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Polysaccharides from fruit calyx of *Physalis alkekengi* var. *francheti*: Isolation, purification, structural features and antioxidant activities

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ABSTRACT

The conditions for extracting and purifying polysaccharides from fruit calyx of *Physalis alkekengi* var. *francheti* were investigated, including hot water extraction, ultrasonic wave-assistant extraction and enzyme extraction methods. Four polysaccharide fractions (PAVF I, II-a and III) were separated from the extracts of fruit calyx of *P. alkekengi* var. *francheti* using a DEAE anion-exchange column and Sephadex G-200 column. Their chemical compositions were determined in this study. On the basis of hydroxyl radical assay (**.**OH), superoxide radical assay, 1,1-dipheny-l-2-picrylhydrazyl (DPPH) assay, the antioxidant activities of crude polysaccharide from fruit calyx of *P. alkekengi* var. *francheti* (FCP), PAVF I, II-a, -b and III were investigated. Among these contents, PAVF I has higher scavenging effects on DPPH, **.**OH and superoxide anion-scavenging activities.

1. Introduction

Physalis alkekengi var. *francheti* (Solanaceae) is a well known edible and medicinal plant in oriental countries. Studies on *P. alkekengi* var. *francheti* have attracted more attention in recent years due to the potential biological functions. Its fruit calyx has been used in traditional Chinese medicine as a therapeutic agent for removing heat and toxic materials, relieving sore-throat and was proved to have obvious clinical effect (The China Pharmacopeial Convention, 2005). Its fruit calyx is bitter and the fruit is sweet and slight acidic. So *P. alkekengi* var. *francheti* has great exploitation value and has been widely applied as an important material for food industry. In recent years, physalin from *P. alkekengi* var. *francheti* has been widely studied.

However, to the best of our knowledge, up to now, studies on polysaccharide from *P. alkekengi* var. *francheti* have not been reported. Polysaccharides are generally present in the cellular walls of fruits and vegetables (Mazumder, Morvan, Thakur, & Ray, 2004) and these can give a thickening, stabilizing or gelling effect to maintain fruit texture. In addition, most polysaccharides that are used in the industry are from plant origins (Benhura & Chidewe, 2002). Published data indicate that plant polysaccharides in general have strong antioxidant activities and can be explored as novel potential antioxidants (Hu & Xu, 2003; Jiang, Jiang, Wang, & Hu, 2005). Studies are widely conducted on isolation, purification and structural analysis of polysaccharides. Several reports have indicated that chemical components and molecular weight (MW) of polysaccharide are among the major important factors responsible for biological activities (Zhou et al., 2004). Identification of the polysaccharides is the first and necessary for step the effective exploitation of the structure and functional properties.

However, use of polysaccharides is restricted for various reasons, including a lack of simple methods for isolating them from extracts. So the objectives of this study are to further define the conditions for extracting and purifying the polysaccharide products and shorten the procedure time and improve the quality of product, providing a foundation theory for applying the technology to mass production. The molecular weights (MW), chemical compositions, physical and chemical characters and structural features of purified polysaccharides from fruit calyx of *P. alkekengi* var. *francheti* were measured and the antioxidant activities of these major polysaccharides were evaluated.

2. Materials and methods

2.1. Materials and preliminary treatments of P. alkekengi var. francheti

Fresh *P. alkekengi* var. *francheti* at the mature stage were picked from a commercial orchard in A'cheng, China. *P. alkekengi* var. *francheti* were selected for uniformity of shape and color. Its fruit calyx was treated with petroleum ether (12 h) in a Soxhlet apparatus to remove lipids. The defatted fruit calyx was air dried and then stored in tightly closed glass jars at 4 °C until used.

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2.2. Chemicals

The following chemicals were used: Alkaline protease (62 U/mg) were obtained from Novozyme biological Co. (Tianjin, China); Neutral proteinase (60 U/mg) was obtained from Beijing Auboxing biotech. Co. (Beijin, China); Papain (15 U/mg) was obtained from Guangxi Papain industry (Guangxi, China); Vitamin C (Vc), Nitro blue tetrazolium (NBT), phenazine methosulfate (FMS), dihydromicotineamidadenine dinucleotide (NADH), thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA); DEAE-52 was purchased from Whatman International Ltd. (Maidstone, Kent, UK); Sephadex G-200 was purchased from Pharmacia Co. (Sweden). Other reagents were of analytic reagent grade.

