Structural Characteristics of Pumpkin Pectin Extracted by Microwave Heating

Sang-Ho Yoo, Byeong-Hoo Lee, Heungsook Lee, Suyong Lee, In Young Bae, Hyeon Gyu Lee, Marshall L. Fishman, Hoa K. Chau, Brett J. Savary, and Arland T. Hotchkiss Jr.

Abstract: To improve extraction yield of pumpkin pectin, microwave heating was adopted in this study. Using hot acid extraction, pumpkin pectin yield decreased from 5.7% to 1.0% as pH increased from pH 1.0 to 2.0. At pH 2.5, no pectin was recovered from pumpkin flesh powder. After a pretreatment at pH 1.0 and 25 °C for 1 h, pumpkin powder was microwave-extracted at 120 °C for 3 min resulting in 10.5% of pectin yield. However, premicrowave treatment at 60 °C for 20 min did not improve extraction yield. When microwave heating at 80 °C for 10 min was applied after premicrowave treatment, final pectin yield increased to 11.3%. When pH was adjusted to 2.0, the yield dropped to 7.7% under the same extraction conditions. Molecular shape and properties as well as chemical composition of pumpkin pectin were significantly affected depending on extraction methods. Galacturonic acid content (51% to 58%) of pumpkin pectin was lower than that detected in commercial acid-extracted citrus pectin, while higher content of neutral sugars and acetyl esters existed in pumpkin pectin structure. Molecular weight (M_w) and intrinsic viscosity (η_w) determined for microwave-extracted pumpkin pectins were substantially lower than acid-extracted pectin, whereas polydispersity was greater. However, microwave-extracted pectin at pH 2.0 had more than 5 times greater M_w than did the pectin extracted at pH 1.0. The η_w of microwave-extracted pectin produced at pH 2.0 was almost twice that of other microwave-extracted pectins, which were comparable to that of acid-extracted pectin. These results indicate that extraction yield of pumpkin pectin would be improved by microwave extraction and different pectin structure and properties can be obtained compared to acid extraction.

Keywords: intrinsic viscosity, microwave extraction, molecular weight, pumpkin pectin

Practical Application: Pumpkin is a promising alternative source for pectin material. Pumpkin pectin has a unique chemical structure and physical properties, presumably providing different functional properties compared to conventional commercial pectin sources. Depending on the conditions to produce pumpkin pectin, diverse molecular structures can be obtained and utilized in various food applications.

Introduction

Pectin is a complex heteropolysaccharide in plant cell wall, consisting of α-(1,4)-linked homogalacturonan and rhamnogalacturonan highly branched with various neutral sugar glycans (Ovodov 2009). Pectin is a well-known gelling compound used in processed foods such as jams, jellies, and marmalade. Galacturonan methoxylation, galacturonic acid content (GalA), the composition of neutral sugars, and molecular weight of pectin structure all directly affect the pectin's physicochemical properties, and these can vary considerably depending on the pectin source. These factors are intercorrelated and the complex structure of pectin materials is still not well defined. Generally, commercial pectins are classified as high methoxyl (HM) and low methoxyl (LM) pectin, depending on the extent of GalA methoxylation (greater or less than 50%, respectively): HM pectins gel under sugar–acid conditions while LM pectins gel by calcium cross-linking. LM pectin gelling can be improved by chemical amidation on the C6 carboxyl group of GalA. When the pectin structure is dissected into the unique patterns consisting of various sugars, there are at least 3 distinguished structural domains (Ridley and others 2001). One is linear homogalacturonan backbone that is connected by another one, rhamnogalacturonan I. The RG-I fragment in the pectin structure basically consists of disaccharide units, [→4)-α-D-galacturonosyl-α-L-(1→2)-rhamnose-(1→], which sometimes may have long side chains of arabinan and galactan (Oosterveld and others 1996). The other one is an extremely complex structure, rhamnogalacturonan II, consisting of wide range of monosaccharides such as GalA, rhamnose, arabinose, galactose, xylose, fucose, and even glucuronic acid, apiose, aceric acid, 3-deoxy-D-manno-2-octulosonic acid (KDO), and 3-deoxy-D-lyxo-2-heptulosaric acid (DHA) (O'Neill and others 1990, 1996). In the gelling mechanism, the possible role of rhamnogalacturonan II remains virtually unstudied (Hwang and others 1993), yet

