ELSEVIER

Food Chemistry 65 (1999) 461–467

Food Chemistry

Preparation of soy hull pectin

Ravin Gnanasambandam*, A. Proctor

Department of Food Science, University of Arkansas, 272 Young Avenue, Fayetteville, AR 72704, USA

Received 20 April 1998; accepted 15 September 1998

Abstract

Soy hull, a co-product of soybean processing, was investigated as a source of pectin. Pectin content of soy hull extracts expressed as percent galacturonic acid content, varied from 76.7% to 88.3%. Enzymatic pretreatment of soy hulls increased content of alkali soluble pectins. X-ray diffraction patterns of soy hull pectins were similar to those of commercial citrus pectin; however, enzymatic pretreatment increased X-ray diffraction intensities corresponding to interatomic distances of 17.5, 7.5, 4.3, 3.0, 2.9, and 2.7 Å. Fourier transform infrared spectroscopy of soy hull pectins revealed differences in the region between 1800 and 1600 cm^{-1}. Size exclusion high performance liquid chromatography was useful in identifying soy hull pectins.

Keywords: Value added; Soy hull; Pectins; FTIR; X-ray diffraction; HPLC

1. Introduction

Pectins play an important role in food processing as food additives and as a source of dietary fiber. Pectin gels are very important in creating or modifying the texture of jams, jellies, confectionery, and in low fat dairy products. They are also used as ingredients in pharmaceutical preparations such as antidiarrheal, detoxicant and demulcent formulations and they affect glucose metabolism by lowering the glucose response curve (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Pectin is considered a safe additive with no limits on acceptable daily intake (FAO, 1969).

In order to understand their type and content, pectins are separated based on their solubility by sequential extraction in water or buffer solutions, solutions of chelating agents, dilute acids, or dilute sodium hydroxide or sodium carbonate (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Enzymes are also used in the extraction of pectins (Saulnier, & Thibault, 1987; Renard, Searle-van Leeuwen, Voragen, Thibault, & Pilnik, 1991). Two major approaches of enzymatic extraction include (i) degradation of the rhamnogalacturonan backbone using enzymes such as endo-polygalacturonase, or a combination of pectin esterase/endo-polygalacturonase, and, (ii) use of non-pectolytic enzymes (non-degradative) such as endo-arabinase, and endo-galactanase. Less degradative non-pectolytic enzymes might be useful in preparation of pectin ingredients in their native state without substantially altering their properties.

Factors that influence the functionality of pectins include their composition, DM, solubility, pH, temperature, and presence of soluble solids. Typically, the objective of commercial extraction of pectins is to obtain water-soluble preparation of pectins of high molecular weight with specified (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Commercial extraction of pectin utilizes citrus peels containing 25% pectin, and dried apple pomace containing 15–18% pectin (Walter, 1991a). Other sources of pectin include sugar beets, sunflower heads, onion skins, tobacco leaves, residues of mango, guava, papaya, coffee, and cocoa processing. Currently, more than half of the pectins used in the food industry are extracted from citrus peels (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995).

The seed coat of soybeans, also known as soy hulls, are a major co-product of the soybean processing industry. Soy hulls constitute about 8% of the whole seed and contain about 86% complex carbohydrates thus making them a source of dietary fiber. The insoluble carbohydrate fraction of soy hull cell walls consist of 30% pectin, 50% hemicellulose, and 20% cellulose. (Snyder, & Kwon, 1987). Soy hulls, as a source of dietary fiber, have been shown to reduce blood serum cholesterol (Mahalko, Sandstead, Johnson, Inman, Milne,

* Corresponding author. Tel.: +1 501 575-6823; fax: +1 501 575-6936; e-mail: raving@comp.uark.edu

0308-8146/99/$ - see front matter
PII: S0308-8146(98)00197-6

Warner, & Haubnz, 1984). Johnson, Berry, & Weaver (1985) reported use of soybean hulls as a fiber supplement in bakery products. Increased consumption and potential health benefits of soy-based foods has created a need for studies on the pectins of soy hull as an ingredient, as well as their role in soy-based food systems. Currently, soy hulls are worth about $67 a ton (Farmland Industries, Kansas City, KS, personal communication, 1997). Soy hulls due to their pectin content and availability are a natural, inexpensive source of food grade pectins.

The objectives of this study were to (i) to characterize the pectic fraction of soy hull by sequential extraction, (ii) prepare soy hull pectins with maximum pectin yield and investigate an enzymatic pretreatment for soy hull pectin extraction, and (iii) elicit the structure of pectins using techniques including X-ray diffraction and infrared spectroscopy (FTIR).

