J Food Sci Nutr
Vol 15, p 57~66 (2010)
DOI: 10.3746/jfn.2010.15.1.057

J Food Science and Nutrition

Pectin from Passion Fruit Fiber and Its Modification by Pectinmethylesterase

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Abstract

Passion fruit fiber pectin gels represent a new alternative pectin source with potential for food and non-food applications on a commercial scale. Pectic polysaccharides were extracted from passion fruit (*Passiflora edulis*) fiber using citric acid as a clean catalyst and autoclaved for 20 to 60 min at 121°C. The best condition of pectin yield with the highest molecular weight was obtained with 1.0% of citric acid (250 mg/g dry passion fruit fiber pectin) for 20 min of autoclaving. Spectroscopic analyses by Fourier transform infrared, enzymatic degradation reactions, and ion-exchange chromatography assays showed that passion fruit pectin extracted for 20 min was homogeneous high methoxylated pectin (70%). Gel permeation analysis confirmed that the pectin extract obtained by autoclaving by 20 min showed higher molecular weights than those autoclaved for 40 and 60 min. Passion fruit pectin extracted for 20 min was enzymatically modified with fungal pectinmethylesterase to create re-structured gels. Short autoclave treatment (20 min) with citric acid as extractant resulted in a significant increase of gel strength, improving pectin extraction in terms of functionality. The treatment of solubilized material (pectic polysaccharides) in the presence of insoluble material (cellulose and hemicellulose) with pectinmethylesterase and calcium led to the creation of a stiffer passion fruit fiber pectin gel, while syneresis was not observed.

Key words: *Passiflora edulis*, pectins, pectic substances, pectinesterase, autoclave

INTRODUCTION

Passion fruit processing generates large quantities of peel and seed waste, which creates a disposal problem. Wastes are composed of 51% peel and 11% seed by mass of yellow passion fruit (1). Studies on how to improve the use of passion fruit wastes have identified a number of different possibilities, such as: animal feed (2), pectin liquid-extract (3), dietary fiber (4,5) and pectolytic enzyme production (6). Methods of pectin extraction from passion fruit peel include: conventional heating (7-12), microwaving (13,14), and ultrasound (15).

An alternative to pectin manufacture is the production of crude pectin or fiber pectin (FP), which can be used for the same purposes of pectin. FP can be obtained by various thermal (16) or thermomechanical (17) treatments, whose aim is to increase the soluble fraction of the fiber. The main difference between FP and purified pectin (standard pectin) is the high level of fiber (18). FP manufacture is a promising technique because it would obviate the long, costly, dangerous and environmentally-detrimental techniques, namely strong acid extraction and most notably solvent precipitation (isopropyl-alcohol or ethanol), which are classically used for pectin recovery (19).

Passion fruit pectin possesses characteristics comparable to commercial pectins with degrees of methylation higher than 50% (8,10). A high concentration of sugars must be present for gel formation by high methoxyl pectins. Sugar decreases hydration of the pectin by competing for water molecules. Upon cooling, an irreversible gel is formed, which can be liquefied by heating. Meanwhile low methoxyl pectins (DM$<$50) form gels with divalent cations (Ca^{++} in food-related processes) through ionic interactions between the ions and the free α-(1→4)-linked galacturonic acid (GalA) residues of two pectin chains. This phenomenon has been described with the term of "egg-box" (20). These gels can be thermo-reversible and do not require either low water activities or low pHs; consequently, a wide

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range of products can be generated.

Enzymatic modification in dietary fiber can alter its chemical structure specifically to improve the flavor, sensory and/or texture attributes of foods and food ingredients. A grape skin product from grape press cakes after an enzymatic modification with pectinases has been development (21). Dietary fibers from yellow pea hulls, apple pomace and olive cake have been modified with pectinases and cellulases to improve the sensory properties due to a change in the chemical composition of the samples (22,23).

Pectinmethylesterase (PME; EC 3.1.1.11), a demethoxylant enzyme of pectin, is commonly found in fruits and vegetables, and can be synthesized by various microorganisms. PME removes methoxyl groups in pectin by a nucleophilic attack of the enzyme on the ester bond, resulting in the formation of an acyl-enzyme intermediate with the release of a methanol molecule. This is followed by deacylation, which results in the hydrolysis of the intermediary to regenerate the enzyme and a carboxylic acid yielding low-methoxyl pectin or polygalacturonic acid (24). For 25 years, the commonly accepted hypothesis concerning to the mode of action of PME on pectin-homogalacturonans was that they could act either randomly (as in fungi) or linearly (as in plant) along the chain of pectins (25). Each of these mechanisms might be exploited industrially. With the random mechanism, low-ester pectins are produced that have relatively reduced calcium sensitivity. PMEs free from depolymerases are of special interest for preparing low methoxyl pectins (26), french cider (27), vegetable infusion (28) and development of "*in situ*" gelation in the jam industry (29).