2.3. Isolation of crude polysaccharides

2.3.1. Extraction with hot water

The polysaccharide extraction parameters are shown in Table 1. The dried and defatted fruit calyx (5 g) was put in certain temperature of distilled water, and effect of time, temperature and the ratio of material and water on the yields of FCP were investigated.

2.3.2. Extraction with ultrasonic wave-assistant

The polysaccharide extraction parameters are shown in Table 2. The dried and defatted fruit calyx (5 g) was mixed with distilled water. The cells were disrupted by ultrasonic waves ACQ-600 Ultrasound cell breakage apparatus. The aim of the study was to determine effect of intensity and duration of ultrasound on the yields of FCP (Liu, Yang, & Lin, 2001; Oliveira, Marques, & Azeredo, 1999).

Table 1
Polysaccharide yields under the conditions indicated using $L_9(3^4)$ orthogonal design.

Run	The temperature of incubation	The time of incubation	The ratio of material and water (g/mL)	Polysaccharide yield (%)
1	I (70 °C)	I (4 h)	I (1:10)	1.392 ± 0.219
2	I (70 °C)	II (5 h)	II (1:15)	1.654 ± 0.481
3	I (70 °C)	III (6 h)	III (1:20)	1.733 ± 0.367
4	II (80 °C)	I (4 h)	II (1:15)	1.645 ± 0.269
5	II (80 °C)	II (5 h)	III (1:20)	1.863 ± 0.411
6	II (80 °C)	III (6 h)	I (1:10)	2.246 ± 0.552
7	III (90 °C)	I (4 h)	III (1:20)	1.744 ± 0.31
8	III (90 °C)	II (5 h)	I (1:10)	2.416 ± 0.501
9	III (90 °C)	III (6 h)	II (1:15)	2.920 ± 0.429
K_I[a]	4.77900	4.78100	6.05400	
K_{II}[a]	5.75400	5.93300	6.21900	
K_{III}[a]	7.08000	6.89900	5.34000	
R[b]	0.7600	0.7000	0.2867	

[a] Average responses (K_I, K_{II} and K_{III}) of each level about extraction yield.
[b] R value means range between three average responses of each level about extraction yield.

Table 2
Polysaccharide yields under the conditions indicated using $L_9(3^4)$ orthogonal design.

Run	Intensity of ultrasound (W)	Duration of ultrasound (min)	The ratio of material and water (g/mL)	Polysaccharide yield (%)
1	I (200)	I (15)	I (1:10)	2.74 ± 0.449
2	I (200)	II (20)	II (1:15)	2.812 ± 0.621
3	I (200)	III (25)	III (1:20)	2.908 ± 0.33
4	II (250)	I (15)	II (1:15)	3.000 ± 0.596
5	II (250)	II (20)	III (1:20)	3.068 ± 0.611
6	II (250)	III (25)	I (1:10)	3.095 ± 0.385
7	III (300)	I (15)	III (1:20)	3.267 ± 0.43
8	III (300)	II (20)	I (1:10)	3.35 ± 0.513
9	III (300)	III (25)	II (1:15)	3.67 ± 0.711
K_I[a]	8.46	9.007	9.185	
K_{II}[a]	9.163	9.23	9.482	
K_{III}[a]	10.287	9.673	9.243	
R[b]	0.609	0.222	0.099	

[a] Average responses (K_I, K_{II} and K_{III})of each level about extraction yield.
[b] R value means range between three average responses of each level about extraction yield.

Table 3
Influence of vary of enzyme on the polysaccharide yield.

Enzyme species	Temperature (°C)	pH	Dosage (%)	Polysaccharide yield (%)
Alkaline protease	50	8.0	1	4.153 ± 0.689
Papain	60	6.5	1	8.581 ± 1.32
Neutral proteinase	43	7.4	1	2.597 ± 0.411
blank	60	7.0	–	1.1 ± 0.121

2.3.3. Extraction with enzyme

The same quality of dried and defatted fruit calyx (5 g) was put in distilled water with different enzyme Neutral proteinase, Papain and alkaline protease, respectively, in their suitable pH and temperature (Table 3), using the following extraction conditions: extract with enzyme for 1 h, followed by 1 h at 90 °C (inactivation of enzyme). The aim of the study was to determine effect of different enzyme on the yields of FCP.

