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Research Article

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Promising polysaccharide fractions from jujube (*Ziziphus Jujuba* Mill.) fruit

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ABSTRACT

Jujube, the most important economic fruit for edible-medicinal use, is an important member of the Chinese herbs. Jujube polysaccharides are the most promising components in its fruit. As apart of a continuing study on the biological activities of jujube, we have researched on the fractionation of the polysaccharides from jujube by sequential extraction. Jujube polysaccharides were sequentially extracted with water at 22 °C (fraction 1 (F1)) and 60 °C (F2), and with 0.1 M HCl (F3) and 2 M KOH (F4) at 37 °C. Soluble fractions (16.8% yield) were composed of neutral sugars, uronic acids, and small amounts of protein. Galactose and arabinose are the main constituents of jujube polysaccharides. F3 showed the highest potential to be antioxidant, followed by F4 and F2. Jujube polysaccharides could be used as potential natural antioxidants in food industry.

Keywords: Jujube, Sequentially extraction, Polysaccharides, FT-IR, Antioxidant activity

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INTRUDUCTION

Epidemiological studies have shown that intake of fruits and vegetables is related with reduced risk of chronic diseases. In consequence, increased consumption of products from fruit which are rich in dietary fibre and phytochemical constituents has been recommended [1,2]. Therefore, various natural products are receiving continuous attention as antioxidants. Polysaccharides and their fractions with polyhyroxyl groups are a promising group of antioxidative compounds [3,4].

Jujube, the most important economic fruit for edible-medicinal use, is an important member of the Chinese herbs. Jujube belongs to the Rhamnaceae family, and is widely distributed in the temperate and subtropical areas of the North Hemisphere, especially the inland region of North China [5]. Traditionally, it is a popular fruit in many eastern Asian countries. In addition, it has been applied in Traditional Chinese Medicine as a home remedy for anorexia, lassitude, and loose stools in deficiency syndromes of the spleen and of hysteria in women for 4000 years [6]. In recent years, many researches on jujubes have reported its health-promoting effects, including anticancer effects [7-9], immune stimulating activity [10], hepatoprotective effects [11,12], gastrointestinal protective effects [9], anti-inflammatory action [13], and antioxidant properties [14-16]. Polysaccharides are one of the main components of jujube and exhibit many biological activities including anti-proliferation capability on melanoma cell [17], immune stimulating effects [18] and antioxidant activity [19].

Thus as apart of a continuing study on the biological activities of jujube, we have researched on the fractionation of the polysaccharides from jujube by sequential extraction. The chemical composition and structural features from FT-IR spectra, along with the antioxidant activities of the polysaccharide fractions *in vitro* are reported.

EXPERIMENTAL SECTION

2.1. Sequential extraction of jujube polysaccharides

The procedure was based on the different solubilities of the polysaccharides from jujube. Jujube (80 g) was extracted

with distilled water (500 mL) under constant stirring at 22 °C for 1 h (fraction 1 (F1)) and, again, with water (500 mL) at 60 °C for 1 h (F2), to remove the bulk of the water-soluble polysaccharides. The water-insoluble residue was then sequentially extracted with 0.1 M HCl (500 mL) (F3) and with 2M KOH (500 mL) (F4), each treatment at 37 °C for 16 h. The insoluble residue was washed with 2 M HCl (100 mL) and distilled water until neutral pH, and then dialyzed against running tap water (7 L/h) for 48 h and then freeze-dried (F5: 7.1752 g). Each soluble fraction (F1 to F4) was filtered through a No. 3 sintered glass funnel (9.5 cm diameter) under reduced pressure. Next, the pH of the acid and alkali extracts was adjusted to 5.5, either with 2 M KOH or with concentrated HCl, as appropriate, and all of the extracts were dialyzed against water for 48 h (molecular weight cutoff 12-14 kDa). The dialyzed solutions were concentrated at 40 °C under reduced pressure and kept at -20 °C for the further analysis. Each supernatant solution (F1 to F4) was freeze-dried for the further chemical analyses, and the infrared (IR). Recoveries of the soluble fractions (g): F1, 0.4738; F2, 1.1008; F3, 1.5280; F4, 3.1479.

2.2. Chemical Analysis of Polysaccharide Fractions

Protein. Total nitrogen in jujube polysaccharide samples was determined according to the method of Bradford [20]. Uronic acids were determined by Blumenkrantz et al [21].