Attempts have been made to obtain gels with citrus and beet pectins, improving the gelification properties by enzymatic treatment using PME and pectin-acetylesterase, either from orange peel (30) or from *Aspergillus niger* (31). More recently, a system was developed where heat-denaturated PME was used to reticulate pectins and thus form a gel (32). Also, an alkaline treatment to stimulate pectin demethylation by endogenous PME in fruit wastes, which were then autoclaved in presence of guar gum, a calcium sequestrant and a sparingly soluble calcium salt was studied (33). This process was prone to β-elimination (high pHs used for pectin demethylation, partly chemical and partly enzymatic, followed by autoclaving at a relatively high pH), and therefore required fruit wastes with high quality pectin and very high levels of endogenous PME activities.

Our work attempts to use passion fruit waste for the preparation of a gelified FP-system through a two-step process: solubilization of pectins by autoclaving in acid conditions, to retain high molecular weight, followed by a treatment with a fungal PME at a pH more compatible with food processes. The main objectives of this work were (1) to extract and characterize pectin from passion fruit FP and (2) to study gelification process of FP by using released pectic polysaccharide in joint with PME.

MATERIALS AND METHODS

Materials

Yellow passion fruit (*Passiflora edulis*) were obtained from a local supermarket (Porto Alegre, Brazil). This material was stored in a cold room at 5°C until it was used. CPE Rapidase®, a commercial PME preparation from *Aspergillus niger* was a gift from Gist-Brocades (38.9±2.7 g/L protein, Batch number: 1364; Seclin, France) and showed no polygalacturonase (PG), pectin-lyase or rhamnogalacturon-hydrolase and -lyase activities under our experimental conditions. Pectin (P 9153) and GalA monohydrate were purchased from Sigma-Aldrich (Mexico City, Mexico). Citric acid, sodium hydroxide, and sulfuric acid were supplied by CTR (Monterrey City, Nuevo Leon State, Mexico) and *m*-hydroxybiphenyl was from Eastman Kodak Co. (Kingsport, Tennessee, USA).

Preparation of passion fruit-FP

Passion fruit-FP was prepared according to the procedure presented in Fig. 1. The fruits were washed thoroughly in running tap water. Upon arrival, the flavedo was stripped from skin with a potato peeler, followed by removal of the albedo with a knife. After cutting the albedo into small pieces it was blanched at 90°C for 7 min (4). Blanched albedo was washed twice with tap water and then two times with distilled water to leach out soluble solids and then dried by solvent exchange (ethanol and acetone, 3 times each) and, finally, oven-dried (60°C) overnight.

Autoclave extraction of pectic polysaccharides

The fiber (0.5 g) was mixed with 40 mL of 1% (w/v) citric acid solution and placed in a 50 mL glass vessel covered with an aluminum sheet. The pH of the suspension was 2.3. The fiber was then autoclaved for 20, 40 and 60 min at 121°C (2 atm). The autoclave was pre-heated to 100°C before introduction of the samples, requiring about 10 min to reach 121°C. After treatment, the slurry was cooled in an ice-water bath and filtered through Whatman filter paper No. 1 under vacuum for insoluble solids separation. The pectic filtrate was pre-