2. Materials and methods

2.1. Sequential extraction of soy hull pectins

Soy hulls were obtained from Riceland Foods (Stuttgart, Arkansas), ground to a particle size of < 100 mesh, and extracted sequentially with O.1 N HNO_3, 0.5% sodium hexametaphosphate, and 0.05 N NaOH. Each extraction was done by mixing ground soy hulls and the solvent (1:20) in a 1000 ml round bottom flask 90°C for 40 min in a Rotavapor (BUCHI R-114, Switzerland), cooling to room temperature in a water bath, and centrifuging at $2700 \times g$ for 15 min (CRU 5000, IEC, Needham Heights, MA). Supernatants obtained from individual extractions were dispersed in equal volumes of 2-propanol to precipitate the pectins, and allowed to settle for about 4 h. The precipitate was collected, centrifuged, dispersed in 2-propanol, stirred for 30 min, and centrifuged. This was repeated with 2-propanol one more time and finally with 70% 2-propanol. The sediment was dispersed in a small amount of distilled deionized (dd) water and freeze dried.

The sequential extraction was repeated using ground soy hulls treated with a cell wall degrading enzyme as follows: ground soy hull was incubated with 0.1% (v/v) mannanase (Mannanase and Xylanase, Enzeco, New York; Activity 40,000 mannanase Units (MNU) per ml; one MNU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one nmol mannose/s, under assay conditions) in 50°C water at pH 5.5 for 60 min before acid extraction with 0.1 N HNO_3. The pH was maintained at pH 5.5 throughout by adding 0.01 N HNO_3. Sequential extraction of enzyme- pretreated ground soy hulls was carried out as detailed above.

2.2. Preparation of total soy hull pectins by acid extraction

Ground soy hulls were extracted with 0.1 N HNO_3, (1:20), at 90°C for 40 min in a rotary evaporator (Rotavapor, BUCHI R-114, Switzerland), cooled to room temperature in a waterbath, and centrifuged ($2700 \times g$, 15 min, CRU 5000, IEC, Needham Heights, MA). The supernatant was collected, and the sediment was extracted twice more in 0.1 N HNO_3. All the three supernatants were combined and dispersed in equal volumes of 2-propanol to precipitate the pectins, and allowed to settle for about 4 h. The precipitate was collected, centrifuged, dispersed in 2-propanol, stirred for 30 min and centrifuged. This was repeated one more time with 2-propanol and, finally, with 70% 2-propanol. The sediment was dispersed in a small amount of dd water and freeze dried. Acid-extracted soy hull pectins were also prepared from ground soy hull samples that were enzyme pretreated using the protocol as described before. The pectins obtained by sequential extraction and the total pectin extracts were each subjected to the following analyses:

2.3. Pectin and methoxyl contents

Pectin contents of the sample was determined by a colorimetric method using m-hydroxydiphenyl (Kintner, & Van Buren, 1982). Degree of esterification was expressed as percent methoxy groups as determined by a titration method (Gould, & Davis, 1954).

2.4. Galacturonic acid content by high performance liquid chromatography

A high performance liquid chromatography (HPLC) method was also developed for identifying soy hull pectins. Twenty mg of soy hull pectins were dispersed in 100 ml of 0.01 N Phosphoric acid and stirred for 1 h at room temperature. The samples were adjusted to pH 4.5, 100 mg of pectinase (Enzeco, New York, NY) was added, incubated at 55°C for 18 h, centrifuged, and the supernatants filtered through 0.22 μm filters for HPLC analysis.

A HPLC (Spectra SYSTEM AS 1000, Spectra Physics, Fremont, CA) with a size exclusion column (Ultrahydrogel 2000, Waters Corporation, Milford, MA) and a guard column (Ultrahydrogel Guard Column, Waters Corporation, Milford, MA) with a forward optical scanning detector (Spectra Focus, Spectra-Physics, Fremont, CA) was used to determine galacturonic acid content of the soy hull pectins. D-galacturonic acid monohydrate (Sigma) was used as standard. Phosphoric acid (0.01 N) with a flow rate of 0.70 ml/min was used as the mobile phase. Ten μl samples were injected and the detection wavelength was 200 nm.

2.5. Pectin solubility

Pectin samples (1.6% w/v) were dissolved in phosphate buffer (pH 6.0), and percent galacturonic acid contents of insoluble and soluble fractions determined (Kintner, & Van Buren, 1982).

