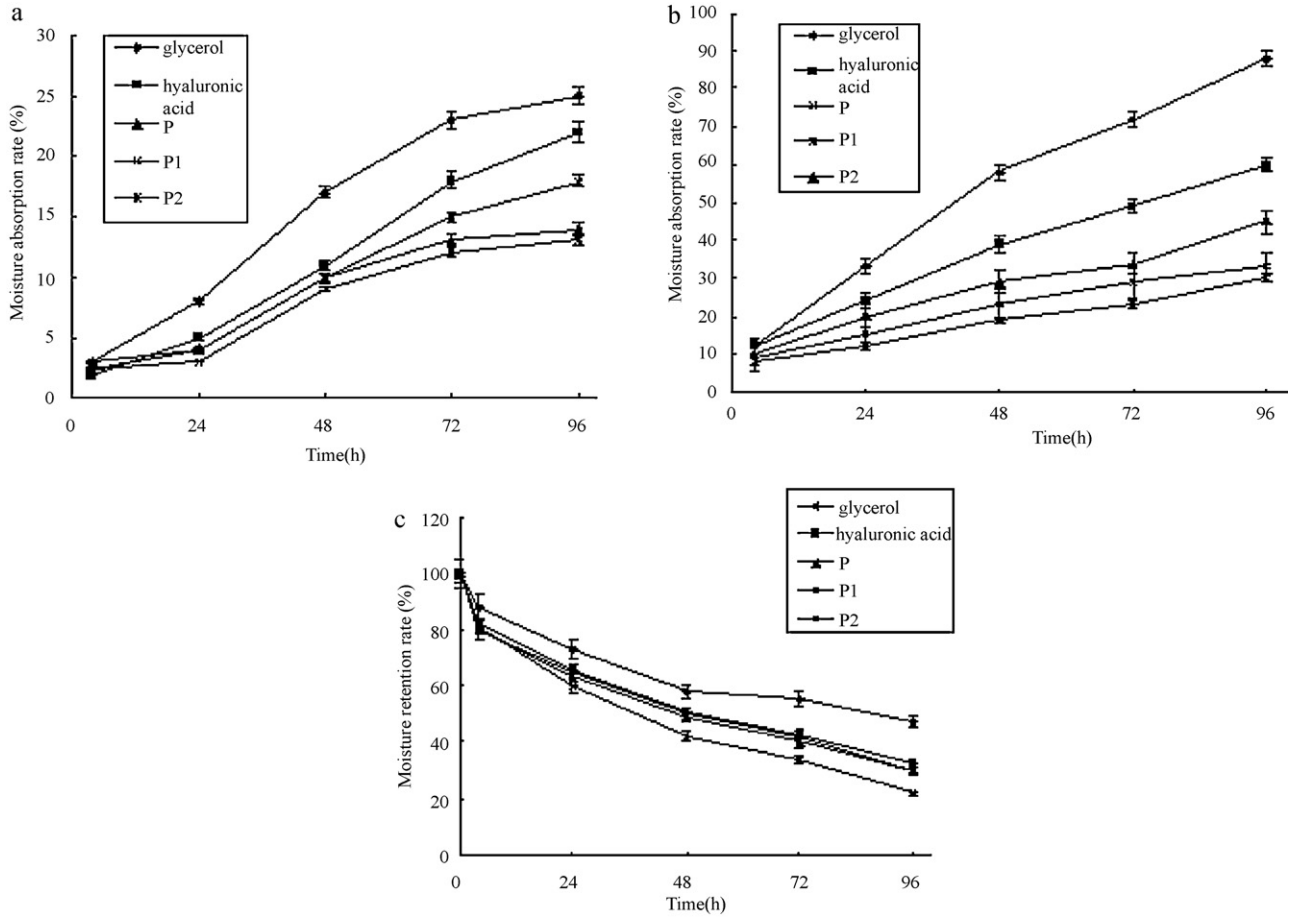

Fig. 5. Moisture absorption by samples at RH = 43% (a) and 81% (b) and moisture retention by samples at RH = 43% (c).

