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Biochemical Characteristics and Gelling Capacity of Pectin from Yellow Passion Fruit Rind as Affected by Acid Extractant Nature

BEDA M. YAPO*

Unit of Training and Research in Food Science and Technology, University of Abobo-Adjamé, 02 BP 801 Abidjan 02, The Ivory Coast

The effects of acid extractant type on the yield and characteristics of pectin from yellow passion fruit (*Passiflora edulis* flavicarpa) rind was investigated by using citric, nitric, or sulfuric acids at different concentrations (10 mM and 30 mM) and pH (1.8 and 2.5). The results showed that not only concentration, but also acid type influenced the extracted pectin yields (3−14%, w/w). The yield of pectin extracted with citric acid was the lowest. Acid type and concentration affected the molecular characteristics of pectin, notably, the degree of esterification (29−73), galacturonic acid to rhamnose ratio (14−35), weight average-molecular weight (100−250 kDa), gel strength (127−179), and setting time (841−1236 s). Citric acid-extracted pectin had a higher degree of esterification and weight average-molecular weight and better gelling properties. At 30 mM concentration, nitric and sulfuric acids solubilize pectins having a degree of esterification <50, contrary to citric acid. The results indicate that the latter acid exerts the least deesterifying action on pectin solubilization from the cell wall material. Citric acid-extracted pectin was closer to lemon pectin of similar degree of esterification in terms of gelling properties.

KEYWORDS: ***Passiflora edulis*** **flavicarpa rind; acid extractant; pectin; biochemical characteristics; gelling properties**

INTRODUCTION

Pectins are natural polymers in all land plants, which have been discovered in the 18th century (*1*) and coined as such in the 19th century after being crudely characterized as the active fruit component responsible for gel formation (*2*). Pectic substances are structural polysaccharides of the plant cell walls, which play an important role as cementing material in the middle lamellae (*3*) and form one of the two independent but interactive polysaccharide networks of the primary cell walls in flowering plants (*4*). Their widely believed macrostructure encompasses copolymer blocks of homogalacturonan covalently linked to type I rhamnogalacturonan bearing neutral sugar side chains, although type II rhamnogalacturonan may also be present. Depending on the extraction mode or plant source, homogalacturonan can predominate over type I rhamnogalacturonan or vice versa, but much higher proportions of the former are generally reported in most of the pectin sources hitherto investigated. To examine the structural features and functional properties of pectins, researchers have generally extracted them from plant cell walls using various approaches including chemical and/or enzymatic methods. The chemical agents used for pectin extraction can be divided into groups of four: water and buffers, calcium-ion chelators, acids, and bases. As successively used in the above cited order (from water to base), these different chemical agents may selectively extract, from the same starting cell wall material, pectins showing different chemical structures (*5*) supposed to differ in solubility and ease of isolation (*6*, *7*). Nevertheless, the selectivity can appear less evident when the extraction procedure does not abide by this sequential order, especially when acid is utilized as the first extractant or when these agents are individually used to extract pectins from starting cell wall materials of the same kind. Acids are generally the strongest extracting agents as regards the yield of extracted pectin. The often used acids are acetic, citric, lactic, malic, tartaric (organic), and hydrochloric, nitric, phosphoric, and sulfuric (mineral) acids (*8*). However, commercially, pectin from either apple pomace or citrus peel is extracted by treating the raw material with hot dilute mineral acid (*9*), nitric acid being the one most often used to acidify hot water in order to achieve this purpose (*10*). The extraction parameters, i.e., dry raw material to solvent weight ratio, temperature, time, and pH, are generally in the range of 1:35−1:15, 60−100 °C, 20−360 min, and 1.4−3, respectively (*10, 11*), but laboratory extractions are sometimes based on the extracting solvent acid concentration in lieu of pH. The best known property of pectin is that it forms gels with sugar and acid (*9*). The main aim of the pectin industry is therefore to obtain water-soluble pectin preparations of high molecular weight and specified degree of methoxy-esterification (DM) and degree of amidation which are able to form gels under specified

* To whom correspondence should be addressed. Tel: (+225) 03 12 85 71. E-mail: bedamarcel@yahoo.fr.

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conditions (*11*), in addition to good pectin yields. The main factor governing the gelling mechanism of pectin is its DM. Thus, pectin with a DM > 50, called high-methoxy pectin (HMP), needs sugar (such as sucrose) at a concentration ≥55 wt % and acid (pH 2.0−3.5) to form gels, whereas pectin with a DM < 50, called low-methoxy pectin (LMP), needs calcium ions to form gels within a larger pH range (2.0−7.0) whether sugar is present or not.