2.6. X-ray diffraction

X-ray diffraction patterns of soy hull pectin samples were obtained using a Philips X-ray diffractometer (X'pert, Philips Electrical, The Netherlands) at a generator voltage of 45 kV, and diffraction angle of 4–50° 2θ. A commercial citrus pectin (Sigma P-9135, galacturonic acid content, 79%, and methoxyl content 8%, Sigma Chemical St. Louis, MO) was used for comparison.

2.7. Fourier transform infrared spectroscopy

Diffuse reflectance Fourier transform infrared (FTIR) spectra of soy hull pectin samples were obtained using a Nicolet Model 410 FT-IR instrument (Nicolet Analytical Instruments, Madison, Wisconsin). Spectra of the samples were obtained by co-adding 100 scans at a resolution of 4 cm^{-1} and compared with the commercial citrus pectins.

2.8. Statistical analysis

A minimum of three replications of the experiment were performed in completely randomized design and least square means procedures were used to analyze the data (SAS, 1994). The differences reported were significant ($P < 0.05$) unless specified otherwise.

3. Results and discussion

3.1. Sequential extraction of soy hull pectins

Data on the sequential extraction of soy hull pectins are presented in Table 1. Enzyme pretreatment resulted in an increased water soluble fraction of pectins extracted (54.98% and 64.25% for control and enzyme pretreatment, respectively) ($P < 0.05$). However, galacturonic acid content of other fractions were not significantly affected. Enzymatic pretreatment resulted in significant changes in the proportions of pectins extracted in different solvents. Extractions without enzyme pretreatment had a higher proportion of water soluble pectins, while enzymatic pretreatment resulted in a significant increase in alkali soluble pectins with a proportionate decrease in water soluble portion. Enzymatic degradation of cell wall hemicellulose would facilitate the solubilization of high molecular weight protopectins (alkali soluble), thereby increasing their content in the final product. There appeared to be a slight decrease (non significant) in the methoxyl content of aqueous and SHMP extracted pectins from enzyme pretreatments. The enzyme used in the present study (mannanase) has primary activities of mannanase and xylanase and trace amounts of acid protease, according to the manufacturer's specification. However, it was observed by these authors that hydrolysis of the substrate (soy hull) by mannanase possibly produced galacturonic acid as indicated by a HPLC peak at elution times matching those of galacturonic acid standards. Trace amounts of pectolytic activity of mannanase might account for the differences between samples. Although acid extraction alone might be effective in the preparation of pectin ingredients, enzymatic pretreatment would help to manipulate the quality and contents of different pectin fractions, thus be useful in the preparation of specific food ingredients.

3.2. Total soy hull pectin content by acid extraction

Yield, percent galacturonic acid, and methoxyl contents of soy hull pectins are presented in Table 2. Differences were observed in both yield and content of soy hull pectins (expressed as percent galacturonic acid). Acid extraction of soy hull (0.1 N HNO_3, 40 min at 90°C) resulted in a 76.7% pectin sample with 15% yield. However, enzymatic pretreatment resulted in a pectin

Table 1
Sequential extraction of soy hull pectins: galacturonic acid and methoxyl contents, and percent of the total pectins of control and enzyme pretreatment

Extraction	Galacturonic acid (%)	Methoxyl content (%)	Percent of the total pectins
Aqueous extraction	54.98 [a]	7.53	59 [a]
Aqueous extraction with enzyme pretreatment	64.25 [b]	5.98	34 [b]
SHMP [b] extraction	67.41	6.12	6.8
SHMP extraction with enzyme pretreatment	61.78	4.21	1.6
NaOH extraction	65.6	6.87	34 [a]
NaOH extraction with enzyme pretreatment	63.2	6.44	56.8 [b]

[a,b] Mean values with different superscripts in the same column in each extraction are significantly different ($P < 0.05$). SHMP and NaOH are sodium hexametaphosphate (0.5%) and sodium hydroxide (0.05 N), respectively.

Table 2
Yield, percent galacturonic acid and methoxyl contents of acid extracted and enzyme pretreated acid extracted soy hull pectins

Sample	Yield (%)	Galacturonic acid (%)	Methoxyl content (%)
Acid extraction with 0.1 N HNO_3 (1:20) 90°C, 40 min	15.0	76.7 [a]	4.055 [a]
Acid extraction [*] (enzyme pre-treated, 1:20, 90°C, 40 min)	14.75	88.3 [b]	3.89 [b]

[*] Enzyme pre-treated with Mannanase (Enzeco, S-9074, Enzyme Development Corporation, New York, NY), at 0.1% v/v in dd water, pH 5.5, 50°C for 60 min.