MS 20120369 Submitted 3/9/2012, Accepted 8/23/2012. *Authors Yoo, B.-H. Lee, H. Lee, and S. Lee are with Dept. of Food Science & Technology and Carbohydrate Bioproduct Research Center, Sejong Univ., 98 Gunja-dong, Gwangjin-gu, Seoul 143-747, Korea. Authors Bae and H.G. Lee are with Dept. of Food and Nutrition, Hanyang Univ., 17 Haengdang-dong, Seongdong-gu, Seoul 133-791, Korea. Authors Fishman, Chau, Savary, and Hotchkiss Jr. are with Dairy and Functional Foods, Eastern Regional Research Center, Agricultural Research Service, U.S. Dept. of Agriculture, 600 E Mermaid Lane, Wyndmoor, PA 19038, U.S.A. Direct inquiries to author Yoo (E-mail: shyoo@sejong.ac.kr).*

Journal of Food Science A Publication of the Institute of Food Technologists

doi: 10.1111/j.1750-3841.2012.02960.x

Table 1–Effect of the extraction method on the pumpkin pectin yield.

Extraction method	Pretreatment condition			Microwave heating condition			Extraction yield[A] (%)
	Temperature (°C)	Time (hr)	pH	Temperature (°C)	Time (min)	pH	
A	65	2	1.0	NA[B]	NA	NA	4.2 ± 0.4^{d}
B	4	16	1.0	120	3	1.0	10.5 ± 0.2^{b}
C	25	1	1.0	60/80	20/10	1.0	11.3 ± 0.2^{a}
D	25	1	2.0	60/80	20/10	2.0	7.7 ± 0.3^{c}

[A]Values superscripted with different letters are statistically different ($P < 0.05$).
[B]NA, not applied.

rhamnogalacturonan II is known to form cross-links through borate-diol diester bonds (Ishii and Matsunaga 1996; Kobayashi and others 1996). Thus, fairly large pieces of information on pectins isolated from a wide range of plant sources should be obtained to gain better insight of pectin structure and how the structure affects its physical properties and functional properties.

Pumpkins are traditionally used as a medicinal food in many countries worldwide (Popovic 1971; Jia and others 2003; Adolfo and Michael 2005). They contain bioactive compounds such as polysaccharides, sterols and oils, carotenoids, and γ-aminobutyric acid (Kuhlman and others 1999; Murkovic and others 2002; Zhang and Yao 2002; Zhang 2003). Especially, pectin from pumpkin peel was reported to activate growth of several lactic acid bacteria, while it inhibited growth of *Escherichia coli* and *Clostridium perfringens* (Jun and others 2006). A pectin preparation from pumpkin pulp contained RG-I, and it had a comparable glucose binding capacity to other hydrocolloids (Ou and others 2001; de Escalad Pla and others 2007; Fissore and others 2009). Pumpkin pectin forms gels at concentrations much lower than commercial citrus pectin (Ptitchkina and others 1994). Depending on the extraction methods of pumpkin pectin, the yield and chemical structure of pectin were quite different. When pumpkin pectin was isolated by treating with several strains of *Bacillus polymyxa*, the yield was more than twice compared with mineral acid extraction (Matora and others 1995). Accordingly, much higher degree of acetyl esterification (DAE) was observed from the enzymatically extracted pumpkin pectin, which did not form gels as was the case for highly acetylated sugar beet pectin (Rolin 2002; Evageliou and others 2005). In order to improve the extraction yield of pumpkin pectin, various cell wall degrading enzymes, cellulase from *Trichoderma viride*, hemicellulase from *Aspergillus niger*, and a glycosidase complex from *Xanthomonas campestris* (Shkodina and others 1998) were utilized. In addition, the extraction method was optimized by preliminary treatment of the pumpkin pulp with an enzyme preparation from *Aspergillus. awamori* (Ptitchkina and others 2008).

Our present study is the first report of applying microwave heating to extract pumpkin pectin and we show the effect of this extraction method on the molecular structure and properties of isolated pumpkin pectin as well as on yield.

Materials and Methods

Materials

Pumpkins (*Cucurbita maxima*) were purchased from a local market in Philadelphia, Pa., U.S.A. After removing the outer skin and inner core, the flesh was diced to small rectangular pieces (about 2 cm^3), then lyophilized and ground to a fine powder. Tri-fluoroacetic acid (TFA; Sigma-Aldrich Chemical Co., Mo., U.S.A.) and other chemicals were reagent grade and used without further purification. Solvents were of analytical grade and purchased from Burdick and Jackson (Muskegon, Mich., U.S.A.). Acetic-d_3 acid-d (99.9 atom% D) and methyl-d_3 alcohol-d (99.8 atom% D) were from Sigma-Aldrich Chemical Co. Solid-phase microextraction fibers, 75 μm Carboxen-PDMS (5-7318), and 65 μm Carbowax-DVB (5-7312) were purchased from Supelco (Bellefonte, Pa., U.S.A.), which were conditioned as recommended by the manufacturer prior to use.