The acid extraction of commercial pectin is advantageous, apart from high extraction yield, by the fact that the pectin obtained is generally enriched in galacturonic acid, its main constituent (*12*), following a substantial hydrolysis of side chains-containing neutral sugar linkages, while remaining methoxy groups esterifying (at *C-6*) galacturonic acid units within pectin (homogalacturonic) backbone can allow good gelation in the presence of high soluble solids (e.g., sucrose) content and acid under specified conditions. Indeed, in an acid medium (especially at pH < 2), linkages between galacturonic acid residues (glycosiduronyl bonds) are more stable than linkages between galacturonic acid and rhamnose (aldobiuronic and pseudoaldobiuronic linkages), which are in turn more resistant than linkages between neutral sugars to acid hydrolysis (*13, 14*). Various studies have shown the effects of acid extractant strength on pectin yield, chemical, and/or physicochemical characteristics (*12, 15−17*). In contrast, as regards the acid extractant kind, the few available reports are somewhat contradictory. Earlier studies indeed reported no significant effects of extracting acid type (hydrochloric versus nitric acids) on the characteristics of extracted pectins from fresh sugar beet (*17*), whereas two more recent studies have reported large influences of acid type (hydrochloric against nitric acids) on the characteristics of pectins from apple pomace (*18*) and buttercup squash flesh (*19*). In either of the two latter studies, citric acid (as an extracting agent) has been reported to have more positive effects on the yield (*18*) and less damaging effects on the degree of esterification (*19*) of extracted pectin compared to other acids, in particular nitric acid.

Yapo and Koffi (*20, 21*) have recently reported that yellow passion fruit (YPF) rind, an unexploited byproduct from the juice industry in many tropical and subtropical regions, is a pectin-rich material (14−23% galacturonic acid weight equivalent) and may stand as a suitable local source for the production of industrial pectin (*20, 21*). However, the extraction of pectin from YPF rind with hot water or oxalate acidified with nitric acid under conditions optimizing the yield resulted in pectin isolates characterized by DM <50% (*20, 22*). As a consequence, extracted pectins were only good for the preparation of Ca^{2+}-mediated LMP gels. This may constitute a snag in the promotion of YPF rind as an alternative industrial pectin source to apple pomace or citrus peel in tropical countries. We thus deemed it necessary to experiment with different kinds of acid extractants in order to find out the more suitable one(s) regarding the extracted pectin characteristics, especially DE and yield. The goal of this study was to examine the influence of acid extractant type on the biochemical features and gelling properties of pectin from YPF rind.

MATERIAL AND METHODS

Pectin Production. Fresh YPF rinds were obtained from a local juice factory (*21*). Because of high soluble solids content, fresh rinds (crushed into ∼0.8−1.0 mm) were first alcohol-extracted prior to oven-drying at 35 °C for 24 h. Lemon (*Citrus limon*) fruits were obtained from a local market. The skin (flavedo) was peeled off, and the lemon peel was produced from the albedo as described for YPF rind (*21, 22*) with slight modifications. YPF rind pectins were extracted with hot distilled water acidified with nitric acid (strong monoprotic mineral acid), sulfuric acid (strong diprotic mineral acid), or citric acid (weak triprotic organic acid). Preliminary experiments on a small scale using a composite experimental design to optimize extraction conditions revealed that a solid to liquid ratio of 1:20−1:30 (w/v), a number of two extractions, at 75 ± 5 °C, and for 60−90 min were needed to obtain optimum pectin yields from YPF rinds whatever the acid type (unpublished results). Pectin extraction on a larger scale was thus carried out by keeping consistent temperature (75 °C), duration (60 min), dry rind to solvent ratio (1:25, w/v), and number of peel extraction (2), and (i) varying (at two levels), the initial concentration (0.01 or 0.03 M) or pH (1.8 or 2.5) of extracting acid solvent, (ii) using monosodium citrate-containing nitric acid solvents at pH 1.4 and 1.8. Lemon peel pectins were extracted with hot distilled water acidified with nitric acid solution to an initial pH of 1.4 under the following conditions: dry peel to extractant ratio of 1:30 (w/v); temperature of 75 °C, and extraction time of 120 min. These extraction conditions are the optimum conditions to extract a high amount of lemon pectin having DE comparable to YPF pectin. At the end of each extraction, the slurry was centrifuged and filtered on G-3 sintered glass, and the pectin solution obtained was rapidly brought to pH ∼4 for the sake of its stability. The extracts from the first and second extractions were combined, concentrated, and dialyzed (12000 *Mw* cutoff tubing) against water, prior to pectin precipitation in 3 volumes of 95% ethanol at 5 °C for 2 h. The pectin precipitate was washed (twice) with 70% ethanol followed by 95% ethanol and acetone, kept for a while under a fume extractor to let residual acetone evaporate, and then oven-dried at 35 °C for 24 h and weighed. Pectin extraction was performed in three runs for each acid type. All pectin extracts from a given extraction type were pooled, finely ground, and sieved to pass through 60 mesh (0.25 mm) size sifters. The resulting homogeneous pectin flours were canned in plastic containers and kept at room temperature in a desiccator under airless and moisture-free conditions until used.