Carbohydrate analysis. The four soluble fractions from the sequential extraction were hydrolyzed with 1 M H_2SO_4 (100 ^oC, 90 min), whereas the final residue was pre-treated with 12 M H_2SO_4 (30 ^oC, 1 h), followed by hydrolysis with 1 M H_2SO_4 (100 ^oC, 90 min) and the neutral sugars and uronic acids released in the five fractions were analyzed. Neutral sugars composition was determined by gas chromatography (GC) [22]. A Shimadzu gas chromatograph model GC-2014C was used. The column was a DB-17 capillary fused silica, 30 m×0.25 mm i.d., 0.25 μm film thickness. The injector, and detector temperatures were 250 ^oC, and 280 ^oC, respectively. The whole process applied the following temperature programme: 130 ^oC -160 ^oC，30 ^oC /min, 0 min; 160 ^oC -180 ^oC,10 ^oC /min，2 min; 180 ^oC -210 ^oC，3 ^oC /min，2 min; 210 ^oC -220 ^oC，1 ^oC /min，4 min.

2.3. Fourier transform infrared spectroscopy (FT-IR)

Jujube polysaccharide fractions were incorporated into KBr and pressed into a 1 mm disk. FT-IR spectra of jujube samples were obtained at a phase resolution of 4 cm^{-1} and averaging 32 scans/min. Spectra were recorded in the transmittance mode from 4000 to 400 cm^{-1} using a Vetex 70 Fourier transform infrared spectrometer [22]. Five replicate spectra were obtained for each jujube polysacchrides fraction.

2.4. *In vitro* antioxidant activity

2.4.1. Preparation of the extracts

Each polysaccharide soluble fraction (0.2 g) was placed in a centrifuge tube; 30 mL of water were added, and the tube was constantly and thoroughly shaken for 1 h at room temperature. Next, it was centrifuged (2500 × g, 10 min), and the supernatant was recovered. The triplicate extracts were produced to measure the *in vitro* antioxidant activity [22].

2.4.2. DPPH radical scavenging activity

DPPH radical scavenging capacity was estimated according to He et al. with slight modification [23]. One milliliter of diluted jujube polysacchrides extract was mixed with 1 mL of DPPH solution, the mixture was kept in the dark for 30 min and the absorbance at 517 nm was determined.

2.4.3. Reduction power

The reducing power of jujube polysacchrides sample was determined according to the reported method [24]. Jujube polysacchrides samples (1 mL) were mixed with 2 mL of 0.2 mol/L phosphate buffer (pH 6.6) and 2 mL of 1% potassium ferricyanide. After the above mixture was incubated at 50 ^oC for 20 min, 2 mL of 10% trichloroacetic acid was added. Then, 2 mL of the reaction mixture was added to the test tubes. Next, 0.4 mL of 0.1% ferric chloride and 2 mL of distilled water were added and then vortexed. After 30 min, absorbance of the mixtures was determined at 700 nm, and the vitamin C equivalent was calculated using a standard curve. Results were expressed as milligrams of vitamin C equivalent antioxidant capacity per milligrams (mg vitamin C eq./mg).

2.5. Statistical analysis

Results are expressed as mean values ± standard deviation (SD). Comparison of means of at least three determinations, using a significance level of $P < 0.05$, was performed by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

3.1. Sequential extraction and chemical analyses of jujube polysaccharide fractions

The sequential extraction process was based on the different solubilities of polysaccharides from jujube. The yield,

uronic acids and protein contents of polysaccharide fractions from jujube are shown in Table 1. Total recovery (16.8%) corresponded to nondialyzable compounds, as free minerals and low-molecular-weight substances were removed during exhaustive dialysis of the fractions. Recovery of soluble fractions (F1-F4) amounted to 7.8% of the jujube dry weight. F3 showed the highest uronic acids content. The uronic acids and protein contents in the insoluble residue (F5) were quite low, which could be related to the high values of lignin associated to the insoluble dietary fiber of jujube fruits.

Table 1. Yields, and the contents of uronic acids of and protein of polysaccharide fractions from jujube (g/100 g dry weight)

Fractions	Yield （%）	Uronic acids	Protein
F1	0.6	1.82 ± 0.01 d	2.47 ± 0.01 b
F2	1.4	2.01 ± 0.01 c	1.45 ± 0.02 c
F3	1.9	2.85 ± 0.02 a	0.23 ± 0.02 d
F4	3.9	2.56 ± 0.1 b	4.36 ± 0.01 a
F5	9.0	Trace	Trace

Value of three determinations±SD; values within the same column followed by different superscript symbols are significantly different (P≤0.05); F1, soluble in water at 22 °C; F2, soluble in water at 60 °C; F3, soluble in 0.1 M HCl at 37 °C; F4, soluble in 2 M KOH at 37 °C; F5, insoluble residue.

