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Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin

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Abstract

Soy hull pectin was prepared by hydrochloride acid extraction, followed by alcohol precipitation, and the effect of extracting acid concentration and the pH of precipitating solvent on the yield and purity of pectin were investigated. Strength of acid used for extracting pectin from soy hull and the pH of precipitation had significant effects on the pectin yield. Highest yields of 26 and 28% were obtained when the acid strength was 0.05 and 0.1 N, respectively, and the pH of the precipitating solution was 3.5. Extraction of pectin with 0.2 and 0.3 N acid, or precipitation of pectin at pH 2.0, reduced pectin yield. Strength of acid used for extraction or pH of the solution during precipitation did not significantly affect the purity or degree of esterification of the product. Galacturonic acid content and degree of esterification of the products varied from 68–72% and 56–60%, respectively. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Soy hull; Pectin; Yield; Purity

1. Introduction

Pectins are used as gelling agents in jams and jellies and as a filler or stabilizer in confections, dairy products, fruit preparations, bakery fillings, icings and frostings. Development of pectin-based fat replacers has resulted in a wide range of applications in products such as spreads, salad dressings, ice cream and emulsified meat products. Pectins are complex carbohydrate molecules found in cell walls and middle lamellae of plants. Commercial pectins are galacturonoglycans with varying methyl ester contents. Pectins are commercially produced by acid extraction of citrus peel or apple pomace, followed by filtration and precipitation by 2-propanol (Whistler & BeMiller, 1997). Citrus peel, and apple pomace contain about 25 and 12% pectin, respectively, and commercial extraction yield for citrus peel is about 25% (May, 1986; Walter, 1991).

Sugar beet, and sunflower head residues, containing about 10–20% pectin, have also been considered as sources for pectin. Extraction and characterization of pectin from sugar beet and sunflower head residues have been reported (Michael, Thibault, Mereier, Heitz & Pouolladue, 1985; Miyamoto & Chang, 1992). Soy hull is a good source of pectin (about 25%) that is readily available (Gnanasambandam & Proctor, 1999). Gnanasambandam and Proctor (1999) have shown that soy hull pectin could be extracted by adapting the acid extraction method used for citrus pectin production. Dried pectin preparations (extracts), containing over 76% pectin with 15% yield, were produced by acid extraction. A pretreatment of soy hull with an enzyme, mannanase, containing mannanase and xylanase, increased the pectin content to 88.3% without significant affect on pectin yield. Commercially dried citrus pectin extracts, containing over 70% pectin are produced at a yield of 20–26% (Kravtchenko, Vorgan & Pilnik, 1992). Soy hull, a co-product of the soybean industry, may be an inexpensive pectin source and available in large quantities. Soy hull can be stored and transported without further processing, in contrast to citrus peels and apple pomace, which require a drying process to prevent deterioration during storage and transportation. For production of commercially marketable pectin from soy hull, pectin content and yield should be comparable to that of citrus products. Hence, it is necessary to optimize the pectin extraction and precipitation conditions for improved yield with desirable purity and functional properties. The objective of this research was to (1) investigate the effect of strength of the acid used for

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pectin extraction and pH of precipitating solvent, on pectin yield and purity, and (2) to establish an optimum condition for soy hull pectin production.

2. Materials and methods

Soy hull was obtained from Riceland Foods (Stuttgart, AR) and ground in a centrifugal grinding mill (Model ZM-1, Retsch/Brinkmann, Westbury, NY), three times with each pass lasting for 3 min.