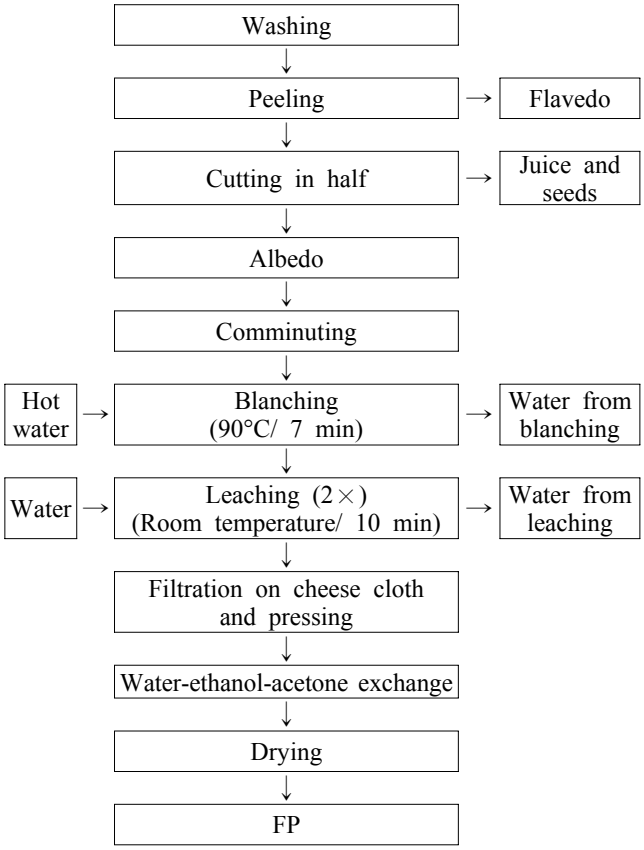

Fig. 1. Preparation scheme of passion fruit-fiber pectin (FP).

cipitated by adding 2 volumes of 95% ethanol to 1 volume of extract. The precipitate was recovered by centrifugation at 6,500×*g* for 20 min and re-dissolved in deionized water before freeze-drying and kept at room temperature until use. Treatments were carried out in duplicate.

Preparation of gelified passion fruit FP-system treatment and gel strength

Unless otherwise stated, passion fruit FP solid : solvent ratio (1:80) was autoclaved basically in the same manner as was described above, except insoluble fiber was not separated. The slurry was immediately cooled and adjusted to pH 4.5 with NaOH (20% w/v). Then 200 μL of PME enzyme (200 units) and 3 mL $CaCl_2$ (0.4 M) were added and mixed prior to incubation in a water bath at 45°C for 30 min, followed by an overnight stay (16 hr) at room temperature until determination of gel strength. The enzyme concentration covered and exceeded the levels recommended by the manufacturer. Treatments were carried out in duplicate. The effect of different solid-solvent ratio (1:26 to 1:80 w/v) on the gel strength of gelified FP-system was also studied. Texture tests were carried out using an Instron (Model 4301, Norwood, Massachusetts, USA) texturometer fitted with a cylinder (diameter=25 mm, height=35 mm). The conditions of measurement were: speed of pre-test 2 mm/seg, speed of test of 1 mm/seg and distance of penetration of 15 mm. The measurement force was expressed in Newtons (N). Treatments were carried out in duplicate.

High-performance size-exclusion chromatography (HPSEC)

The molecular weight distribution of passion fruit pectins were determined using an HPLC system involving a laboratory data control (LDC) programmable pump equipped with four Bio-Gel TSK columns (300×7.8 mm each) in series (50, 40, 30 and 25 PWXL; Tosohaas, Stuttgart, Germany), in combination with a TSK XL guard column (40×6 mm) at 35°C. Solutions (300 μL) of the pectic extracts (2 mg/mL) were injected. They were eluted with 0.4 M sodium acetate buffer (pH 3.6) at 0.8 mL/min. The eluate was continuously monitored using the automated *m*-hydroxydiphenyl (34) and orcinol (35) assays on an Alliance instruments (Méry/Oise, France) autoanalyzer. The system was calibrated with pectins for which molecular weights were determined by viscosimetric measurements using a reported relation (36).

High-performance ion-exchange chromatography (HPIEC)

Anion-exchange column ProGel TSK DEAE 5PW (75×7.5 mm, Sigma, St. Louis, MO, USA) connected to an HPLC system (Waters 625 LC, Milford, MA, USA) was used for high-performance anion-exchange chromatography. The column was eluted with 0.05 M ammonium acetate buffer (pH 6.0), followed by a linear gradient 0.05～0.5 M of ammonium acetate buffer (pH 6) at 0.6 mL/min. Solutions (300 μL) of the pectic extracts (2 mg/mL) were injected. The eluate was continuously monitored as above.

Fourier transform infrared (FTIR)

A FTIR spectrometer (Spectrum GX, PerkinElmer, Waltham, Massachusetts, USA) equipped with a Goldengate single reflection attenuated total reflectance (ATR) accessory (part number 071-1305, SensIR Technologies, Watford, Herts, UK) placed with the sample compartment was used for the pectin analysis. The FTIR spectral regions used were 4000～670/cm. Spectra were collected by co-adding 35 scans at a resolution of 4.0/cm.