Table 2. Neutral sugars determined by GC (g/100 g dry weight) in hydrolyzed fractions of jujube

Neutral sugars	F1	F2	F3	F4	F5
Rhamnose	0.39 ± 0.01 c	0.34 ± 0.01 d	0.98 ± 0.03 a	0.67 ± 0.02 b	0.70 ± 0.01 b
Arabinose	2.96 ± 0.08 c	3.02 ± 0.13 c	13.43 ± 0.81 a	8.12 ± 0.29 b	13.53 ± 0.41 a
Xylose	0.27 ± 0.01 d	0.33 ± 0.01 d	1.04 ± 0.09 c	10.39 ± 0.71 a	2.35 ± 0.17 b
Mannose	0.48 ± 0.01c	1.28 ± 0.09 b	1.37 ± 0.11 b	2.25 ± 0.10 a	0.59 ± 0.01 c
Glucose	0.63 ± 0.07d	2.28 ± 0.11 b	1.65 ± 0.08 c	2.62 ± 0.08 a	0.75 ± 0.08 d
Galactose	9.63 ± 0.35c	6.21 ± 0.23 d	21.12 ± 1.29 a	14.46 ± 0.63 b	20.94 ± 1.69 a
Total sugar（%）	14.36	13.46	39.59	38.51	38.86

Mean value of three determinations±SD; values within the same line followed by different symbols are significantly different (P≤0.05); F1, soluble in water at 22 °C; F2, soluble in water at 60 °C; F3, soluble in 0.1 M HCl at 37 °C; F4, soluble in 2 M KOH at 37 °C; F5, insoluble residue.

Table 3. Antioxidant power values of soluble polysaccharide fractions from jujube

Fractions	DPPH（%）	Reducing power （mg Vc eq./100 g DW）
F1	36.6 ± 0.4 c	413.9 ± 2.9 d
F2	43.0 ± 0.6 b	435.4 ± 1.9 c
F3	47.7 ± 0.1 a	541.9 ± 4.1 a
F4	43.3 ± 1.1 b	453.3 ± 5.9 b

Value of three determinations±SD; values within the same column followed by different superscript symbols are significantly different (P≤0.05); F1, soluble in water at 22 °C; F2, soluble in water at 60 °C; F3, soluble in 0.1 M HCl at 37 °C; F4, soluble in 2 M KOH at 37 °C; F5, insoluble residue.

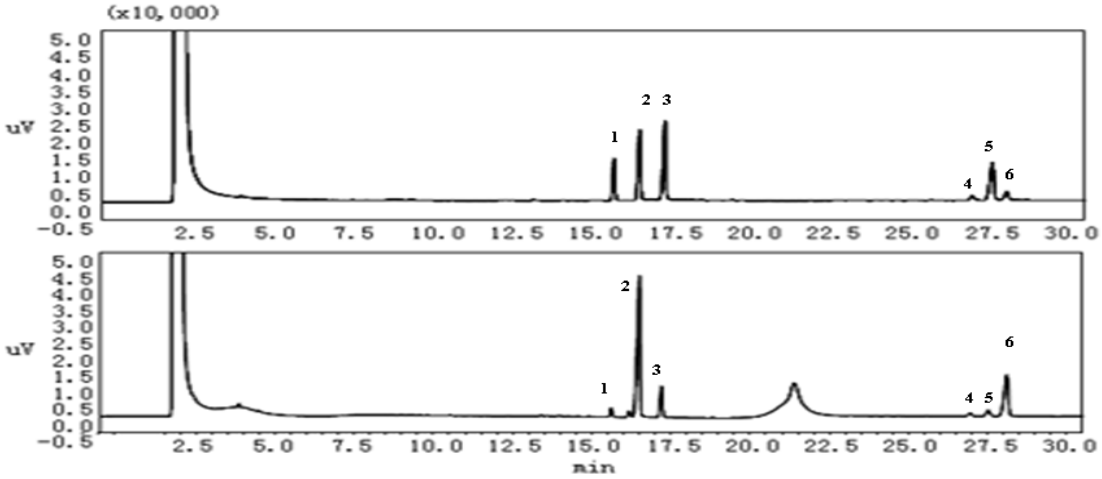

Figure 1. GC chromatogram of the monosaccharide standards and jujube sample. The peaks represent : (1) rhamnose, (2) arabinose, (3) xylose, (4) mannose, (5) glucose, and (6) galactose