2.1. Pectin production

Ground soy hull (100 g) was extracted using 0.05, 0.1, 0.2, and 0.3 N HCl (300 ml) under reflux at 90°C for 45 min. The extracts were allowed to cool to room temperature, and centrifuged at 2700×***g*** for 15 min. The supernatants were collected, and dispersed in an equal volume of 2-propanol. Pectin was precipitated by adjusting the pH of these dispersions to either 2.0 (pH of the dispersion of 0.1 N HCl extract at which pectin is highly soluble, and used for pectin precipitation in the previous study; Gnanasambandam & Proctor, 1999) or 3.5 (the pH at which pectin solubility is minimum) and allowed to settle for 4 h. The precipitate was collected, centrifuged, dispersed in 70% 2-propanol, stirred for 30 min and centrifuged. The washing was repeated with 70% 2-propanol and then with 100% 2-propanol. The product was dispersed in 25–30 ml deionized water, freeze-dried, and stored at room temperature (23–25°C).

2.2. Pectin content

The pectin content of the sample, as percent galacturonic acid, was determined by a colorimetric method using m-hydroxydiphenyl (Kinter & Van Buren, 1982). All pectin samples were solubilized by dispersing a 10-mg sample in 50 ml 1 N H_2SO_4, and stirring for 30 min at room temperature and then for 1 h at 90°C in a water bath.

2.3. Ash and moisture content

The ash content of pectin samples was determined by ashing at 660°C for 8 h. Moisture contents of the pectin samples were determined using an air oven method (AACC, 1997). Weight loss due to drying at 110°C for 12 h was reported as moisture content.

2.4. Degree of esterification

Diffuse reflectance Fourier Transform Infrared spectroscopic method, developed in our laboratory, was used to determine the degree of esterification of the soy hull pectins (Gnanasambandam & Proctor, 1999).

2.5. Structural analyses

Diffuse reflectance FTIR spectra of pectins were used to obtain information on chemical structure. Fourier transform infrared data were obtained by adding 100 inteferograms, using an Impact 410 Nicolet instrument (Analytical Instruments, Madison, WI).

2.6. Statistical analyses

Pectin yield and content were determined from triplicate measurements. Data were analyzed by analysis of variance and means were separated by the least significant difference when significant $F(P<0.05)$ values were observed (SAS, JMP IN, 1997).

3. Results and discussion

3.1. Pectin yield

The extraction temperature and time used in this study were identical to that which were used for citrus pectin extraction. A preliminary study, conducted using 0.1 N HCl extraction, indicated that, while lower temperature or shorter extraction time resulted in lower pectin extraction yield, higher temperature or longer extraction time did not affect the pectin extraction yield. Acid strength used for extracting pectin from soy hull, and the 2-propanol solution pH during pectin precipitation, had significant effects on the pectin yield (Table 1). For acid-extracted pectins, precipitation of pectin at pH 3.5 gave a significantly higher yield compared to precipitation at pH 2.0 (Table 2). The pectin yield decreased with increasing acid strength. Highest yields of 26 and 28% were obtained when the strength of the acid was 0.05 and 0.1 N, respectively, and the 2-propanol solution pH was 3.5. Extraction with 0.2 and 0.3 N acid gave lower pectin yields (18 and 21%, respectively). Strong acid solution could lead to highly soluble smaller pectin molecules, due to partial hydrolysis, and these

Table 1
Pectin content, pectin yield, and ash content of soyhull pectins, produced by extraction with HCl at varying strength, followed by precipitation with 2-propanol at pH 3.5[a]

Strength of acid used for extraction (N)	Pectin yield (% w/w)	Pectin content (% w/w galacturonic acid)	Ash (% w/w)
0.05	26a	70a	1.24a
0.10	28a	72a	1.81b
0.20	21b	68a	2.72c
0.30	18b	71a	2.88c

[a] Values are means of triplicate measurements. Values with different letters in each column are significantly ($P<0.05$) different from each other.