3.4. DPPH radical-scavenging assay

The extracts showed maximum hydrogen-donating ability in the presence of DPPH stable radicals at high concentrations. As shown in Fig. 4(a), the scavenging activities of the extracts on DPPH radicals were similar to the results of the scavenging activities on superoxide radicals. The scavenging activity of the fraction P1 was ($p < 0.05$) lower ($8.59 \pm 0.93\%$) at 0.023 mg/mL and, at a concentration of 3.57 mg/mL, reached a plateau of $78.5 \pm 0.36\%$. The DPPH scavenging activities of the extracts, expressed as an EC_{50} value, ranged from 0.48, 0.62 to 1.07 mg/mL. P and P2 exhibited the strongest antioxidant activity than P1 at the high concentration.

These results suggested that the fractions P and P2 contained the strongest free radical scavenging compounds. So, they can be used as effective DPPH free radical scavengers.

3.5. Reducing power assay

In the reducing power assay, the presence of antioxidants in the extracts results in the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form. Fig. 4(b) shows the extent of the reduction, in terms of absorbance values at 700 nm, for the extracts ranging in concentration from 0.5 to 7 mg/mL. As shown in the figure, the reducing power of the samples correlated well with increasing concentrations except that of P1, which showed extremely weaker reducing power even at higher concentration. It has been previously reported that there was a direct correlation between antioxidant activities and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductant, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductant is also reported to react with certain precursors of peroxide, thus preventing peroxide formation. In this assay, P2 contain the more content of uronic acid, which resulted in the increase of the reducing power.

3.6. Moisture absorption and retention properties

The in vitro moisture absorption and retention properties of polysaccharide were examined gravimetrically and compared with those of hyaluronic acid and glycerol, which are frequently used as hygroscopic and humectant agents (Shi et al., 2010). As shown in Fig. 5(a), the moisture absorption rate (Ra) of all the samples at 43% RH increased in the first 12 h, and the Ra of polysaccharides increased much fast. After exposed to 43% RH for 96 h, the Ra of P2 (18.2%) was much higher than that of P (14.3%) and P1 (13.8%). The Ra of all the samples at 81% RH was higher than that at 43% RH, and continued to increase until 96 h; the Ra of P, P1 and P2 at 96 h was 45.0%, 33.2% and 30.6%, respectively (Fig. 5(b)).

To evaluate the moisture-retention properties, all the samples were placed in a water-humidified chamber to absorb moisture for 96 h at 43% RH. As shown in Fig. 5(c), water was lost gradually in all the samples but much slower in glycerol than in other samples. After test for 96 h at 43% RH, the moisture retention rate (Rr) of P and P2 was 30.5% and 30.6% respectively, which were slightly lower than hyaluronic acid (32.1%). Together these results demonstrate the strong moisture-retention capacity of papaya polysaccharide as compared to hyaluronic acid and glycerol.

The moisture content is very important to the skin health, and so the guarantees of the water moisturizer have been one of the

skin care cosmetics most main research subjects. The hydroxyl, the carboxyl group and other polar group in the polysaccharide may form the hydrogen bond with the H_2O to unify the massive moisture contents, and simultaneously, the polysaccharide chain can also mutually interweaves to the lattice, which plays very strongly a role in guarantying the moisture content. Based on this mechanism, the polysaccharides from papaya could be useful as moisturizing agents in future.

The results of the present work indicated that all samples possessed antioxidant activities and moisture absorption and retention properties in certain assays. Of the three samples, P2 had the strong radical scavenging effect in four systems of assay. The reason was possible that P2 has the higher total sugar content, the higher uronic acid content and the lower molecular weight. These results were consistent with our previous reports (Zhang et al., 2009). On the other hand, we reveal that the P2 has strong in vitro moisture absorption and retention capacities as compared to hyaluronic acid and glycerol. P2 may have a use as a possible supplement in the food and pharmaceutical industries. The radical scavenging effect was stable at high temperatures so that these samples may be used as resources of medicine. However, factors effecting and attributing to radical scavenging effect and moisture absorption and retention capacities need to be further studied.

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References

Banerjee, A., Dasgupta, N. & De, B. (2005). In vitro study of antioxidant activity of *Syzygium cumini* fruit. *Food Chemistry*, *90*, 727–733.

BeMiller, J. N. & Dikko, S. B. (1986). Structural analysis of papaya polysaccharide II. *Carbohydrate Research*, *158*, 173–181.

Biswas, A. B., Mukherjee, A. K. & Rao, C. V. N. (1969). Pectic acid from the pulp of unripe papaya (*Carica papaya*) fruit. *Indian Journal of Chemistry*, *7*, 588–591.

Bitter, T. & Muir, H. M. (1962). A modified uronic acid carbazole reaction. *Analytical Biochemistry*, *4*, 330–334.