Extraction of pumpkin pectin

Traditional hot acid and microwave-assisted acid treatments were compared for extracting pectin from pumpkin powder. To extract pectin by traditional hot acid treatment, 1.5 g of the ground pumpkin flesh powder were dispersed in a 0.1 N HCl solution at 65 °C for 2 h with constant stirring. To extract pectin by the microwave-assisted method, the lyophilized flesh powder was microwave-heated with or without pretreatment of 0.1 or 0.01 N HCl. The pretreatment was done at 4 or 25 °C for the designated time periods. Microwave temperature (60, 80, and 120 °C) and time (20, 10, and 3 min) were varied to evaluate the effect of extraction conditions on extraction yield and pectin structure. The microwave-assisted heating method for pectin extraction has been described previously (Fishman and others 2000). Briefly, microwave treatment was performed using a model MarsX microwave heating system (CEM Corp., Matthews, N.C., U.S.A.). Pumpkin samples were irradiated with 1200 W of microwave power at a frequency of 2450 MHz. The oven contained a rotating circular carousel that holds up to 6 vessels and each vessel was loaded with 1.5 g of lyophilized pectin powder. One of the vessels was equipped with temperature and pressure sensors that measured and controlled the temperature and pressure within the cells. This treatment was immediately followed by rapid cooling in a cold ice water bath. The specific treatment conditions were described in Table 1. Once acid and/or microwave treatment was accomplished, the extracted solution was filtered with Miracloth and precipitated with 2 vol isopropyl alcohol (95%, v/v). The resulting precipitates were washed with 95% and 100% isopropyl alcohol (IPA) sequentially and dried in a vacuum oven. Moisture content of extracted pectin was determined after drying at 45 °C for 12 h in a vacuum oven.

Gas chromatography–mass spectroscopy analysis of methyl and acetyl substitution levels

Methyl and acetyl ester contents of pectin were analyzed by following a gas chromatography–mass spectrometry (GC-MS) method (Savary and Nuñez 2003). Briefly, a fresh pectin suspension was prepared at 5 mg/mL by heating at 60 °C and sonication. For pectin analyses, vials received 0.200 mL of pectin (1 mg), 0.200 mL of 1.0 M NaOH, and 0.100 mL of acetic acid and methyl alcohol d_3-standards (2.00 μmol of MeOH and 0.500 or 0.050 μmol of HOAc). The vials were immediately capped and heated at 40 °C for 1 h, and then placed on ice. Vials then received 0.5 mL of 0.4 M sulfuric acid (final pH < 2.0). Headspace-SPME sampling was performed as described previously (Savary and Nuñez 2003).

The GC–MS system consisted of a 5890 Series II Plus gas chromatograph with a Mass Selective Detector (Hewlett-Packard, San Fernando, Calif., U.S.A.) fitted with a PoraPLOT Q capillary column, 25 mL × 0.25 mm i.d., film thickness 8 μm (Chrompack, Raritan, N.J., U.S.A.), and a narrow bore (0.75 mm) SPME injection liner (Supelco). Methanol concentrations were calculated by plotting the peak area ratios (normal to deuterated forms) for base ion pairs (m/z 29/30$_d$) over the indicated range of concentration ratios. Acetic acid concentrations were similarly calculated using peak area ratios of base ion pairs (m/z 43/46$_d$) over the indicated concentration range.

Galacturonic acid content and esterification degree

Galacturonic acid content was determined with a colorimetric method described by Filisetti-Cozzi and Carpita (1991) with some modifications (Yoo and others 2006). To 0.4 mL of a pectin sample (100 μg/mL), 40 μL of 4 M sulfamate solution and 2.5 mL of 75 mM sodium tetraborate in concentrated sulfuric acid were added and mixed vigorously. The mixture was cooled to room temperature in an ice bath before it was heated in a boiling water bath for 15 min. The sample was immediately cooled in an ice bath for 1.5 min to bring it to room temperature. Finally, 80 μL of 0.15% 3-phenylphenol (w/v) in 0.5% NaOH (w/v) was added and the sample was mixed well prior to reading its absorption at 525 nm within 3 to 5 min. The GalA concentration was divided by the initial sample concentration to give the GA content (%) of the pectin. The degree of methyl esterification (DME) and degree of acetyl esterification (DAE) values (%) were calculated from the molar ratio of methanol and acetic acid, respectively, to GalA. To calculate the DE (%), the μmol of ester equivalents are simply divided by the μmol of GalA for the given pectin sample.