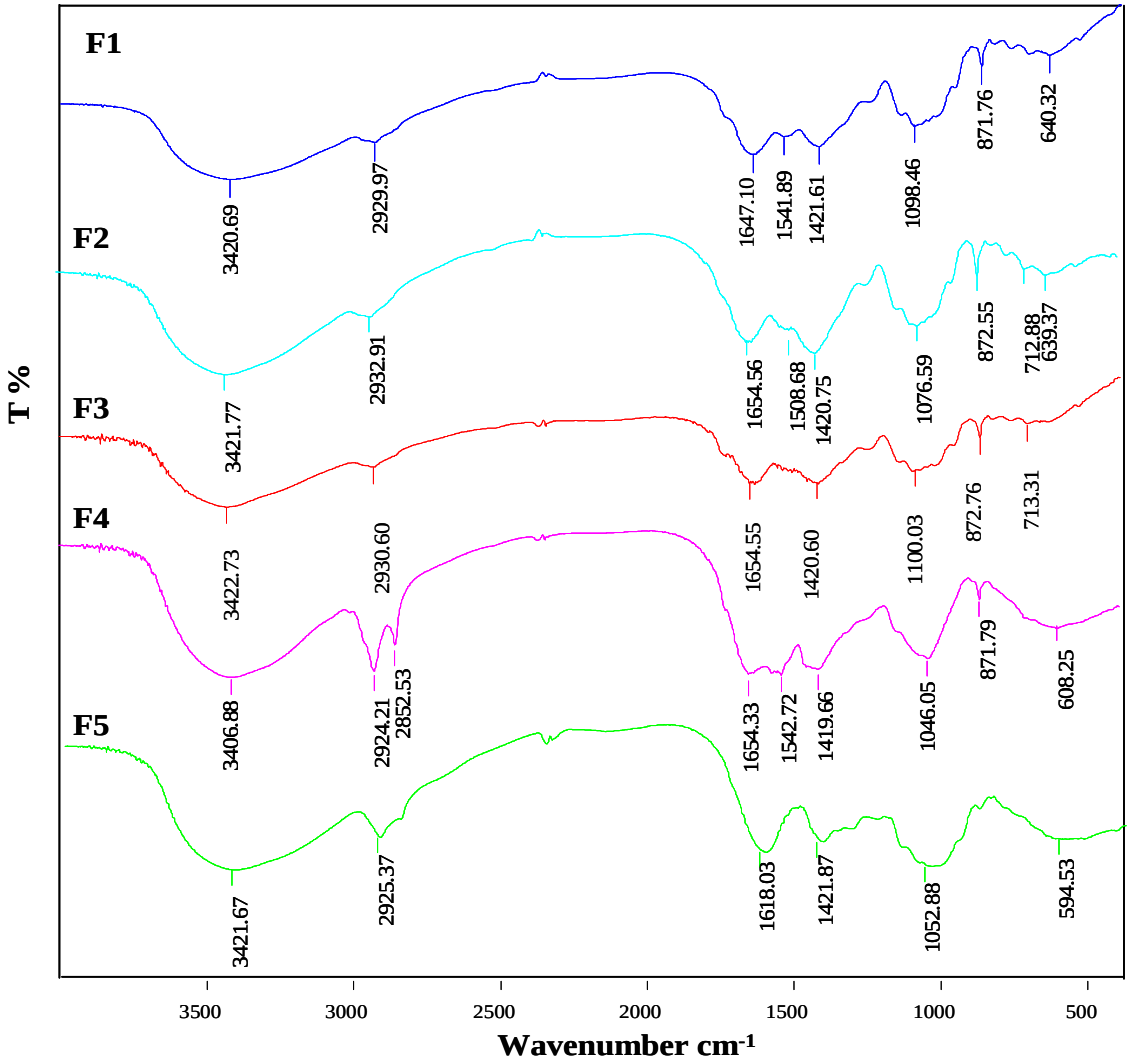

Figure 2. Infrared spectra of polysaccharide fractions from jujube fruit. F1, soluble in water at 22 °C; F2, soluble in water at 60 °C; F3, soluble in 0.1 M HCl at 37 °C; F4, soluble in 2 M KOH at 37 °C; F5, insoluble residue