The resulting sample used for three extraction ways above was centrifuged at 5000 rpm for 10 min. The supernatant was concentrated and then precipitated by the addition of ethanol in 1:4 (v/v) at room temperature. The precipitate was dissolved in distilled water and the solution was then washed with sevag reagent (isoamyl alcohol and chloroform in 1:4 ratio) (Navarini, Gilli, & gombac, 1999), which were centrifuged at 5000 rpm for 15 min and the protein was removed. The supernatant was dialyzed against deionized water for 24 h before concentration under vacuum evaporator at 55 °C. The mixture was precipitated by the addition of ethanol in 1:4 (v/v) at room temperature and the precipitate was freeze dried. Total sugars were determined by the phenol–sulfuric acid assay using glucose as standard (Dubious, Gilles, Hamilton, Rebers, & Smith, 1956). The yields of the crude polysaccharide from its fruit calyx (FCP) was calculated.

2.4. Separation and purification of the polysaccharides

Considering the possible effect of ultrasonic wave and enzyme to the structure and biological activities of FCP, ultrasonic wave-assistant's method and enzymic hydrolysis method were not applied to the research on structural features and the antioxidant activities of FCP and hot water extract method was adopted. Dissolved FCP was fractionated on DEAE-52 Cellulose anion-exchange column (2.5 × 65 cm, Cl^-) (Tan, 2002). The column was eluted first with distilled water, and then with gradient solutions (0.1 M, 0.25 M, 0.5 M NaCl and 0.5 M NaOH), at a flow rate of 0.6 mL/min. The major polysaccharide fractions were collected with a fraction collector and concentrated using a rotary evaporator at 55 °C and residues were loaded onto a Sephadex G-200 gel column (2.5 × 65 cm). The column was eluted with 0.1 M NaCl at a flow rate of 0.3 mL/min. The major fraction was collected and then freeze dried. All of these fractions were assayed for sugar content by the phenol–sulfuric acid method using glucose as standard (Dubious et al., 1956).

2.5. Determination of the polysaccharides purification

The sample were dissolved in distilled water, centrifuged, filtrated and the filtrate was applied to a Sephadex G-200 column (1.1 × 100 cm), which was eluted with 0.1 M sodium chloride at 1.2 mL/10 min/tube. Polysaccharides were detected by the phe-

nol–sulfuric acid method using glucose as standard (Dubious et al., 1956). Elution curve was drawn by tube number as abscissa and absorbance as vertical coordinate. In addition, the polysaccharides purification was also identified by cellulose acetate pellicle electrophoresis (borax-sodium hydroxide buffer, pH 8.0) at 300 V for 10 min with detection using Toluidine Blue.

2.6. Determination of molecular weight of the purified polysaccharide

The molecular weight of samples were determined by Gel-Filtration Chromatography (GFC) using the method of Yamamoto, Numome, Yamauchi, Kato, and Sone (1995) on Sephadex G-200 gel column (1.1 × 100 cm). The column was eluted with 0.1 M of NaCl at a flow rate of 0.3 mL/min, and the elution was monitored by the phenol–sulfuric acid method. The column was calibrated with standard Dextran from Pharmacia (Molecular weight: 10,000, 40,000, 70,000, 133,000, 482,000, 2,000,000 Da) and a standard curve was established before sample analysis.

2.7. Physical and chemical characters of purified polysaccharides

Examination of the solubility of purified FCP was carried out as following: the purified polysaccharides (10 mg) and distilled water (2 mL) were vigorously mixed and incubated in a 37 °C water bath and the time was recorded when the polysaccharides were completely dissolved. Starchy polysaccharide was determined by iodine reaction (Lee, Yu, & Chen, 1994). Phenolic hydroxyls substance was assayed by the ferric chloride method (Zhou, 1978). Reducing sugar was determined by Fehling reagent (Schneider, 1979). Uronic acids were assayed colorimetrically by the uronic acid–carbazole reaction according to the procedure outlined by Bitter and Muir (1962). Protein was measured by bradford technique (Bradford, 1976) and the absorptivity at 280 nm. Each experiment was performed for three times.