Analysis of neutral sugar composition

The isolated pumpkin pectin was hydrolyzed with 2 M trifluoroacetic acid (TFA) at 121 °C for 2 h. TFA was then removed by evaporation under gentle N_2 gas flow. Sugar components of the pumpkin pectin were analyzed by high-performance anion exchange chromatography (HPAEC). HPAEC analysis was done using a Dionex Series 4500 HPLC system equipped with an analytical CarboPac PA1 column (Dionex Co., Sunnyvale, Calif.) and PAD. Separation of individual monosaccharide constituents was conducted isocratically using eluent A (15 mM NaOH) up to 20 min, and then followed by a linear gradient from 20 to 40 min with eluent B (600 mM sodium acetate + 150 mM NaOH) (Seo and others 2004).

Structural properties of pumpkin pectin

In order to determine the molecular properties of the pumpkin pectin, the lyophilized pectin sample was stirred in 50 mM $NaNO_3$ until dissolved, and filtered with a 0.2 μm Millex HV filter (Millipore Corp., Billerica, Mass., U.S.A.) prior to high-performance size exclusion chromatography (HPSEC) analysis. HPSEC used 2 PL-Aquagel OH-60 columns and 1 OH-40 column (Polymer Labs) in that order, an injection volume of 200 μL, and samples were run in triplicate. Weight-average molecular weight (M_w), intrinsic viscosity ([η_w]), and the z-average radius of gyration (R_{gz}) for pectin samples were determined using 50 mM $NaNO_3$ as an eluent (0.7 mL/min) for HPSEC combined with multiangle laser light scattering (Dawn DSP, Wyatt Technology Corp., Santa Barbara, Calif.), refractive index (Optilab DSP, Wyatt Technology Corp.), and differential pressure viscometer detectors (Viscotek Corp., Houston, Tex., U.S.A.) (Hotchkiss and others 2002).

Statistical analysis

A one-way analysis of variance (ANOVA) procedure of SPSS (Version 17, SPSS Inc., Chicago, Ill., U.S.A.) was applied. Means were used to compare differences. Duncan test was applied to compare the mean values of each group.

Results and Discussion

Extraction yield of pumpkin pectin

From 1.5 g of fine powder of lyophilized pumpkin flesh, the pectin was extracted using various conditions and the extraction yield was determined. As a preliminary experiment of simple hydrochloric acid extraction, 1.0 g of pumpkin flesh powder was used to evaluate the effect of pH and temperature on pectin extraction yield. While the extraction yield at pH 1.5 increased from 4.3% to 5.2% with the temperature increase from 65 to 85 °C (Figure 1), this increase was not evident at pH 1.0 and 2.0. Thus, temperature was not a critical factor to affect the pectin yield using hydrochloric acid within this range. In contrast, pH strongly