[a,b] Mean values in the same column with different superscripts are significantly different ($P < 0.05$).

content of 88.3%, with yields comparable to that of control acid extractions (14.75% vs. 15.0%). The solubility of pectins from enzyme pre-treated and control samples in pH 6.0 phosphate buffer was 100% and 40%, respectively. Previous studies on extraction of pectins using pectolytic enzymes include use of endo-polygalacturonase, a combination of pectin esterase/endo-polygalacturonase (Renard, Searle-van Leeuwen, Voragen, Thibault, & Pilnik, 1991), endo-pectinlyase (Massiot, & Thibault, 1989), endo-pectatelyase (Konno, & Yamasaki, 1982), or rhamnogalacturonase (Renard, Thibault, Voragen, van der Broek, & Pilnik, 1993). Endo-arabinase, endo-galactanase, cellobiohydrolase, and various endo-glucanases are some of the non-pectolytic enzymes used in the pectin extraction (Renard, Searle-van Leeuwen, Voragen, Thibault, & Pilnik, 1991).

Methoxyl contents of control acid extractions were higher compared to those of enzyme pretreated acid extractions. Percent methoxyl groups refer to the quantity of methoxyl groups in a sample whereas degree of esterification (DE) refers to the ratio of carboxyl groups esterified with methanol to free carboxyl groups. Methoxyl content in pectins is an important chemical property that determines the pectin functionality. In the present study, increased extractability of pectins due to enzymatic degradation of soy hull might have rendered the pectins more prone for demethylation during acid hydrolysis, resulting in the reduction of methoxyl groups. It is also possible that trace amounts of methyl esterases present in mannanase might contribute to demethylation.

3.3. X-Ray diffraction studies

X-ray diffraction patterns of soy hull pectins are presented in Fig. 1. Samples from acid extraction showed similar X-ray diffraction patterns to those of commercial pectin samples. Increased diffraction intensities were observed for both samples in the region between 10° and 30° 2θ, corresponding to interatomic distances of approximately 7 and 31 Å. This profile is similar to the X-ray diffraction patterns of cellulose. Differences between these two samples are attributed to their source. Enzymatic pretreatment resulted in major changes in X-ray diffraction patterns of soy hull pectins. The typical cellulose pattern that is observed in control extractions was replaced by well defined peaks. The susceptibility of non-pectic cell wall polysaccharides to the enzymatic treatment might make the galacturon backbone more susceptible for acid hydrolysis. Disruption of hydrogen bonds and hydrophobic interactions of pectin molecule cross-links might have resulted in the depolymerization of pectin molecules. Increased X-ray diffraction intensities between 5° and 35° 2θ, corresponding to an interatomic distances of 17.5, 7.5, 4.3, 3.0, 2.9, and 2.7 Å indicates the presence of such shorter galacturon chains with possibly lesser crosslinking. Other possibilities for occurrence of these peaks are the presence of cellulose and residual hemicellulose as contaminant non-galacturonic polysaccharides.

3.4. FTIR spectra of soy hull pectin samples

The infrared (Diffuse Reflectance FTIR) spectra of soy hull pectin samples are presented in Fig. 2. Bands in the region between 1000–2000 cm^{-1} are typically used to identify pectin (Walter, 1991b). Bands around 1650 and 1750 cm^{-1} are indicative of free and esterified carboxyl groups which may be useful in identifying pectin samples. The FTIR spectra of soy hull pectins had features similar to that of a commercial pectin sample, except at regions corresponding to free and esterified carboxyl groups (1650 and 1750 cm^{-1}, respectively). The acid extracted soy hull pectins had similar FTIR spectra to that of enzyme pretreated samples, except the 1750 cm^{-1} stretch was shorter in case of enzyme pretreated samples. Enzymatic degradation of soy hull would loosen the cell wall matrix thereby increasing its susceptibility to acid hydrolysis of the pectin molecule resulting in a relative increase in free carboxyl groups (1650 cm^{-1} band) vs. esterified carboxyl groups (1750 cm^{-1} band). Infrared absorbance corresponding to esterified carboxyl groups was consistent with the methoxyl content of the samples (8.0%, 4.05%, and 3.89% for commercial pectins, acid extracted soy hull pectins, and enzyme pretreated acid extracted soy hull pectins, respectively). Infrared spectroscopy of uronic acid polymers is typically carried out in D_2O-phosphate buffer (Bociek, & Welti, 1975; Casu, Scovenna, Cifonelli, & Perlin, 1977). In the present study, powdered pectin samples were used in a diffuse reflectance sample kit. This procedure would eliminate laborious sampling procedures and interference due to diluents.