Enzymatic hydrolysis of passion fruit pectins

Nelson-Somogyi reagents were used to measure the reducing groups released by different enzymes from passion fruit pectins (37). The following highly purified enzymes were used: PG from *A. niger* (Lot MPG00301;

800 U/mg protein, 5000 U/mL, Megazyme, Ireland), PME from *A. niger* (Batch number: 1364; Gist-brocades, France, 1000 U/mL) and pectate-lyase from *A. sp.* (PAL; Lot 10801; 89 U/mg protein; 580 U/mL; Megazyme, Ireland). Pectinex Ultra SPL., a commercial pectinase from *A. aculeatus* with homogalacturonan- and rhamnogalacturonan-degrading activity preparation (36.8±2.1 g/L protein, Novozymes, Bagsvaerd, Denmark), was also used. Enzymatic pectin degradability with PG, PG+PME and P-SPL were evaluated with a substrate stock solution of 500 mg/L in 100 mM sodium acetate buffer, pH 4.5. Enzyme degradations were initiated by mixing 245 μL of substrate stock solution and 5 μL of correspondent enzyme diluted 1:300 and incubated during 12 hr at 40°C. D-galacturonic acid monohydrate was used to prepare a standard curve. Results are expressed as mg of reducing sugars per 100 mg of substrate under described conditions. Enzymatic pectin (500 mg/L) degradations with PAL were carried out at pH 8.0 according to a procedure described by Hansen et al. (38). Treatments were carried out in triplicate.

Thermal analysis

Thermogravimetric (TGA) analysis was carried out in air using a thermogravimetric (TGA-50, Shimadzu Corporation, Kyoto, Japan) analyzer connected to a computer. Before loading of powder samples, the instruments were calibrated with aluminum and zinc standards. The passion fruit pectins were analyzed with a linear heating rate of 2.5°C/min using 7～10 mg samples in an aluminum container.

Other assays

Total-GalA and polymeric-GalA in pectic extracts were determined by a previous reported method (39). Protein was estimated by Micro-Kjeldahl method (4). The protein concentration was determined according to the microassay of Biuret (Randox Laboratories, Antrim, UK) using bovine serum albumin as standard. The GalA content and degree of methylesterification for passion fruit pectins were determined according to the method previously described (40). PME activity was measured titrimetrically, estimating free carboxyl groups formed in pectin as result of enzyme demethylation. The amount of 0.1 M NaOH required to maintain the media of reaction at pH 4.5 (37°C) was measured by the method described by Fish and Dustman for fungal PME (41). To start the assay 100 μL (diluted 1:10) of commercial PME were added to 20 mL citrus pectin solution (0.5%, w/v). The reaction was continuously monitored at correspondent pH for 20 min. One unit of PME was defined as the amount of enzyme that released 1 μmol of carboxyl groups/min.

Table 1. Yields of peel, pulp and seeds from passion fruit

Material	Yields (% w/w, whole fruit)
Albedo	49.23
Flavedo	12.69
Juice and seeds	41.53
Total	100.00

RESULTS AND DISUSSION

Partition of the fruit

The yields of the various tissues in the passion fruit are given in Table 1. The proportion of rind material (albedo and flavedo) is in agreement with previously published data by Nakasone and Paull (1). The whole dry passion fruit FP had an unevenly distributed light yellow color and a bitter taste was not detected. Passion fruit FP represented～9% (dry base) of wet albedo passion fruit. The content of total-GalA (as indicator of pectin) in passion fruit-FP was 250 mg/g of FP and the protein content was (N×6.25) 30 mg/g. The total-GalA content estimated in FP was higher than reported in isolated yellow passion fruit cell walls (42).

Pectin extraction from the fiber: effect of autoclaving time

In this study, a weak acid (citric acid) combined with an autoclave process was used to extract pectic polysaccharides from passion fruit-FP. Total-GalA in the extracts did not vary significantly with extraction time, while polymeric-GalA decreased with increased extraction time (Table 2). On the other hand, a maximum pectin yield (as ethanol precipitate) of 30% (dry basis) was obtained after 40 min and plateaued. This yield is somewhat higher than those reported by other researchers when citric acid was used as an extractant agent (7,12) and comparable with lime pomace (43). Oosterveld et al. (44) developed a pectic polysaccharide extraction procedure from sugar beet pulp that included two sequential autoclave treatments, 121°C and 40 min. Pectin-starch fraction from potato pulp was solubilized by 60 min autoclave treatment (2 atm, pH 3.3 and at 134°C) (45). The development of alternative methods to avoid equipment and environmental deterioration has prompted the use of clean technologies, such as physical and enzymatic methods (46,47), to extract the pectin. The extraction of pectin from okara has also been carried out through autoclaving treatments, however the extraction times used were also long (48). Lima (8) extracted pectin from passion fruit peels in 15 min using