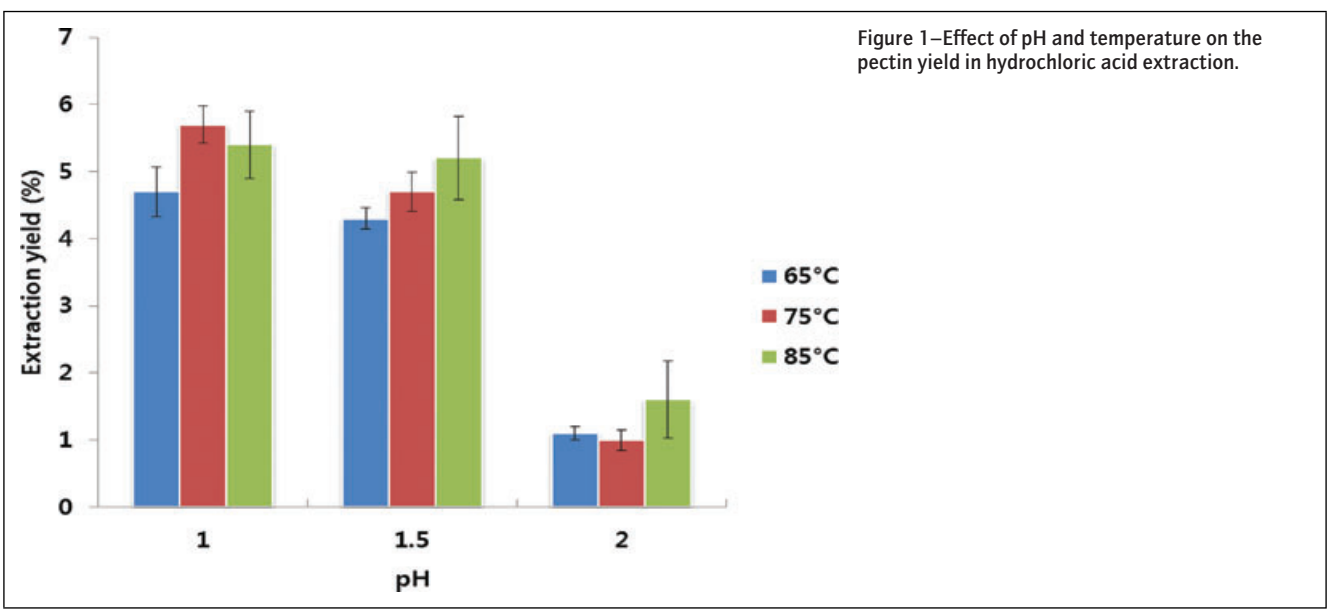

Figure 1–Effect of pH and temperature on the pectin yield in hydrochloric acid extraction.

Table 2–Neutral sugar composition and galacturonic acid content of pumpkin pectin samples obtained using different extraction methods (% wt).

Pectin sample	Rhamnose (Rha)	Arabinose (Ara)	Galactose (Gal)	Glucose (Glu)	Xylose (Xyl)	Galacturonic acid (GalA)
A	1.26 ± 0.01^{c}	0.95 ± 0.01^{b}	5.18 ± 0.01^{b}	14.8 ± 0.1^{c}	0.43 ± 0.03^{a}	58.6 ± 1.5^{ab}
B	1.88 ± 0.05^{a}	0.86 ± 0.01^{c}	5.37 ± 0.03^{a}	15.3 ± 0.0^{b}	0.26 ± 0.01^{b}	54.7 ± 1.9^{bc}
C	1.43 ± 0.01^{b}	0.45 ± 0.01^{d}	3.46 ± 0.01^{c}	9.43 ± 0.06^{d}	0.21 ± 0.02^{c}	58.9 ± 2.8^{a}
D	1.40 ± 0.02^{b}	2.36 ± 0.02^{a}	3.05 ± 0.02^{d}	20.3 ± 0.3^{a}	0.21 ± 0.01^{c}	51.0 ± 1.9^{c}

Values superscripted with different letters in each column are statistically different ($P < 0.05$).

Table 3–Methanol and acetic acid contents, and GalA esterification in pumpkin pectin samples.[A]

Pectin sample	Methanol content[B]	Acetic acid content[B]	Methanol composition (%)[C]	Acetic acid composition (%)[C]	Degree of methyl esterification (DME, %)[D]	Degree of acetyl esterification (DAE, %)[D]
A	1.89 ± 0.02^{a}	ND[E]	6.05 ± 0.06^{a}	0^{b}	63.0 ± 0.67^{a}	0^{b}
B	1.56 ± 0.12^{b}	0.093 ± 0.019^{a}	5.00 ± 0.4^{b}	0.56 ± 0.11^{a}	55.3 ± 4.3^{b}	3.30 ± 0.67^{a}
C	1.71 ± 0.1^{b}	0.076 ± 0.011^{a}	5.47 ± 0.3^{a}	0.46 ± 0.07^{a}	56.3 ± 3.2^{b}	2.50 ± 0.33^{a}
D	1.37 ± 0.06^{c}	0.081 ± 0.018^{a}	4.38 ± 0.19^{c}	0.49 ± 0.11^{a}	52.1 ± 2.3^{b}	3.08 ± 0.68^{a}

[A]Each pectin sample was prepared in duplicate and subsequently analyzed in triplicate.
[B]Methanol and acetic acid contents were determined as μmol per 1.0 mg of pectin (±standard deviation).
[C]Methanol and acetic acid compositions were determined as mg per 1.0 mg of pectin (±standard deviation).
[D]Degree of methyl and acetyl esterification is percent molar ratio with galacturonic acid equivalents.
[E]ND, not detectable.
Values superscripted with different letters in each column are statistically different ($P < 0.05$).