may not be precipitated by the addition of alcohol. Hence, a higher strength of acid resulted in lower pectin yield. The acid dissociation constant, pKa, of pectin is 3.5 (Hoefler, 1999). Hence, at pH 3.5, pectin is in a 50% ionized state, and produces a strong gel network, due to a desirable balance between hydrophilic and hydrophobic character, which might have led to lower solubility in 50% or higher 2-propanol solution. Generally, at pH 3.2–3.5, pectin gels give the highest gel strength (Hoefler, 1999; Thakur, Singh & Handa, 1997). At pH 2.0, only 3% pectin is in the ionized state. As a result of the differences in molecular interaction due to the differences in the extent of ionization, precipitation by 2-propanol at pH 2.0 resulted in a significantly lower yield compared to precipitation at pH 3.5. Increasing the precipitation pH above 3.5 did not have any significant effects on pectin yield (data not shown). Further, increasing the extraction temperature (to 100°C) or time, also did not affect the pectin yield.

3.2. Pectin, ash and moisture content

The strength of acid used for pectin extraction or pH at which pectin was precipitated, did not significantly affect the pectin content of the product. Pectin and moisture contents of the products were about 68–72 and 6–7%, respectively. Pectins extracted by 0.05 N HCl had lowest ash contents of 1.24 and 1.21%, for pH 3.5 and 2.0 precipitation, respectively. Ash content of pectins increased with increasing strength of acid used for pectin extraction. This could be due to an increased ability of acid to solubilize indigenous minerals in the hull, with increased acid strength. The solubilized mineral would then precipitate with pectin during alcohol precipitation. Ash obtained from pectin products was acid-soluble. No acid-insoluble material was detected in measurable quantities in any pectin products.

Hence, based on yield and purity, extraction of pectin with 0.05 N or 0.1 N acid and precipitation at pH3.5 would be the optimum conditions for soy hull pectin production. However, 0.05 N acid extraction has the advantage of producing a lower ash content of the pectin, with lower acid cost, when compared to 0.1 N acid extraction. The pectin content and yield of the soy hull product was comparable to those of citrus pectin, indicating the potential of soy hull as an alternate source for commercial production of pectin. Soy hull has an additional advantage over citrus peel in that soy hull can be stored and transported without further processing until used for pectin extraction.

3.3. Degree of esterification

Degree of esterification of soy pectins, as measured by FTIR, was from 53 to 60%, but with no statistically significant differences. Acid strength or 2-propanol pH did not have a significant effect on degree of esterification (Table 3).

3.4. Soy hull pectin structure

FTIR spectra in the region between 1000–2000 cm^{-1} identified the major chemical groups in the pectins (Gnanasambandam & Proctor, 1999; Wellner, Kacurakova, Malovikaova, Wilson & Belton, 1998) and provided structural information that could be used to compare different types of pectin. The FTIR spectra indicated that there were no major structural differences in pectins that were produced using different extraction conditions. The diffuse reflectance infrared spectra of pectins, produced by extraction with 0.05 N HCl followed by alcohol precipitation at pH 3.5 and 2.0, are compared to two commercial citrus pectins in Fig. 1. The carbonyl absorption bands at 1650 and 1750 cm^{-1} were from free and esterified carboxyl groups, respectively. The broader band, from 2400 to 3600 cm^{-1}, was due to adsorbed moisture in the pectin samples. The absorption peak, at 3000 cm^{-1}, was due to methyl group stretching vibrations from the methyl ester. The absorption band at 1500 cm^{-1}, in the soy hull pectin spectra, was due to OH bending vibrations. The absence of OH bending vibration in commercial citrus pectin

Table 2
Pectin content, pectin yield, and ash content of soyhull pectins, produced by extraction with HCl at varying strength, followed by precipitation with 2-propanol at pH 2.0[a]

Strength of acid used for extraction (N)	Pectin yield (% w/w)	Pectin content (% w/w galacturonic acid)	Ash (% w/w)
0.05	19a	72a	1.21a
0.10	17a	68a	2.06b
0.20	13b	69a	2.93c
0.30	12b	69a	3.21c

[a] Values are means of triplicate measurements. Values with different letters in each column are significantly ($P < 0.05$) different from each other.