2.8. Infrared spectral analysis of the polysaccharides

The IR spectrum of the polysaccharides purified from FCP was carried out using a Fourier transform infrared spectrophotometer (FTIR, Bruker, Germany) equipped with OPUS 3.1 software. The purified polysaccharide was ground with KBr powder and then pressed into pellets for FTIR measurement in the frequency range of 4000–500 cm^{-1} (Ganesh Kumar, Joo, Choi, Koo, & Chang, 2004).

2.9. Analysis of monosaccharide compositions

The polysaccharide (10 mg) was hydrolyzed with 10 mL of 2 M trifluoroacetic acid (TFA) at 120 °C for 6 h in a sealed glass tube (Erbing, Jansson, Widmalm, & Nimmich, 1995). Derivation was then carried out using the trimethylsilylation reagent (Guentas et al., 2001). The trimethylsilylated derivatives were analyzed using gas chromatography (GC) with an OV-17capillary gas chromatography (GC) column equipped with a flame-ionization detector (FID). As references, the following standard sugars were converted to their trimethylsilylated derivatives and analyzed: rhamnose, arabinose, xylose, mannose, glucose, fructose, L-sorbose and galactose.

The GC operation was performed using the following conditions: N_2: 46 mL/min; injection temperature: 280 °C; detector temperature: 300 °C; column temperature programmed from 180 to 220 °C at 6 °C/min.

2.10. Assays for antioxidant activity

2.10.1. Evaluation of DPPH-scavenging activity

The free-radical-scavenging activity was measured by the method of Yamaguchi, Takamura, Matoba, and Terao (1998). Samples were dissolved in 10 mL of distilled water at 0 (control), 0.5, 1 or 1.5 mg/mL. Then 2 mL of 0.2 mM DPPH in EtOH was added to 1 mL of the sample solution. The absorbance at 517 nm was measured after 20 min of incubation at 25 °C. In the study, Vc at 0.5, 1 or 1.5 mg/mL was used as a positive control. The inhibition of DPPH radicals by the samples was calculated according to the following equation:

$$\text{DPPH-scavenging activity}(\%) = [\text{1-absorbance of sample}/\text{absorbance of control}] \times 100.$$

2.10.2. Evaluation of •OH scavenging activity

The •OH scavenging activity was investigated by the method of Ghiselli et al. with a minor modification (Ghiselli, Nardini, Baldi, & Scaccini, 1998). Samples were dissolved in 10 mL of distilled water at 0 (control), 0.5, 1 or 1.5 mg/mL. The sample solution (0.1 mL) was mixed with 0.8 mL of reaction buffer 0.2 M phosphate buffer (pH 7.4), 1.75 mM deoxyribose, 0.1 mM ferrous ammonium sulfate and 0.1 mM EDTA and 0.1 mL of 1.0 mM ascorbic acid, and 0.1 mL of 10 mM H_2O_2 was then added to the reaction solution. The reaction solution was incubated for 10 min at 37 °C and then 0.5 mL of 1% thiobarbituric acid and 1 mL of 2.8% trichloroacetic acid were added to the mixture. The mixture was boiled for 10 min and cooled on ice. The absorbance of the mixture was measured at 532 nm. Percent inhibition of deoxyribose degradation was calculated as (1-absorbance of sample/absorbance of control) × 100. In the study, Vc at 0.5, 1 or 1.5 mg/mL was used as a positive control.

2.10.3. Determination of superoxide anion-scavenging activity

The superoxide anion-scavenging activity was measured by the method of Robak and Gryglewski (1988) with a minor modification. Samples were dissolved in 10 mL of distilled water at 0 (control), 0.5, 1 or 1.5 mg/mL. A 1 mL aliquot of each sample solution was mixed with 1 mL of 0.1 M phosphate buffer (pH 7.4) containing 150 μM NBT, 60 μM PMS and 468 μM NADH. After 5 min of incubation at 25 °C, the absorbance was measured at 560 nm. The superoxide anion-scavenging activity was calculated as scavenging activity (%) = (1-absorbance of sample/absorbance of control) × 100. Vc at 0.5, 1 or 1.5 mg/mL was used as a positive control in the study.