affected the yield, and extraction yield was better at lower pH. The pectin yield drastically dropped from 5.7% (pH 1.0) to 1.0% (pH 2.0) as the pH increased. When the pH increased to 2.5, no pectic material was solubilized from pumpkin flesh powder. Microwave extraction was then evaluated for improving extraction yield of pumpkin pectin. In the initial experiment, microwave heating at 74 °C for 10 min resulted in only 4.0% extraction yield, and this was not improved with pretreatment at 4 °C and pH 1.0 for 16 h. This resulted in a lower yield (6.2%) than that by of conventional acid extraction at pH 1.0 and 65 °C for 2 h with the same pretreatment. Various microwave heating conditions were tested (data not shown, Supplement 1), and 3 programmed extraction methods were further compared with the traditional hot acid method. The extraction methods are summarized in Table 1. After the pretreatment of pumpkin powder at pH 1.0 and 4 °C for 16 h, a flash microwave-assisted extraction at 120 °C for 3 min resulted in a 10.5% pectin yield (Table 1). Premicrowaving at 60 °C for 20 min did not improve the extraction yield for this flash microwave-assisted extraction. When microwave heating at 80 °C for 10 min was applied after this premicrowave treatment, the final pectin yield was 11.3%. When the pH was adjusted to 2.0, the yield dropped to 7.7% under the same extraction conditions, as shown in Table 1. However, this extraction yield was much higher than that obtained from the traditional hot acid extraction procedure.

Galacturonic acid content and esterification degree of pumpkin pectin

The structural characteristics of the extracted pumpkin pectins were determined by analyzing GalA content and DME and DAE. As shown in Table 2, the GalA content in the pectin C (58.9%) was greatest and was not significantly different from that in the pectin A (58.6%), and then followed by pectin B (54.7%) and D (51.0%) in that order. Thus, the microwave heating may be an efficient tool to extract pumpkin pectin without quality loss.

While the GalA content in pumpkin pectin (51% to 58%) was lower than other pectins such as apple (73.5%), citrus (72.1%), methylated lime (89.0%), and sugar beet (65%) (Ptitchkina and others 1994; Savary and Nuñez 2003), the DME values of the extracted pumpkin pectins A, B, C, and D were 63%, 55%, 56%, and 52%, respectively (Table 3). Previously, it was reported that the HCl-extracted pumpkin pectin at 65 °C and pH 1.0 for 2 h had 50% and 60% GalA content and unusually high content of neutral monosaccharides (Ptitchkina and others 1994; Shkodina and others 1998). In this study, the composition of neutral sugars in the pectin backbone was analyzed by HPAEC after 2 M TFA hydrolysis (Table 2). As a result, the contents of glucose and galactose in the pumpkin pectin were significantly higher than those of commercial citrus pectin (Shkodina and others 1998), while less than 2.5% of other neutral monosaccharides (Rha, Ara, and Xyl) existed as constituents in the pectin structure. Unlike the previous study (Shkodina and others 1998), mannose was not detected from the pumpkin pectins extracted from our study. When the DAE was determined, there was no detectable amount of acetyl esters on the pectin backbone from the acid-extracted pectin A. The pectins from microwave-assisted extraction (B-D) had DAE levels of 2.5% to 3.3% with no significant difference (Table 3). Previously, it was reported that enzymatically extracted pumpkin pectin contained significantly high amount of acetyl group (26%), which is even higher than DAE of sugar beet pectin (19%) (Matora and others 1995). Possibly, exposure to strong acidic condition at high temperature in the present study led to greater hydrolysis of acetyl esters.

Molecular weight and intrinsic viscosity of pumpkin pectin

The molecular structure and physical property of the isolated pumpkin pectins were determined using HPSEC equipped with light scattering and viscometry detectors. Weight-average molecular weight (M_w) and intrinsic viscosity (η_w) of microwave-extracted pectins B and C were substantially lower than those of the traditional hot-acid-extracted pectin A, whereas polydispersity (that is, M_w/M_n and M_z/M_n) was greater (Table 4). However, the pectin D extracted at pH 2.0 had more than 5 times greater M_w than did the pectin C that was extracted at pH 1.0 when microwave heating was applied to extract pectin. The η_w of pectin D was almost twice higher than other microwave-extracted pectins, which is comparable to that of acid-extracted pectin A. Molecular weight of pectin is one of the important factors to determine its quality because low molecular weight results in an undesirable weak gelling property.

Table 4–Molecular properties of pumpkin pectin samples.