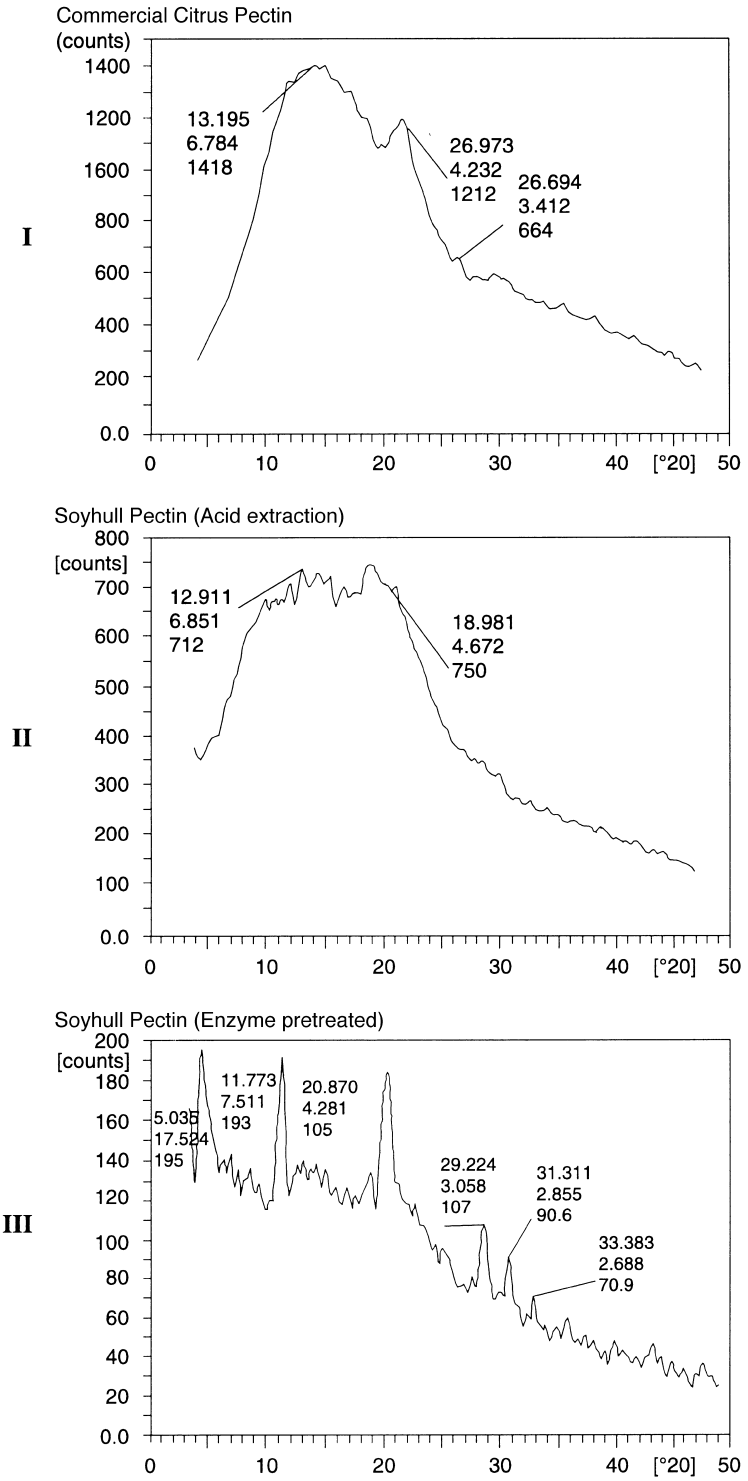

Fig. 1. X-ray diffraction patterns of soy hull pectin samples (Generator voltage of 45 kV and diffraction angle 4–50° 2θ). (I) Commercial citrus pectin. (II) Soy hull pectins from acid extractions. (III) Soy hull pectins from enzyme pretreated acid extractions.