Table 3
Degree of esterification of soyhull pectins, produced by extraction with HCl at varying strength, followed by precipitation with 2-propanol at pH 2.0 and 3.5[a]

Strength of acid used for extraction (N)	Degree of esterification (%) pH during 2-propanol precipitation:	
	2.0	3.5
0.05	56a	54a
0.10	53a	58a
0.20	53a	55a
0.30	60a	59a

[a] Values are means of triplicate measurements. Values with different letters are significantly ($P < 0.05$) different from each other.

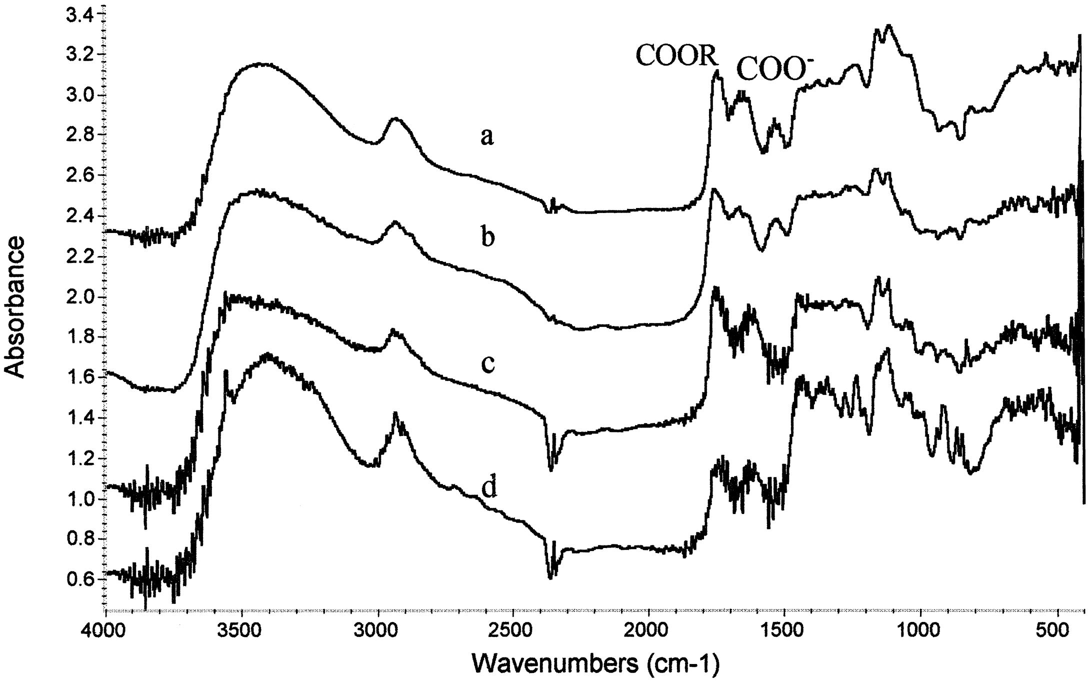

Fig. 1. FTIR spectra of (a) soy hull pectin produced by 0.05 HCl extraction, followed by 2-propanol precipitation at pH 3.5, (b) soy hull pectin produced by 0.05 HCl extraction, followed by 2-propanol precipitation at pH 2.0, (c) citrus pectin, from Danisco Inc., and (d) citrus pectin from Hercules Inc.

may be due to the differences in the surface structure of the pectin products, resulting from the differences in laboratory freeze-drying or commercial spray-drying. The absorption bands between 1100 ando 1200cm were from ether (R-O-R) and ring C-C bonds in the pectin molecule.

This study shows that pectin containing over 70% pectin, with a yield of over 26%, could be produced from soy hulls using optimum acid extraction with 0.05 N or 0.1 N HCl ,followed by alcohol precipitation at pH 3.5. The pectin content, as percent galacturonic acid, and yield of soy hull pectin, were similar to those of commercial citrus pectin.

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