Neutral sugars determined by GC in partially and completely hydrolyzed fractions of the polysacchrides are shown in Table 2. All the fractions contained rhamnose, arabinose, xylose, mannose, glucose and galactose (Figure 1). Galactose and arabinose are the main constituents of jujube polysacchrides. The acid-soluble fraction (F3) was mainly composed of arabinose (13.43 g/100 g) and galactose (21.12 g/100 g) as well as small amounts of xylose. The general composition of jujube depended on their extraction sequence [25]. The monosaccharide contents in the five fractions were different, for the variety in branching degree and even the difference in the main chain. Jujube polysacchrides with uronic acid and amounts of arabinose and galactose, which suggested the main type of jujube polysacchrides is pectic polysaccharide. In addition, jujube polysaccharides also contain a certain amount of xylose and glucose, which give us an idear of the existence of hemicellulose component in them, such as xyloglucan.

3.2. Fourier transform infrared spectroscopy

For more detailed information of the polysaccharides present in jujube and its cell-wall fractions the acquisition of FT-IR spectra in the region 3750–400 cm^{-1} was performed (Figure 2). With mid-infrared FT-IR spectroscopy, we can monitor the main functional groups and glycosidic linkages present in different polysaccharide fractions obtained from plant cell-walls [26], which represents an important improvement in comparison with the traditional chemical – instrumental methods [25].

All the fractions showed a broadly stretched intense peak around 3500–3400 cm^{-1} (sample concentration broads this absorption band and moves it to 3400 cm^{-1}), and a weak band around 2930 cm^{-1}, which is the characteristic absorption of hydroxyl groups, and the C–H bond, respectively in the polysaccharide molecule [27,28].In addition, some weak bands from 1400 to 1200 cm^{-1} were also the characteristic absorptions of a polysaccharide from FT-IR [27]. The amide band at 1650 cm^{-1}, is proposed for identification of proteins by IR spectroscopy [26]. The amide II band at 1540 cm^{-1} indicates the presence of secondary amides, which are also related to the presence of proteins [29].

Bands around 1618.03~1654.56 cm^{-1} and 1508.68~1542.72 cm^{-1}. Mateos-Aparicio et al. [30] suggested the present of protein in jujube polysacchrides. In our work, the absorption bands at 1650 and 1540 cm^{-1} due to proteins found in all the fractions, although with different relative intensity. The presence of FT-IR absorption bands at 2924.21~2932.91 cm^{-1} suggested the prevalence of the β-glycosidic linkage in the fractions tested [26]. The presence of FT-IR absorption bands at 1419.66~1421.61 cm^{-1} suggested the present of the uronic acid. A large absorption band at 1425 cm^{-1} in all soluble fractions was due to uronic acids, in agreement with the certain content of uronic acid in these fractions. The fractions tested showed specific α-D-glucopyranoside bands in the 871.76~872.76 cm^{-1} region.

3.3. Potential antioxidant activity of soluble polysaccharide fractions

In the past, it has been found that the plant polysaccharides are not only energy resources but also play important biological roles in many life processes. The bioactivities of polysaccharides can be affected by several factors including chemical components, molecular weight, structure, conformation, even the extraction and isolation procedures [28]. Ethanolic extracts from jujube exhibited DPPH radical scavenging activity and reducing power (Table 3). The antioxidant activity of different polysaccharide fractions correlates directly to the increasing sulfate group content or to the decreasing molecular weight of the polysaccharides [22]. The jujube polysacchrides extracts presented a similar change in the trend of antioxidant activity. According to Rupérez et al. [22], differences in antioxidant abilities of the soluble fractions might be related to molecular mass. Moreover, anionic groups in jujube polysacchrides would display a significant antioxidant effect. Different molecular mass and neutral sugar composition of jujube polysacchrides would also play a role inhibiting human LDL oxidation *in vitro* [22].

CONCLUSION

Jujube polysacchrides contained rhamnose, arabinose, xylose, mannose, glucose and galactose. Galactose and arabinose are the main constituents. On the basis of FT-IR spectra of the different polysaccharide fractions obtained from the sequential extraction of jujube fruit, a strong link between structural proteins, β-glycosidic linkage and α-D-glucopyranoside networks in this fruit was suggested. F3 exhibited antioxidant potential. Nevertheless, at present, the mechanisms of the polysaccharides extracted by sequential method from jujube exert their antioxidant activity are still unknown. In this sense, it is of great interest to have available highly purified and well characterized jujube polysaccharides with which to elucidate their mode of action. Antioxidant activity may arise not only from phenolics, but from some other phytochemicals, such as polysaccharides from jujube. Jujube polysaccharides, the most promising components in the jujube fruit, could be used as potential natural antioxidants in food industry.