3.5. High performance liquid chromatography

The galacturonic acid content of the soy hull pectin extracts was confirmed using a size exclusion high performance liquid chromatography (SE-HPLC) of galacturonic acid formed by pectin hydrolysis. Soy hull pectin samples had a major peak at elution times around 17.00 min, matching that of galacturonic acid standard (Fig. 3). Detection wavelength of 200 nm was chosen since samples were observed to produce a maximum absorbance

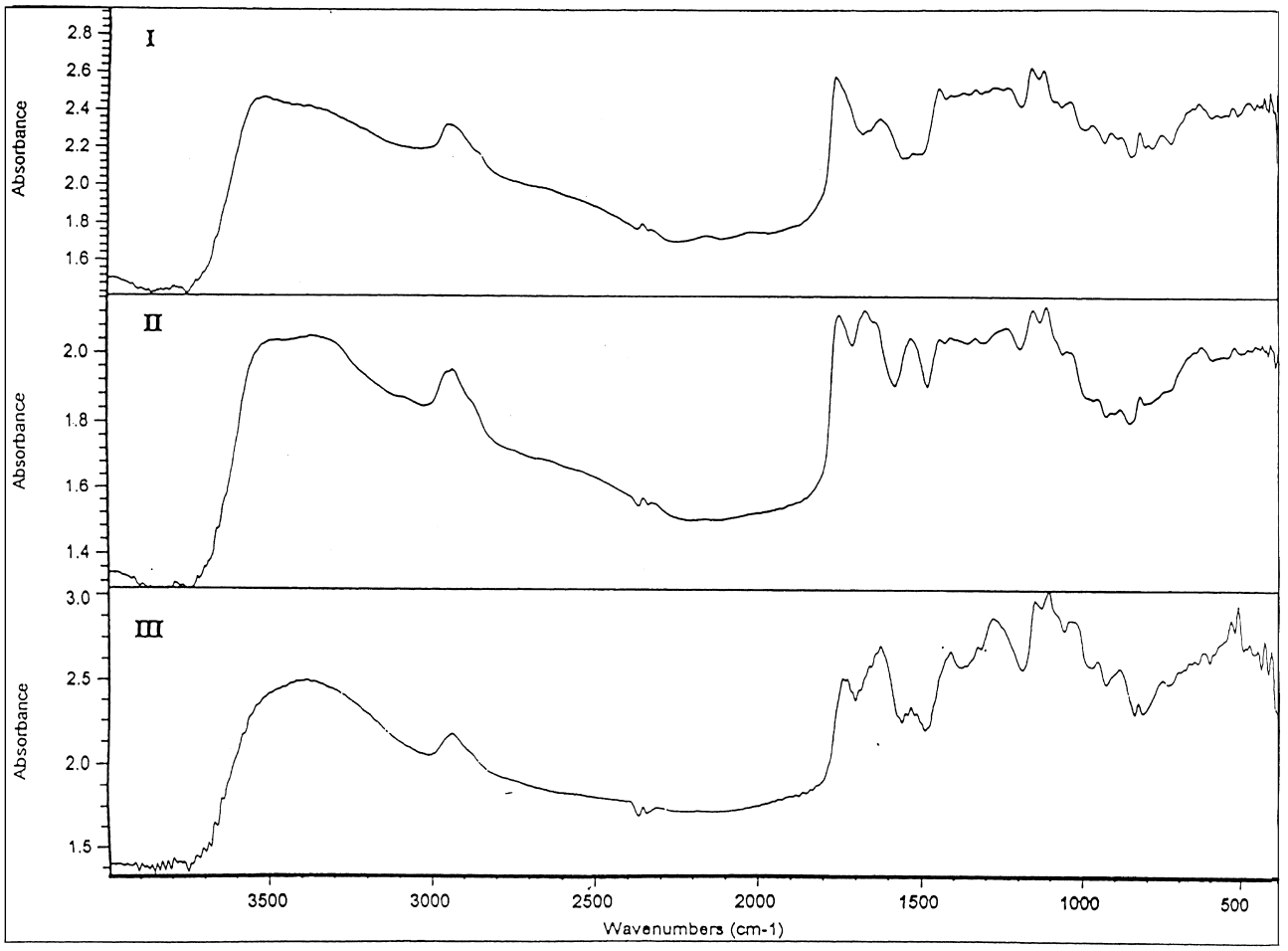

Fig. 2. Fourier Transform Infrared Spectroscopy (FTIR) of soy hull pectin samples. (I) Commercial citrus pectin. (II) Soy hull pectins from acid extractions. (III) Soy hull pectins from enzyme pretreated acid extractions.