Dubois, M., Gillis, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Duh, P. D., Du, P. C. & Yen, G. G. (1999). Action of methanolic extract of mung bean hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. *Food and Chemical Toxicology*, *37*, 1055–1061.

Gordon, M. H. (1990). The mechanism of antioxidant action in vitro. In B. J. F. Hudson (Ed.), *Food antioxidants* (pp. 1–18). London/New York: Elsevier Applied Science.

Li, H. F., Xu, J., Liu, Y. M., Ai, S. B., Qin, F., Li, Z. W., et al. (2011). Antioxidant and moisture-retention activities of the polysaccharide from *Nostoc commune*. *Carbohydrate Polymers*, *83*, 1821–1827.

Nandita, S. & Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, *85*, 611–616.

Nishimiki, M., Rao, N. A. & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*, *46*, 849–854.

Olagunju, J. A., Adeneye, A. A., Fagbohunka, B. S., Bisuga, N. A., Ketiku, A. O., Benebo, A. S., et al. (2009). Nephroprotective activities of the aqueous seed extract of *Carica papaya* Linn. In carbon tetrachloride induced renal injured Wistar rats: A dose- and time-dependent study. *Biology and Medicine*, *1*, 11–19.

Patel, R. P., Cornwell, T. & Darley-Usmar, V. M. (1999). The biochemistry of nitric oxide and peroxynitrite: Implications for mitochondrial function. In E. Cadenas, & L. Packer (Eds.), *Understanding the process of ageing: The roles of mitochondria, free radicals, and antioxidants* (pp. 39–40). New York: Academic Press.

Pirie, N. W. (1987). *Leaf protein and its by products in human and animal nutrition* (pp. 28–55). Cambridge: Cambridge University Press.

Rahmat, A., Rosli, R., Zain, W. M., Endrini, S. & Sani, H. A. (2002). Antiproliferative activity of pure lycopene compared to both extracted lycopene and juices from watermelon (*Citrullus vulgaris*) and papaya (*Carica papaya*) on human breast and liver cancer cell lines. *Journal of Medical Sciences*, *2*, 55–58.

Shi, X. L., Zhang, J. J., Song, H. F., Wang, J. J., Zhang, Z. S. & Zhang, Q. B. (2010). Polysaccharides from *Enteromorpha linza*: Purification and moisture-preserving activity. *Marine Science*, *37*, 81–85.

Ueda, j.-i., Saito, N., Shimazu, Y. & Ozawa, T. (1996). Oxidative DNA strand scission induced by copper (II)-complexes and ascorbic acid. *Archives of Biochemistry and Biophysics*, *333*, 377–384.

Voragen, E. G., Timmers, J. P. J., Linssen, J. P. H., Schols, H. A. & Pilnik, W. (1983). Methods of analysis for cell-wall polysaccharides of fruit and vegetables. *Zeitschrift fur Lebensmittel-Untersuchung und -Forschung*, *177*, 251–256.

Wang, J. C., Xing, G. S., Hu, W. F., Zhu, T. L., Wang, Q. & Zhao, H. (1994). Effects of Ge-132 on oxygen free radicals and lipid peroxidation induced by hydroxyl free radical in vitro. *Journal of Chinese Pharmaceutical Sciences*, *29*, 23–25.

Yamaguchi, T., Takamura, H., Matoba, T. & Terao, J. (1998). HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Bioscience, Biotechnology, and Biochemistry*, *62*, 1201–1204.

Yanagimoto, K., Lee, K. G., Ochi, H. & Shibamoto, T. (2002). Antioxidative activity of heterocyclic compounds found in coffee volatiles produced by Maillard reaction. *Journal of Agricultural and Food Chemistry*, *50*, 5480–5484.

Yen, G. C. & Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry*, *43*, 27–32.

Yismaw, G., Tessema, B., Mulu, A. & Tiruneh, M. (2008). The in vitro assessment of antibacterial effect of papaya seed extract against bacterial pathogens isolated from urine, wound and stool source. *Ethiopian Medical Journal*, *46*, 71–77.

Zhang, Z. S., Zhang, Q. B., Wang, J., Shi, X. L., Song, H. F. & Zhang, J. J. (2009). In vitro antioxidant activities of acetylated, phosphorylated and benzoylated derivatives of porphyran extracted from *Porphyra haitanensis*. *Carbohydrate Polymers*, *78*, 449–453.