Pectin sample	$M_w(\times 10^{-5})$	M_w/M_n	M_z/M_n	R_{gz}(nm)	$[\eta_w]$ (dL/g)	A[A]
A	$8.5 \pm 0.2^{b,B}$	1.0 ± 0.2^{c}	1.2 ± 0.03^{c}	47.2 ± 0.6^{b}	9.5 ± 0.02^{a}	1.2 ± 0.07^{a}
B	4.3 ± 0.01^{c}	1.6 ± 0.0^{b}	2.0 ± 0.0^{b}	43.3 ± 0.4^{c}	5.8 ± 0.2^{c}	1.0 ± 0.01^{b}
C	3.2 ± 0.1^{d}	2.0 ± 0.1^{a}	3.3 ± 0.1^{a}	46.7 ± 0.8^{b}	5.0 ± 0.01^{d}	0.5 ± 0.01^{d}
D	17.1 ± 0.1^{a}	1.0 ± 0.0^{c}	1.0 ± 0.0^{d}	62.3 ± 1.0^{a}	9.0 ± 0.1^{b}	0.66 ± 0.01^{c}

[A]Mark–Houwink exponent.
[B]Standard deviation of triplicate analysis.
Values superscripted with different letters in each column are statistically different ($P < 0.05$).

The "**a**," Mark–Houwink exponent, is correlated with the ratio of the molecular volume to the molecular weight, which is able to provide information of molecular shape. In general, "**a**" decreases as the shape of the polymer becomes more compact, resulting in a spherical form. The "**a**" value for pectin was obtained from the slope of plot of log η_w against log M_w of the best linear least-squares line through each curvilinear data. The pH did not seem to affect the molecular shape of pectins because the compactness of the pectins C and D was very close to each other. Theoretically these pectins can display a random coil shape in aqueous dispersion. On the other hand, the pectins extracted by traditional hot acid extraction (pectin A) and flash microwave-assisted extraction (pectin B) behaved more like a rod shape structure. Thus, the molecular structure of pumpkin pectin can be significantly affected depending on the extraction method.

Conclusions

Pumpkin pectin has its own unique structure that can dictate different physical properties compared to commercial pectins. Galacturonic acid content of the pumpkin pectin is lower than commercial ones, while greater amount of neutral sugars and acetyl esters exists in the pumpkin pectin structure. This suggests that pumpkin pectin has a highly branched structure consisting of neutral sugars, and so-called rhamnogalacturonan I. These structural characteristics have promoted greater gelling (Ptitchkina and others 1994) and bread loaf volume properties (Ptitchkina and others 1998). The extraction yield of pumpkin pectin was greatly improved using microwave heating without the loss of pectin quality; thus, further study should be conducted to better characterize the relationship between chemical structure and physical properties with functional properties of the pumpkin pectin.

Acknowledgments

The study was carried out with the support of On-Site Cooperative Agriculture Research Project (Nr. 200712A01033180), RDA, Republic of Korea. We thank Andre White for his technical assistance.