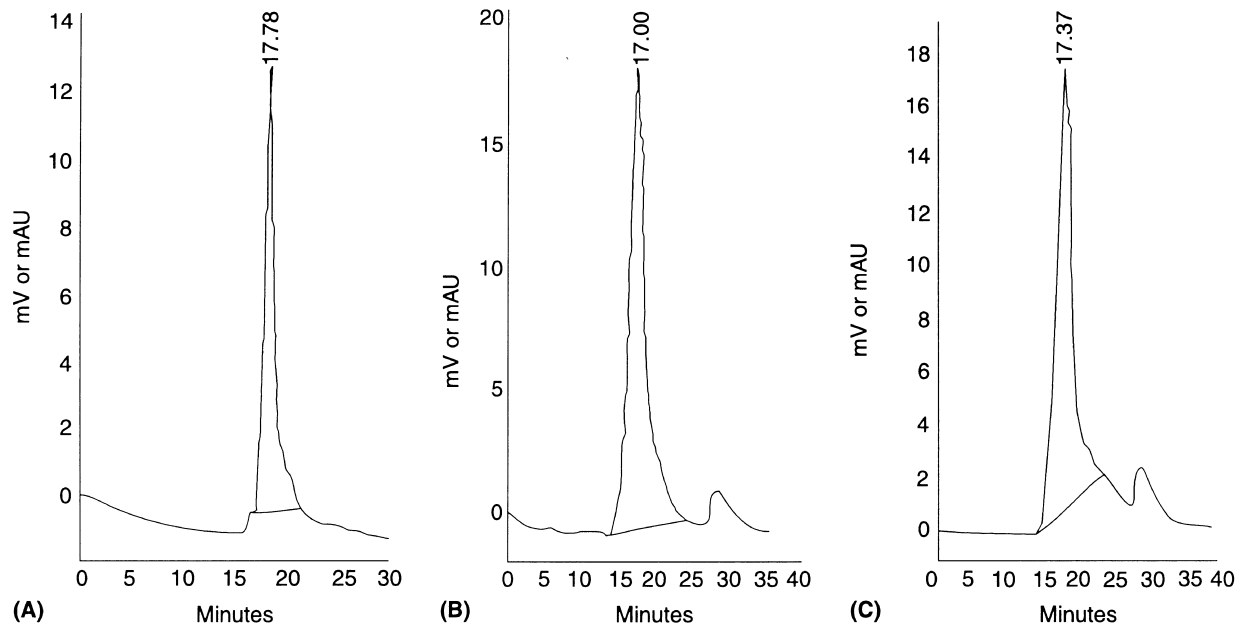

Fig. 3. Chromatogram (SE-HPLC) of pectin samples: (a) Galacturonic acid standard; (b) Pectinase hydrolyzed control soy hull pectins; (c) Pectinase hydrolyzed enzyme pretreated soy hull pectins.

spectra at that wavelength with a forward optical scanning detector (Hernandez, Lozano, & Rodriguez, Blanco, Oderiz, 1993). It has also been shown that HPLC method is more selective and does not suffer from the interferences due to other uronic acids and sugars usually associated with colorimetric techniques (Giangiacomo, Pollesello, & Marin, 1982; Forni, Pollesello, & Braga, 1987). Hence, SE-HPLC may be another reliable method of determination of soy hull pectins which might be useful for the routine analyses of commercial pectins.

Soy hull pectins prepared in the present study varied in their galacturonic acid content from 54.98% in aqueous extractions to 88.3% in enzyme-pretreated acid extraction. The percent methoxyl content showed values ranging 3.89–7.53% depending on the extraction procedure. Based on these results, galacturonic acid and methoxyl contents of soy hull pectins were similar to apple and lemon pectins (galacturonic acid content 60.8% and 76.4%, and mean methoxyl contents 4.4% and 4.6% for apple and lemon pectins, respectively) (Kravtchenko, et al., 1992). Soy hull pectins were also observed to compare well with other pectin sources including sunflower, peach, sugar-beet, and plum for their galacturonic acid and methoxyl contents (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). The pectins from soy hull were extracted using conventional acid extraction procedures used for commercial pectin preparation. In addition, using an enzyme pretreatment was observed to improved the galacturonic acid content of the pectins prepared. These studies demonstrate that soy hull, a high volume co-product of soy processing, is a potentially useful source of commercial pectin.