2.11. Data handling

Results were expressed as means ± standard deviations of three replicated determinations. SPSS 8.0 software (SPSS, Chicago, IL, USA) was used for data analysis.

3. Results and discussion

3.1. Influence of extracting conditions on polysaccharide yield of FCP

3.1.1. Hot water extraction

Basically, the longer time and higher temperature of incubation and the lower ratio of material and water gave a higher yield of polysaccharide. A great increase in yield was observed at temperatures between 70 and 90 °C. A longer incubation in hot water produced a higher yield. However, after 6 h, the increase in yield became insignificant. So an orthogonal design [$L_9(3^4)$] was applied to optimize the temperature and time of incubation and the ratio of material and water. Table 1 shows the yields of the extraction of polysaccharide under the indicated conditions. The orders of impact of the different factors on the yield are: the temperature of incubation > the time of incubation > the ratio of material and water, and the best condition for extraction was determined as fol-

lowings: 90 °C, 6 h, 1:15, and the highest extraction ratio of polysaccharides was 2.920%.

3.1.2. Ultrasonic wave-assistant extraction

On the basis of single experiment of intensity of ultrasound and duration of ultrasound, an orthogonal design [$L_9(3^4)$] was applied to optimize the intensity and duration of ultrasound and the ratio of material and water. Table 2 shows the yields of the extraction of polysaccharide under the indicated conditions. Basically, the stronger ultrasound for a longer time and the lower ratio of material and water gave a higher yield of polysaccharide. The orders of impact of the different factors on the yield are: the intensity of ultrasound applied > the duration of ultrasound > the ratio of material and water. The primary condition for extraction was determined as followings: 300 W, 25 min, 1:15 and the highest extraction ratio of polysaccharides was 3.67%.

3.1.3. Extraction with enzyme

During extraction with special enzyme (Neutral proteinase, Papain and alkaline protease), degradation of the protein chain may occur, which could be responsible for the higher yields obtained under milder condition. As shown in Table 3, the extracting effect of Papain was better than other enzyme and the highest extraction ratio of polysaccharides was 8.581%.

3.2. Identification of polysaccharide components

Chromatography results of PCF are shown in Fig. 1. After fractionation on DEAE-Cellulose Cl^- column, PAVF I, PAVF II, PAVF III and PAVF IV (1.2:2.6:2.4:0.6) were obtained from an aqueous NaCl gradient (0.1, 0.25, 0.5 M) and 0.5 M NaOH. The four fractions were purified by a Sephadex G-200 column, respectively. Both PAVF I and III showed only one symmetrical peak and PAVF II gave two fractions (PAVF II-a, -b). PAVF IV produced many fractions and wasn't studied further. PAVF I, PAVF II-a, -b and PAVF III showed only one symmetrical peak from gel-filtration chromatography on Sephadex G-200 column, and a single spot on cellulose acetate pellicle electrophoresis was observed. The results indicated that no other polysaccharide was present in the sample. It was concluded that PAVF I, PAVF II-a, -b and PAVF III were homogeneous. Average molecular weight of PAVF I, PAVF II-a, -b and PAVF III was estimated to be 107,000, 500,000, 170,000 and 420,000 Da by gel-filtration chromatography, using dextrans of known molecular weight as standards.

The physical and chemical characteristics of PAVF I, PAVF II-a, -b and PAVF III are shown in Table 4. Iodine reaction showed that PAVF III contained starchy polysaccharide. The ferric chloride method proved that PAVF III contained phenolic hydroxyls substance. Fehling reagent methods were minus reactions, indicating

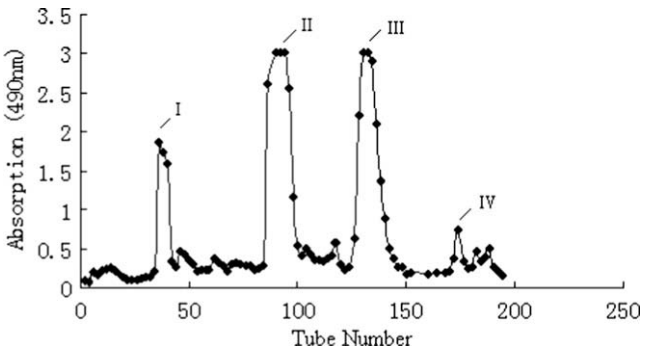

Fig. 1. DEAE-52 Cellulose anion-exchange column chromatogram of the crude polysaccharides extracted from FCP.