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REFERENCES

[1] GB Celli; A Ghanem; MSL Brooks. *Food and Bioprocess Technology*, **2014**, 7, 1541-1554.
[2] F Giampieri; JM Alvarez-Suarez; M Battino. *Journal of Agricultural and Food Chemistry*, **2014**, 62, 3867-3876.
[3] M Jin; K Zhao; Q Huang; P Shang. *International Journal of Biological Macromolecules*, **2014**, 64, 257-266.
[4] J Ou; Z Sun. *Journal of Functional Foods*, **2014**, 7, 90-100.
[5] QH Gao; CS Wu; M Wang. *Journal of Agricultural and Food Chemistry*, **2013**, 61, 3351-3363.
[6] S Guo; JA Duan; YP Tang; ZH Zhu; YF Qian; NY Yang; EX Shang; DW Qian. *Journal of Agricultural and Food Chemistry*, **2010**, 58, 10774-10780.
[7] P Plastina; D Bonofiglio; D Vizza; A Fazio; D Rovito; C Giordano; I Barone; S Catalano; B Gabriele. *Journal of Ethnopharmacology*, **2012**, 140, 325-332.
[8] F Vahedi; MF Najafi; K Bozari. Cytotechnology, **2008**, 56, 105-111.
[9] YL Huang; GC Yen; F Sheu; CF Chau. *Journal of Agricultural and Food Chemistry*, **2008**, 56, 1734-1739.
[10] J Li; L Shan; Y Liu; L Fan; L Ai. *International Journal of Biological Macromolecules*, **2011**, 49, 255-259.
[11] X Shen; Y Tang; R Yang; L Yu; T Fang; JA Duan. *Journal of Ethnopharmacology*, **2009**, 122, 555-560.
[12] D Wang; Y Zhao; Y Jiao; L Yu; S Yang; X Yang. *Carbohydrate Polymers*, **2012**, 88, 1453-1459.
[13]L Yu; BP Jiang; D Luo; XC Shen; S Guo; JA Duan; YP Tang. Phytomedicine, **2012**, 19, 239-244.
[14] YF Sun; ZS Liang; CJ Shan; H Viernstein; F Unger. *Food Chemistry*, **2011**, 124, 1612-1619.
[15] H Zhang; L Jiang; S Ye; Y Ye; F Ren. *Food and Chemical Toxicology*, **2010**, 48, 1461-1465.
[16] QH Gao; PT Wu; JR Liu; CS Wu; JW Parry; M Wang. *Scientia Horticulturae*, **2011**, 130, 67–72.
[17] CF Hung; BY Hsu; SC Chang; BH Chen. Nutrition, **2012**, 28, 98-105.
[18] Z Zhao; J Li; X Wu; H Dai; X Gao; M Liu; P Tu. *Food Research International*, **2006**, 39, 917-923.
[19] J Li; Y Liu; L Fan; L Ai; L Shan. *Carbohydrate Polymers*, **2011**, 84, 390-394.

[20] MM Bradfo. *Analytical Biochemistry*, **1976**, 72, 248-254.
[21] N Blumenkrantz; G Asboe-Hansen. *Analytical Biochemistry*, **1973**, 54, 484-489.
[22]P Rupérez; O Ahrazem; JA Leal. *Journal of Agricultural and Food Chemistry*, **2002**, 50, 840–845.
[23]L He; H Xu; X Liu; W He; F Yuan; ZQ Hou; YX Gao. *Food Research International*, **2010**, 44, 1161–1167.
[24]P Siddhuraju; PS Mohan; K Becker. *Food Chemistry*, **2002**, 79, 61–67.
[25]S Mabeau; B Kloareg; JP Joseleau. *Phytochemistry*, **1990**, 29, 2441-2445.
[26]M Kácurákova; RH Wilson. *Carbohydrate Polymers*, **2001**, 44, 291–303.
[27]MA Coimbra; A Barros; M Barros; DN Rutledge; I Delgadillo. *Carbohydrate Polymers*, **1998**, 37, 241–248.
[28]W Xu; F Zhang; Y Luo; L Ma; X Kou; K Huang. *Carbohydrate Research*, **2009**, 344, 217–222.
[29]A Femenia; M García-Conesa; S Simal; C Rosselló. *Carbohydrate Polymers*, **1998**, 35, 169–177.
[30]I Mateos-Aparicio; C Mateos-Peinado; A Jiménez-Escrig; P Rupérez. *Carbohydrate Polymers*, **2010**, 82, 245-250.