References

Adolfo AC, Michael H. 2005. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. J Ethnopharmacol 99:325–48.
De Escalad pla MF, Ponce NM, Stortz CA, Gerschenson LN, Rojas AM. 2007. Composition and functional properties of enriched fiber products obtained from pumpkin (*Cucurbita moschata* Duch ex Poiret). LWT-Food Sci Technol 40:1176–85.
Evageliou V, Ptitchkina NM, Morris ER. 2005. Solution viscosity and structural modification of pumpkin biopectin. Food Hydrocol 19:1032–6.
Filisetti-Cozzi TMC, Carpita NC. 1991. Measurement of uronic acids without interference from neutral sugars. Anal Biochem 197:157–62.
Fishman ML, Chau HK, Hoagland P, Ayyad K. 2000. Characterization of pectin, flash-extracted from orange albedo by microwave heating, under pressure. Carbohydr Res 323:126–38.
Fissore EN, Matkovic L, Wider E, Rojas AM, Gerschenson LN. 2009. Rheological properties of pectin-enriched products isolated from butternut (*Cucurbita moschata* Duch ex Poiret). LWT-Food Sci Technol 42:1413–21.
Hotchkiss AT, Savary BJ, Cameron RG, Chau HK, Brouillette J, Luzio GA, Fishman ML. 2002. Enzymatic modification of pectin to increase its calcium sensitivity while preserving its molecular weight. J Agric Food Chem 50:2931–7.
Hwang J, Pyun YR, Kokini JL. 1993. Side chains of pectins: some thoughts on their role in plant cell walls and foods. Food Hydrocol 7:39–53.
Ishii T, Matsunaga T. 1996. Isolation and characterization of a boron-rhamnogalacturonan-II complex from cell walls of sugar beet pulp. Carbohydr Res 284:1–9.
Jia W, Gao W, Tang L. 2003. Antidiabetic herbal drugs officially approved in China. Phytother Res 17:1127–34.
Jun HI, Lee CH, Song GS, Kim YS. 2006. Characterization of the pectic polysaccharides from pumpkin peel. LWT-Food Sci Technol 39:554–61.
Kobayashi M, Matoh T, Azuma J-I. 1996. Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. Plant Physiol 110:1017–20.
Kuhlman H, Koetter U, Theurer C. 1999. Sterol contents in medicinal pumpkin (*Cucurbita pepo convar. citrullinina var. styrica*) depending on genotype and location. Acta Horticulturae 492:175–8.
Matora AV, Korshunova VE, Shkodina OG, Zhemerichkin DA, Ptitchkina NM, Morris ER. 1995. The application of bacterial enzymes for extraction of pectin from pumpkin and sugar beet. Food Hydrocol 9:43–6.
Murkovic M, Mülleder U, Neunteufl H. 2002. Carotenoid content in different varieties of pumpkins. J Food Comp Anal 15:633–8.
O'Neill MA, Albersheim P, Darvill AG. 1990. The pectic polysaccharides of primary cell walls. In: Dey DM, editor. Methods in plant biochemistry. London: Academic Press. p 415–41.
O'Neill MA, Warrenfeltz D, Kates K, Pellerin P, Doco T, Darvill AG, Albersheim P. 1996. Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plants, forms a dimer that is covalently cross-linked by a borate di-ester. J Biol Chem 271:22923–30.
Oosterveld A, Beldman G, Schols HA, Voragen AGJ. 1996. Arabinose and ferulic acid rich pectic polysaccharides extracted from sugar beet pulp. Carbohydr Res 288:143–53.
Ou S, Kwok K, Li Y, Fu L. 2001. In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. J Agric Food Chem 49:1026–9.
Ovodov YS. 2009. Current views on pectin substances. Rus J Bioorg Chem 35:269–84.
Popovic M. 1971. On growing squash and pumpkin (*Cucurbita sp.*) in Yugoslavia. Savremena Poljoprivreda 11:59–71.
Ptitchkina NM, Danilova IA, Doxastakis G, Kasapis S, Morris ER. 1994. Pumpkin pectin: gel formation at unusually low concentration. Carbohydr Polym 23:265–73.
Ptitchkina NM, Markina OA, Rumyantseva GN. 2008. Pectin extraction from pumpkin with the aid of microbial enzymes. Food Hydrocol 22:192–5.
Ptitchkina NM, Novokreschonova LV, Piskunova GV, Morris ER. 1998. Large enhancements in loaf volume and organoleptic acceptability of wheat bread by small additions of pumpkin powder: possible role of acetylated pectin in stabilising gas-cell structure. Food Hydrocol 12:333–7.
Ridley BL, O'Neill MA, Mohnen D. 2001. Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochem 57:929–67.
Rolin C. 2002. Commercial pectin preparations. In: Seymour GB, Knox JP, editors. Pectins and their manipulation. Oxford: Blackwell Publ. p 1711–6.
Savary BJ, Nuñez A. 2003. Gas chromatography–mass spectrometry method for determining the methanol and acetic acid contents of pectin using headspace solid-phase microextraction and stable isotope dilution. J Chromatogr A 1017:151–9.
Seo EJ, Yoo SH, Oh K, Cha J, Lee HG, Park C. 2004. Isolation of an exopolysaccharide-producing bacterium, Sphingomonas sp. CS101, which forms an unusual type of sphingan. Biosci Biotechnol Biochem 68:1146–8.
Shkodina OG, Zeltser OA, Selivanov NY, Ignatov VV. 1998. Enzymic extraction of pectin preparations from pumpkin. Food Hydrocol 12:313–6.
Yoo SH, Fishman ML, Hotchkiss Jr. AT, Lee HG. 2006. Viscometric behavior of high-methoxy and low-methoxy pectin solutions. Food Hydrocol 20:62–7.
Zhang Y, Yao H. 2002. Composition analysis of pumpkin polysaccharide and its glucatonic effect. J Wuxi Univ Light Ind 212:173–5.
Zhang H. 2003. Determination of γ-amino-butyric acid and amino acids in pumpkin. Food Res Dev 243:108–9.

Supporting Information

The following supporting information is available for this article:

Supplement 1. Effect of the extraction methods on pectin yield.

Supporting Information may be found in the online version of this article.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.