Pectin Characterization. Prior to characterization, pectin samples were treated with a mixture of 1% (v/v) HCl/60% (v/v) ethanol (three times), and the remaining pectin fractions were exhaustively washed with 60% (v/v) ethanol until the filtrate gave a negative response for chloride ions with silver nitrate. This process aimed at removing free sugars and salts, and converting pectin to the free acid form. Pectins were characterized for their DE, galacturonic acid, neutral sugar, ash, protein, and moisture contents and molecular weight distribution (MWD). DE was determined by a slightly modified titrimetric method (*23*). Briefly, 20 mL of 1% (w/v) pectin aqueous solution was titrated with 0.2 N NaOH in the presence of two drops of phenolphthalein indicator (Titration A). Then, 20 mL of 0.5 N NaOH were added under stirring for 30 min to de-esterify pectin, after which 20 mL of 0.5 N HCl was added to exactly neutralize the NaOH. This mixture was titrated with 0.2 N NaOH in the presence of two drops of phenolphthalein indicator (Titration B). The DE was calculated using eq 1.

$$\mathrm{DE}(\%) = [\text{Titration B}/(\text{Titration A} + \text{Titration B})] \times 100 \quad (1)$$

DE of extracted YPF pectins corresponded to DM, i.e., the number of (galacturonic acid units esterified with) methoxy groups per 100 galacturonic acid units in pectin chains as acetylesterification had been hardly reported (*20, 24*). The galacturonic acid content was titrimetrically determined by a modified Lefevre and Tollens method (*23, 25*). Briefly, pectin (250 mg) was boiled under reflux with 30 mL of 19% HCl in an oil bath at 120 °C for 3 h. Evolved carbon dioxide was absorbed in 25 mL of carbonate-free standard 0.25 N NaOH. After adding 10 mL of 10% barium chloride and two drops of phenolphthalein indicator, the excess of alkali present in the mixture was titrated with standard 0.1 N HCl. Neutral sugar, ash, and moisture contents and MWD were determined as previously described (*22*). The protein content was determined by the Folin−phenol reagent method (*26*) using BSA as standard.

Gelling Properties. The gelling capacity (or power) of pectins was evaluated by the determination of the strength of gels prepared under the following conditions: 65.0% soluble solids (sucrose), 0.70 wt % pectin, and at pH 2.3 using the conventional SAG method (*27, 28*). Although with this method, the breaking (or internal) strength cannot be measured but only the deformation by gravity of demolded gels, it

Table 1. Effect of Acid Extractant Concentration on the Yield (g/100 g of Dried Weight) and DE of Pectins from Yellow Passion Fruit Rind[a]

extraction type	yield	DE
0.01 M HNO_3	6.5 (0.5a)	54 (2a)
0.01 M H_2SO_4	7.9 (0.6ab)	42 (2b)
0.01 M $H_3C_6H_5O_7$	2.8 (0.1c)	73 (4c)
0.03 M HNO_3	13.9 (1.2d)	44 (2b)
0.03 M H_2SO_4	10.2 (0.8b)	29 (1d)
0.03 M $H_3C_6H_5O_7$	5.1 (0.3e)	64 (3e)

[a] Data in parentheses are relative standard deviations ($n \geq 3$). Mean values in the same column with different letters are significantly different ($p < 0.05$).

is simple to handle, reliable, and reproducible as previously observed (*20*). Furthermore, it is the commonly used method to grade most commercial pectins. Briefly, at the end of boiling, the jelly preparation was completely filled in a Ridgelimeter glass, and the surface was covered with a waxed paper disk (to minimize evaporation) and left undisturbed at room temperature for 2 h before aging for a further 22 h in an incubator (water bath) at 30 °C. The gel was then carefully demolded (without damaging) onto a Ridgelimeter glass plate. After exactly 2 min of standing, the pointer of the apparatus (Ridgelimeter) was carefully lowered until it touched the gel surface, and the percentage of sagging under its specific gravity was measured. The gelling power was calculated using eq 2