Table 4
Physical and chemical characteristics of PAVF I, II-a, -b and III.

Parameter	PAVF I	PAVF II-a	PAVF II-b	PAVF III
Color and texture	White loose floccule	White loose floccule	White loose floccule	grayish loose floccule
Solubility	58 s	92 s	60 s	83 s
Iodine reaction	–	–	–	+
The ferric chloride method	–	–	–	+
Fehling reagent	–	–	–	–
The uronic acid–carbazole method	+	+	+	+
Bradford technique	–	–	–	+
The absorptivity at 280 nm	–	–	–	+

Table 5
Components of monosaccharide from *Physalis alkekengi* var. *francheti*.

Sample[a]	Sugar components[c] (relatively mass %)							
	Rha	Ara	Xyl	Man	Glc	Fru	L-Sor	Gal
PAVF I	12.01	60.36	nd[b]	2.95	9.93	nd	nd	9.78
PAVF II-a	nd	nd	41.61	nd	20.73	37.2	nd	nd
PAVF II-b	38.77	nd	nd	19.3	7.39	27.01	6.18	nd
PAVF III	46.45	nd	nd	nd	18.07	21.02	nd	13.26

[a] PAVFI, PAVFII-a, -b and III: NaCl elute fraction by DEAE-52 and Sephadex G-200 column.
[b] nd, not detected.
[c] Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Glc, glucose; Fru, fructose; L-Sor, L-sorbose and Gal, galactose.

that no monosaccharides were present in the sample. The uronic acid–carbazole reaction were plus reaction, respectively, proved that PAVF I, PAVF II-a, -b and III contained uronic acids. Bradford technique and the absorptivity at 280 nm showed that PAVF III was a conjugate with portions of polysaccharide and protein.

Results in Table 5 indicated that PAVF I was mainly consisted of arabinose in terms of the molar proportions. PAVF II-a was mainly consisted of xylose, glucose and fructose. PAVF II-b was mainly consisted of rhamnose, mannose and fructose. PAVF III was mainly consisted of rhamnose, fructose, glucose and galactose.

Both infrared spectrum of the purified PAVF I, II-a, -b and III fraction, as shown in Fig. 2 displayed a broadly-stretched intense peak at 3600–3200 cm^{-1} characteristic of hydroxyl groups and a weak C—H band at around 2930 cm^{-1}. The relatively strong absorption peak at around 1600–1650 cm^{-1} indicated the characteristic of C=O; The peaks at 950–1200 cm^{-1} suggest the presence of C—O—C and C—O—H link bonds (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000). PAVF I displayed the absorption at 870 cm^{-1}, which was due to mannose. Characterization of four glycoconjugates by IR analysis showed the typical absorption of polysaccharide.

3.3. Antioxidant activity of different polysaccharide fractions

Some polysaccharides have exhibited strong antioxidant capability (Hu & Xu, 2003; Jiang et al., 2005). In the investigation, different polysaccharide fractions extracted and purified from FCP showed a dose-dependent, radical-scavenging activity (Table 6). The radical-scavenging activity was enhanced with increased concentration. The DPPH, superoxide anion and **.**OH scavenging activity of the crude polysaccharide and PAVF III were lower than that of Vc, but three purified polysaccharide fractions (PAVF I and II-a, -b) had higher antioxidant activities than Vc in this study. These results indicated that polysaccharides from fruit calyx of *P. alkekengi* var. *francheti* had strong DPPH, Hydroxyl radical and Superoxide