References

Blanco, V. M. E., Oderiz, M. L. V., Hernandez, J. L., Lozano, J. S., & Rodriguez, M. A. R. (1993). HPLC Determination of Pectins in Raspberries as Galacturonic acid and optimization using Forward Optical Scanning. *J. Chromat. Sci., 31*, 477–479.

Bociek, S. M., & Welti, D. (1975). The quantitative analysis of uronic acid polymers by infrared spectroscopy. *Carbohydrate Res., 42*, 217–226.

Casu, B., Scovenna, G., Cifonelli, A., & Perlin, S. (1977). Infrared spectra of glycosaminoglycans in deuterim oxide and deuterium chloride solution: Quantitative evaluation of uronic acid and actamidodeoxyhexose moities. *Carbohydrate Res., 44*.

FAO, (1969). *Nutrition meetings of the FAO*. Report Ser. No. 46A. p. 133.

Forni, E., Polesello, A., & Braga, F. (1987). Studies on the standardization of a combined enzymatic and HPLC method for the evaluation of pectins from their galacturonic acid content. *Food Hydrocolloids, 1*, 531–535.

Giangiacomo, R., Polesello, A., & Marin, F. (1982). Quantitative estimation of galacturonic acid in commercial pectins by HPLC. *Industrie Alimentari, 194*, 386–388.

Gould, W. A., & Davis, R. B. (1954). Effect of added calcium chloride and sodium hexametaphosphate on the pectin content and serum viscosity of tomato puree. *Food Technology, 8*, 220–224.

Johnson, C. D., Berry, M. F., & Weaver, C. M. (1985). Soybean hulls as an iron source for bread enrichment. *J. Food Sci., 50,* 1275–1277.

Kintner, P. K., & Van Buren, J. P. (1982). Carbohydrate interference and its correlation in pectin analysis using the m-hydroxydiphenyl method. *J. Food Sci., 47*, 756–759.

Konno, H., & Yamasaki, Y. (1982). Studies on the pectic substances of plant cell walls. III. Degradation of carrot root cell walls by endo pectatelyase purified from Erwinia carotovora. *Plant Physiol., 69*, 864–868.

Kravtchenko, T. P., Voragen, A. G. J., & Pilnik, W. (1992). Analytical comparison of three industrial pectin preparations. *Carbohydr. Polym. 18:17*.

Mahalko, J. R., Sandstead, H. H., Johnson, L. K., Inman, L. F., Milne, D. B., Warner, R. C., & Haubnz, E. A. (1984). Effect of consuming fiber from corn bran, soy hulls, or apple powder on glucose tolerance and plasma lipids in type II diabetes. *Am. J. Clin. Nutr., 39*, 25.

Massiot, P., & Thibault, J.-F. (1989). Enzymatic analysis of carrot cell wall polysachcharides. *Carbohydrate Res., 190*, 121.

Renard, C. M. G. C., Searle-van Leeuwen, M. J. F., Voragen, A. G. J., Thibault, J.-F., & Pilnik, W. (1991). Studies on apple protopectin. II. Apple cell wall degradation by pure polysaccharidases and their combinations. *Carbohydr. Polym., 14*, 295–299.

Renard, C. M. G. C., Thibault, J.-F., Voragen, A. G. J., van der Broek, L. A. M., & Pilnik, W. (1993). Studies on apple protopectin. VI: Extraction of pectins from apple cell walls with rhamnogalacturonase. *Carbohydrate. Polym. 22*, 203–207.

SAS (1994). *User's Guide to the Statistical Analysis System*.

Saulnier, L., & Thibault, J. F. (1987). Enzymatic degradation of isolated pectic substances and cell wall from pulp of grape berries. *Carbohydr. Polym., 7*, 345.

Snyder, H. E., & Kwon, T. W. (1987). *Soybean Utilization*. (p. 60). New York: Van Nostrand Reinhold.

Voragen, A. G. J., Pilnik, W., Thibault, J. F., Axelos, M. A. V., & Renard, C. M. G. C. (1995). Pectins. In A. M. Stephan (Ed.), *Food Polysaccharides and their Applications* (pp. 287–339). New York: Marcel Dekker.

Walter, R. H. (1991a). In R. H. Walter (Ed.), *The Chemistry and Technology of Pectin* (p. 24). New York: Academic Press.

Walter, R. H. (1991b). *Chemistry and Technology of Pectin* (p. 197). New York: Academic Press.