$$°\mathrm{SAG} = (A/B) \times F \quad (2)$$

where *A*, *B*, and *F* are the amounts of sugar and pectin in gel and the factor of sagging percentage, respectively. Limits of sag range from 10 to 34%. The *F* factor is directly related to the percentage of sagging. A gel of 23.5% sag was considered to be a standard gel and therefore $F = 1$. A gel with a higher sagging percentage (weaker gels) was corrected by a factor <1, and a gel with a lower one (firmer gels) was corrected by a factor >1. Thus, a standard table or graph between the sagging percentage and *F* factor (or gelling power) can be established. The gelling velocity was determined as the setting time, i.e., the duration from the end point of the jelly preparation to the first sign of gelation on cooling at 30 °C in a water bath (*29*). Briefly, a jelly tumbler was prepared with citric acid solution containing coarsely ground pepper and placed in an incubator at 30 °C. The jelly preparation, at the end of boiling, was carefully poured to desired height at time zero. At suitable intervals, the gelling process was controlled by slightly twisting the test glass, and as the jelly was setting, the pepper was seen to swing back slightly before coming to rest. The setting time was recorded when this set reached 6 mm from the gel surface.

Statistical Analysis. Data obtained were statistically evaluated by the global test of analysis of variance (ANOVA) and then by the Fisher's least significant difference (LSD) posthoc test when the former showed significant difference. Means were considered to be significantly different at *p*-value < 0.05. No significant difference was observed in intraextractions, thus confirming the repeatability and reproducibility of each type of extraction method, which allowed to pool fractions of the same kinds in order to have homogeneous pectin samples for assays demanding larger quantities, notably, jelly tests.

RESULTS

Effect of Acid Extractant Concentration on the Pectin Yield and DE. The yield of pectins extracted from YPF rind ranged from 2.8 to 13.9% (w/w) (**Table 1**). For a given acid type, the yield of pectin extracted with 0.03 M acid concentration was significantly ($p < 0.05$) higher than the yield of pectin isolated with 0.01 M acid concentration, with the exception of sulfuric acid. The yield of nitric acid-extracted pectin was significantly ($p < 0.05$) higher than the yield of sulfuric acid-extracted pectin at 0.03 M acid concentration, but both pectin yields were similar at 0.01 M acid concentration. The highest yield was obtained with 0.03 M nitric acid, and the lowest yield was obtained using 0.01 M citric acid.

Table 2. Effect of the pH of Extracting Acid Solvent on the Yield (g/ 100 g of Dried Weight) and DE of Pectins from Yellow Passion Fruit Rind[a]

extraction type	yield	DE
HNO_3, pH 1.8	9.6 (0.7a)	53 (2a)
H_2SO_4, pH 1.8	7.7 (0.5a)	52 (1a)
$H_3C_6H_5O_7$, pH 1.8	5.6 (0.2b)	56 (2b)
HNO_3, pH 2.5	5.9 (0.3b)	58 (3b)
H_2SO_4, pH 2.5	5.3 (0.1b)	56 (2b)
$H_3C_6H_5O_7$, pH 2.5	3.5 (0.1c)	70 (3c)
$HNO_3 + NaH_2C_6H_5O_7$, pH 1.4	12.8 (0.9d)	54 (1b)
$HNO_3 + NaH_2C_6H_5O_7$, pH 1.8	9.1 (0.7a)	63 (2b)
HNO_3, pH 1.4, 75 °C, 120 min, LP	16.7 (1.5e)	62 (5bc)

[a] Data in parentheses are relative standard deviations ($n \geq 3$). Mean values in the same column with different letters are significantly different ($p < 0.05$). LP, lemon pectin; DE, degree of methoxy-esterification.

The DE of pectins varied from 29 to 73 (**Table 1**). For a given acid type, the DE of pectin extracted using 0.01 M acid concentration was significantly ($p < 0.05$) higher than the DE of pectin extracted with 0.03 M acid concentration. The concentration effect was more marked with sulfuric acid extractant than with either nitric or citric acid extractants. At the same extractant concentration, the DE of citric acid-extracted pectin was by far the highest, and the DE of sulfuric acid-extracted pectin was the lowest.