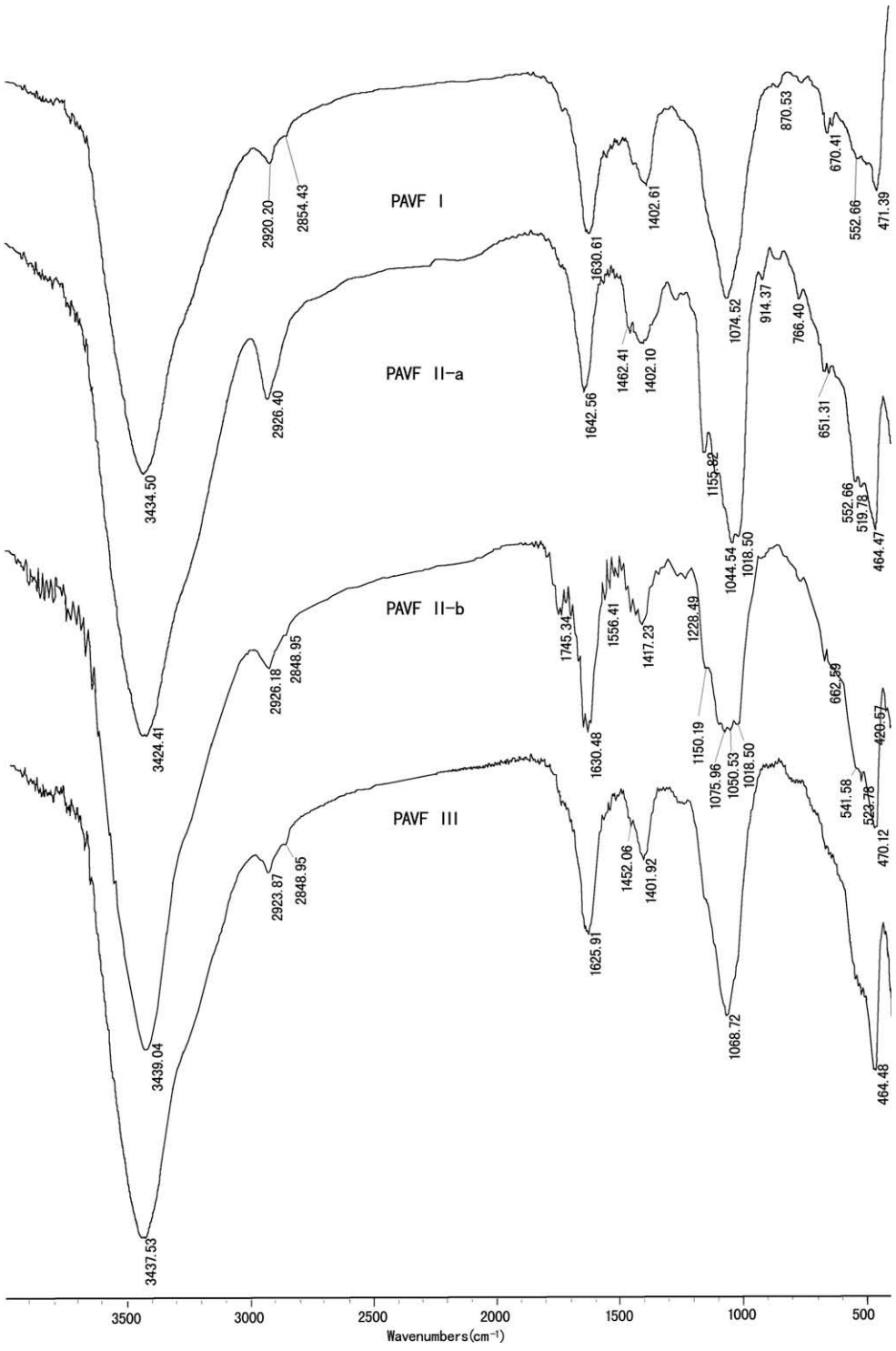

Fig. 2. IR spectrum of PAVF I, II-a, -b and III.

anion-scavenging activity, and most purified polysaccharides were more pronounced than Vitamin C and FCP. Moreover, PAVF I showed the best activities.

4. Conclusions

In this work, the extraction conditions of polysaccharide from fruit calyx of *P. alkekengi* var. *francheti* were compared. Result showed that the highest yield of polysaccharide was observed using enzymatic extraction. The extraction effect of papain is better than that of other enzymes, and the highest extraction ratio of polysaccharides was 8.581%.