Table 2. Effect of extraction time on the characteristics of pectic extracts and pectins from passion fruit-fiber pectin (FP)

Description	Time (min)		
	20	40	60
Pectic extract			
Yield AUA[1)]	206.6±39.2[a]	225.0±26.7[a]	240.3±48.3[a]
Yield polymeric AUA[1)]	134.7±23.0[a]	118.7±14.4[a]	46.2±0.8[b]
Yield pectin[2)]	250.5±13.4[a]	302.0±2.8[b]	303.5±16.7[b]
Gel Strength (N)	0.40±0.07[a]	0.28±0.03[ab]	0.19±0.01[b]
Passion fruit pectin			
Galacturonic acid content	70.2±9.7[a]	70.6±2.8[a]	62.2±5.6[a]
Degree of methoxylation	75.6±0.7[a]	74.8±1.6[a]	71.1±3.9[a]

[1)]Yields of AUA and polymeric AUA in mg/g initial dry matter. AUA: anhydrous-uronic acids.
[2)]Yields of pectin in mg/g initial dry matter.
Different letters mean significant differences (α=0.05).

1% H_2SO_4, while Pruthi (7) used 0.75% citric acid during 60 min. The enzymatic methods require long times of extraction and the enzyme is expensive (47) while physical treatments (extrusion-cooking) have the disadvantage of resulting in colored (brown) products and gels when pectin is extracted from lemon pomace (49). Therefore, the technique of pectin extraction by autoclaving in presence of citric acid represents a novel alternative for extracting pectin in a short period of time and without environmental contamination (43).

The gel strength of restructured gels prepared with FP autoclaved for 20 min were higher than that of FP autoclaved for 60 min (Table 2). This suggested that the increase of extraction time reduced the molecular weight of extracted pectin, as was confirmed by HPSEC (Fig. 2) and the analysis of polymeric-GalA (Table 2). The HPSEC profile obtained for the orcinol (total sugars) and *m*-hydroxydiphenyl (uronic acids) were similar for all three samples; *i.e.*, the molecular weight distribution of

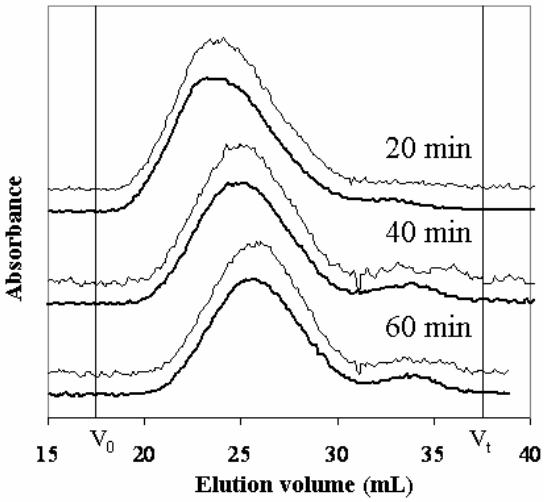

Fig. 2. HPSEC chromatography on combined columns of the passion fruit pectin solubilized by autoclaving for 20, 40 and 60 min. Thin line: total sugars, absorbance at 420 nm; Thick line: uronic acids, absorbance at 520 nm.

the neutral sugars and uronic acids were homogeneous. However, while extraction time increased, the molecular weight of pectin decreased. After 20 min of autoclaving, a single peak was observed with similar distributions of neutral and acidic sugars. After 40 min and even more 60 min of autoclaving, two phenomena were observed: a shift of the main peak towards higher elution volumes, indicating a decrease of the average degree of polymerization, and the apparition of a minor peak close to the total volume. These results are in agreement with Yapo (12), who used citric acid for 60 min for pectin extraction from passion fruit rind. Oosterveld et al. (44) obtained a low molecular weight fraction enriched in uronic acids and a high molecular weight fraction enriched in neutral sugars after autoclaving sugar beet pulp at pH 6. This could be interpreted as a result of β-elimination driven by the (relatively) high pH and high temperatures used (50,51). This phenomenon was avoided here by using a low pH during autoclaving, thus better preserving the functionality of the pectins. Kim et al. (52) reported that the molecular weight of mandarin pectin extracted with citric acid also decreased as extraction time increased. The best condition of pectin yield and high molecular weight from passion fruit-FP was obtained with 1.0% of citric acid (250 mg/g dry FP) for 20 min. Advantages of using a weak instead of a strong acid are two-fold: the opportunity of pectin extraction and buffering of the extraction medium close to the optimal stability pH of 3.5 for pectins (53). The dried pectic polysaccharide was a fine white powder like pectin obtained from lime or lemon pectins by the conventional method (19).

The isolated passion fruit pectins showed a GalA content and degree of methylesterification of over 65 and 70 percent, respectively (Table 2). Pectin extracted for 20 min showed a typical FTIR spectrum comparable with high methoxylated pectin from citrus (data not

shown). To investigate the possibility of demethylation of pectin during extraction, samples were analyzed by FTIR-ATR. No differences in intensity were observed in the IR patterns in pectin samples as shown on Fig. 3 in the region between 1760～1745/cm, and 1640 and 1629/cm; *i.e.*, the ester carbonyl (C=O) groups and carboxylate ion stretching band (COO-), respectively. No significant difference was observed when pectins extracted for different durations (20～60 min) were subjected to enzymatic treatments with purified endo-polygalacturonase from *A. niger*. This confirmed that methyl-esterification was similar in all samples (Table 3). The degradation of the homogalacturonan region from passion fruit-pectin was promoted by the action of the endo-PG and PME. Again, no differences were observed in the liberation of reducing sugars with pectins extracted for different durations. This could indicate that the solubilized pectins present similar homogalacturonan regions independently of their molecular weight as shown by HPSEC. The pectins were also submitted to degradation by pectate-lyase after demethylation by NaOH. All passion fruit pectins showed an increase in absorbance at 235 nm, resulting from the β-eliminative attack of PAL (Table 3), and again no significant differences were observed, further confirming that the size of the homogalacturonans was very similar in all samples. The highest extent of degradation was obtained with the preparation Pectinex Ultra-SPL, which is able to degrade both homogalacturonan and rhamnogalacturonan I (54).

Fig. 4 gives the pattern of the thermal behavior for passion fruit pectins obtained by autoclave-assisted technology at 20, 40 and 60 min. The early minor weight loss in samples was attributed to desorption of water by the polysaccharide sample (55). The main decomposition of pectin samples start above 200°C and shows identical weight loss (70%) pattern until 400°C. The observed stability of passion fruit pectin showed thermal stability, especially in the range of 100 to 180°C. This thermal stability could be contributed to develop biopolymer micro-particles by spray dryer technique.

After pectin extraction, the residual material represented 60 percent of the passion fruit-FP. This residual depectinized material could be used as dietary fiber with high cellulose content for human nutrition or as a source for glucose production by enzymatic methods. One of the advantages of using a weak acid instead of strong

Fig. 3. Comparison of FTIR-ATR spectra of passion fruit pectin extracted by autoclaving for 20, 40 and 60 min.

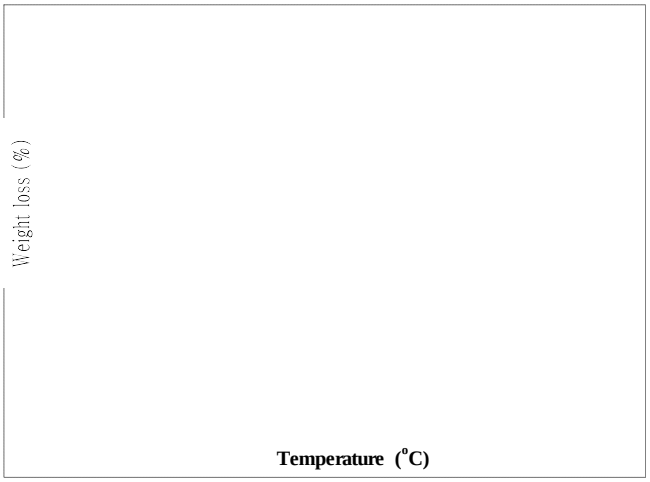

Fig. 4. Thermograms of passion fruit pectins obtained at 20, 40 and 60 min by autoclaving-assisted method.