Four polysaccharide fractions (PAVF I, II-a, -b and III) were separated from the extracts of fruit calyx of *P. alkekengi* var. *francheti* using a DEAE anion-exchange column and Sephadex G-200 column. Results of gas chromatographic analysis, infrared spectra analysis and physical and chemical characters studies indicated that PAVF I mainly contained arabinose and the molecular weight of PAVF I was 107 kDa. PAVF II-a mainly contained xylose, fructose, glucose and the molecular weight of PAVF II-a was 500 kDa. PAVF

Table 6
Radical-scavenging activities of the different polysaccharides extracted and purified from fruit calyx *Physalis alkekengi* var. *francheti*.[a]

Sample[b]	DPPH		Hydroxyl radical		Superoxide anion	
	Amount (mg)	Scavenging effects (%)	Amount (mg)	Scavenging effects (%)	Amount (mg)	Scavenging effects (%)
Crude polysaccharide	0.5	8.7 ± 0.8	0.05	17.1 ± 2.1	0.5	21.6 ± 1.5
	1	11.9 ± 0.5	0.1	29.6 ± 2.6	1	31.2 ± 2.1
	1.5	14.4 ± 2.1	0.15	36.3 ± 3.1	1.5	39.9 ± 2.1
PAVF I	0.5	37.2 ± 2.2	0.05	61.8 ± 2.4	0.5	50.6 ± 2.7
	1	46.1 ± 3.3	0.1	72.5 ± 3.6	1	58.3 ± 2.3
	1.5	53.3 ± 4.1	0.15	80.5 ± 3.2	1.5	68.3 ± 2.6
PAVF II-a	0.5	31.1 ± 2.8	0.05	51.9 ± 2.9	0.5	40.9 ± 2.5
	1	41.9 ± 2.9	0.1	60.3 ± 3.6	1	55.3 ± 2.6
	1.5	49.8 ± 3.5	0.15	68.1 ± 3.2	1.5	66.3 ± 1.9
PAVF II-b	0.5	32.4 ± 2.3	0.05	49.3 ± 3.5	0.5	41.9 ± 2
	1	40.9 ± 2.5	0.1	67.6 ± 3.8	1	50.1 ± 2.7
	1.5	48.2 ± 2.9	0.15	73.4 ± 3.3	1.5	56.3 ± 3.5
PAVF III	0.5	7.3 ± 2.7	0.05	10 ± 2.8	0.5	15.6 ± 2.6
	1	10.5 ± 4.1	0.1	18.3 ± 3.8	1	20.6 ± 3.7
	1.5	13.4 ± 2.9	0.15	29.9 ± 3.1	1.5	24.9 ± 3.6
Vc	0.5	19.4 ± 2.1	0.05	39.3 ± 3.6	0.5	33.2 ± 1.8
	1	19.8 ± 4.7	0.1	41.9 ± 3.9	1	46.4 ± 2.1
	1.5	20.6 ± 5.2	0.15	43.1 ± 3.1	1.5	54.2 ± 1.6

[a] Date are presented as mean ± standard deviations (n = 3).
[b] Crude polysaccharide by hot water extraction; PAVF I, PAVF II-a, PAVF II-b: NaCl elute fraction purified by a DEAE-Cellulose Cl^- column and Sephadex G-200 column; PAVF III: NaOH elute fraction by a DEAE-Cellulose Cl^- column and then NaCl elute fraction by Sephadex G-200 column.

II-b mainly contained rhamnose, mannose and fructose and the molecular weight of PAVF II-b was 170 kD. PAVF III (420 kDa) mainly contained rhamnose, fructose, glucose and galactose and some protein combine with them by covalent bond. Characterization of four glycoconjugates by IR analysis showed the typical absorption of polysaccharide.

Three purified polysaccharide fractions (PAVF I and II-a, -b) exhibited strong DPPH, hydroxyl radical and superoxide anion-scavenging activity, which had a higher antioxidant activity than the crude polysaccharides and PAVF I showed the best activities. In conclusion, the polysaccharide extracted and purified from fruit calyx of *P. alkekengi* var. *francheti* could be explored as a novel potential antioxidant. Further study should be carried out to elucidate bioactivity through animal experiments with the purpose of applying the polysaccharides in the food industry.

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