Table 3. Effect of extraction time on enzymatic degradability of pectins from passion fruit-fiber pectin (FP)

Enzyme[1)]	Time (min)					
	20		40		60	
	Reducing sugars (mg) / 100 mg from passion fruit pectin					
	Blank	Enzyme	Blank	Enzyme	Blank	Enzyme
PG	10.75±0.10	11.71±0.20	10.73±0.34	11.38±0.23	10.85±0.23	12.17±0.09
PG+PME	10.85±0.28	24.44±0.92	10.57±0.18	23.02±0.65	10.57±0.14	24.39±1.20
P-SPL	11.35±0.09	50.59±0.58	11.40±0.52	46.88±2.82	11.11±0.29	48.06±2.27
	Δ [unsaturated product]$\times 10^{-4}$ from passion fruit pectin					
PAL	0.967		1.079		1.044	

[1)]PG, polygalacturonase; PME, pectinmethylesterase; P-SPL, Pectinex Ultra SPL; PAL, Pectatelyase

acid for pectin extraction is the opportunity to use both soluble and insoluble materials either combined or separated in food, agricultural, or pharmaceutical products.

Characteristics of pectin extracted for 20 min before and after PME treatment

The pectin extracted after the 20 min treatment was characterized before and after enzymatic modification with PME by FTIR, charge density (HPIEC) and enzymatic degradations. Large differences in intensity (Fig. 5) were observed in the wavenumber regions between 1760～1745/cm, and 1640 and 1629/cm, indicating loss of methoxyl groups of the pectin molecule due to action of the enzyme. The HPIEC profiles were also very different (Fig. 6). The passion fruit pectic extract before PME treatment showed one main uronic acid peak eluting at a low ionic strength, indicative of the presence of highly esterified pectins (56). A neutral component (reaction with orcinol but not *m*-hydroxybiphenyl) was not retained on the column. Overall, this was the typical ion-exchange pattern for an acid pectin extract from citrus or apple (57,58). The PME-treated pectin showed retained material along the whole range of ionic strength, with more material eluting at high ionic strength. This pectin had a low degree of methylation, which was confirmed here by high charge density. The charge density was also very homogeneous. This is in agreement with the mechanism generally accepted for *Aspergillus* species PMEs (26,59). The modification of the pectin by PME was also confirmed by its higher susceptibility to chain cleavage by endo-PG (data no shown).

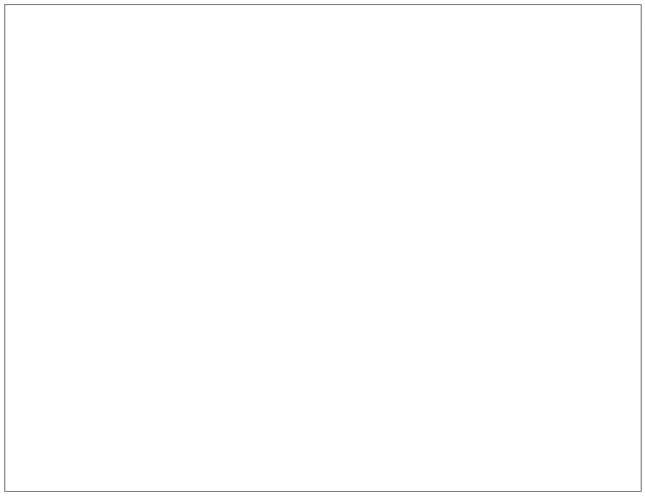

Fig. 5. Comparison of FTIR-ATR spectrum of high methoxyl passion fruit pectin (grey line) and low methoxyl passion fruit pectin obtained by enzymatic modification (black line).

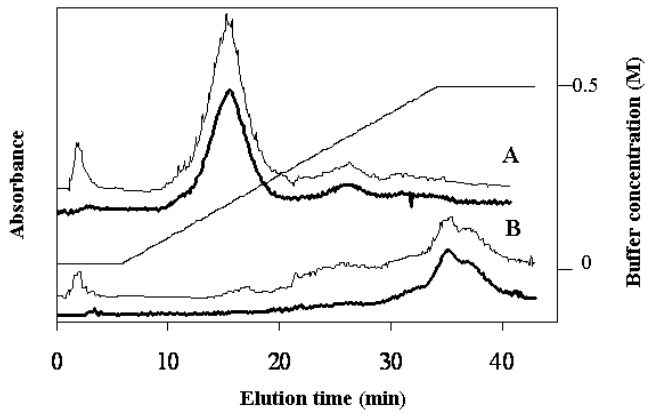

Fig. 6. High performance ion-exchange chromatography of the passion fruit pectin solubilized by 20 min autoclaving before (A) and after (B) PME treatment. Thin line: total sugars, absorbance at 420 nm; Thick line: uronic acids, absorbance at 520 nm.

Effect of passion fruit-FP solid-solvent ratio on gel strength

Effect of passion fruit-FP solid-solvent ratio on gel strength was investigated after 20 min autoclave treatment. Ratios from 1:26 to 1:80 of fiber to acid citric solution were evaluated during gelification tests. The data of Fig. 7 show that the yield strength of the gels increased with the increase of passion fruit-FP content until a maximum value of 7.5 N was reached with solid-solvent ratio of 1:26. The yields in solubilized uronic acid-containing polymers were constant and therefore the concentration of pectins in the medium increased linearly with the concentration of passion fruit-FP. Increased gel strength was thus related to increased pectin concentration. No syneresis was observed in the gels prepared by the enzymatic procedure. This might be related to the random mode of demethylation by fungal PME (25,26). For production of jams and similar types of products, pectin is usually added as a gelling agent. An

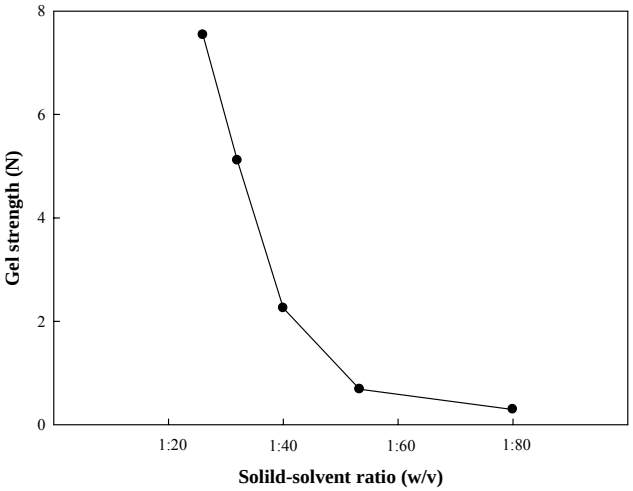

Fig. 7. Effect of solid-solvent ratio of fiber from passion fruit on gel strength after enzymatic gel restructured with fungal PME.

alternative would be to let PME react on the pectin-containing material. This principle has been used in traditional oriental cooking, where the fruits of *Ficus awkeotsang* are used to form gels (60) and to gel homogenized oranges (61). Those authors observed formation of a jam-like appearance in chopped oranges treated with the addition of PME and pectin releasing enzyme mixture during 60 min at 40°C. Norsker et al. (62) described the production of thermo-irreversible gels by enzymatic gelation of sugar beet pectin; however, the principle of gelification of the pectins from sugar beet differed as they used the reactivity of ferulic groups to form covalent bonds. Previous experiments in gelation of fibers relied on high sugar contents and therefore were only usable for jam manufacturing (18,49). Our method, relying on the enzymatic modification of solubilized pectin from FP, can produce gels at high water activities. It can be used to immobilize water (for substitution of synthetic hydro-gels), agrochemicals, enzymes, microorganisms or oligosaccharides used in agriculture.

Passion fruit-FP is an appropriate material for preparation of gelified systems after enzymatic modification; it has potential to be used for food or non-food purposes (agriculture, pharmacy, etc). For food or pharmacy applications, the sole use of ethanol for drying would be recommended. The short autoclave treatments combined with citric acid significantly improved the pectin extraction process from passion fruit-FP, with a higher yield in shorter times, with relatively simple equipment, and comparable physical characteristics to those of pectins extracted by conventional method. Further work is needed on optimization and detailed physical-chemical characterization of pectin extracted by autoclave from passion fruit FP, as well as optimization of time and temperature of gelification in relation to enzyme concentration, aiming at minimizing the costs of preparation of restructured gels.

ACKNOWLEDGEMENTS

The authors would like Agnès Gacel (Le Rheu, INRA, France) for her excellent technical assistant. This work was partially supported by the National Council of Science and Technology (CONACYT, Mexico) from SAGARPA-CONACYT (2005-12532) and National Science Foundation-CONACYT.

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(Received November 25, 2009; Accepted March 3, 2010)