Effect of Extracting Solvent pH on the Pectin Yield and DE. The yield of pectins extracted with pure acids ranged from 3.5 to 9.6% (w/w) depending on the pH of extracting solvent (**Table 2**). The pectin amounts were significantly ($p < 0.05$) higher at pH 1.8 than at pH 2.5 whatever the acid type. At both pH values, similar amounts of pectins were extracted with nitric and sulfuric acids. Here again, the lowest pectin yield was recorded from citric acid extractant at pH 2.5. The yield of pectins isolated with monosodium citrate-containing nitric acid solvents varied significantly ($p < 0.05$) from 9.1 to 12.8% (w/w) as the pH varied from 1.8 to 1.4 (**Table 2**). The pectin amount was not significantly affected when extracted either with pure (monosodium citrate-free) nitric acid or monosodium citrate-containing nitric acid solvents at pH 1.8. The yield of pectin extracted from lemon peel was 16.7% (w/w) under the conditions used and was therefore the highest of all.

The DE of isolated pectins ranged from 52 to 70 depending on the pH of the extracting agent (**Table 2**). Nitric and sulfuric acid-extracted pectins have similar DEs at either pH, contrary to citric acid-extracted pectin whose DE increased significantly ($p < 0.05$) from 56 to 70 as pH increased from 1.8 to 2.5. At the latter pH, citric acid solubilized pectin of a much higher DE than both sulfuric and nitric acids. At pH 1.8, sodium citrate-containing nitric acid extracted pectin of higher DE than pure nitric acid. The DE of isolated lemon pectin was 62, but might be slightly overestimated considering that acetyl groups may be present as has been reported in low amounts in acid-extracted pectin from industrial citrus peel (*5*).

Biochemical, Molecular, and Gelling Characteristics of Selected Pectins. Some of the extracted pectins were selected for further characterization on the basis of extraction type (concentration or pH variants), rather good extraction yields (>5%, w/w), and DE > 50.

Biochemical Composition and Copolymer Block Aspects. The chemical composition of the chosen pectins is shown in **Table 3**. Their galacturonic acid and neutral sugar contents ranged from 64.9 to 77.3% and from 5.6 to 16.1% (w/w), respectively. Pectins extracted with 0.03 M citric acid and 0.01 M nitric acid solvents as well as those solubilized with nitric and citric acids at pH 1.8 had similar galacturonic acid contents, that of sulfuric

Table 3. Chemical Composition and Molecular and Gelling Characteristics of Pectins from YPF Rind and Lemon Peel[a]

	composition (%, w/w)					GalA/Rha		gelling	
extraction type	GalA	Rha	NS	Ash	Protein	ratio	*Mw* (kDa)	power (°sag)	velocity (s)
0.01 M HNO_3	68.4 (1.4a)	2.7 (0.1a)	12.8 (0.4a)	3.1 (0.2)	3.8 (0.3)	23 (2ad)	176 (3.2a)	140 (2a)	1096 (32a)
0.03 M $H_3C_6H_5O_7$	64.9 (3.5a)	3.9 (0.2b)	16.1 (0.7b)	2.7 (0.2)	3.1 (0.1)	14 (2b)	246 (9.5b)	179 (2b)	841 (21b)
HNO_3, pH 1.8	71.9 (1.2a)	2.5 (0.1a)	10.9 (0.4ac)	2.5 (0.1)	3.4 (0.2)	25 (2a)	172 (4.6a)	136 (3a)	1105 (49a)
H_2SO_4, pH 1.8	77.3 (3.8b)	1.8 (0.1c)	5.6 (0.2d)	2.6 (0.1)	2.9 (0.1)	35 (3c)	104 (3.8c)	127 (1c)	1236 (58c)
$H_3C_6H_5O_7$, pH 1.8	70.6 (1.8a)	3.7 (0.2b)	13.5 (0.6b)	2.7 (0.2)	2.4 (0.1)	16 (1b)	218 (7.9d)	165 (1d)	1053 (39a)
$HNO_3 + NaH_2C_6H_5O_7$, pH 1.4	76.3 (3.6b)	2.3 (0.1a)	9.6 (0.3c)	3.1 (0.2)	3.1 (0.2)	27 (2a)	130 (5.7e)	148 (1e)	1078 (43a)
$HNO_3 + NaH_2C_6H_5O_7$, pH 1.8	67.8 (1.2a)	3.0 (0.2b)	11.3 (0.5a)	2.9 (0.1)	3.5 (0.1)	20 (1d)	192 (8.6f)	159 (4d)	894 (18b)
HNO_3, pH 1.4, 75 °C, 2 h, LP	76.9 (4.2b)	1.6 (0.0c)	7.5 (0.3c)	2.1 (0.1)	1.8 (0.1)	41 (2e)	287 (10.2g)	205 (3f)	759 (12d)

[a] Data in parentheses are relative standard deviations ($n \geq 3$). Mean values in the same column with different letters are significantly different ($p < 0.05$). GalA, galacturonic acid; Rha, rhamnose; NS, total neutral sugar; LP, lemon pectin.

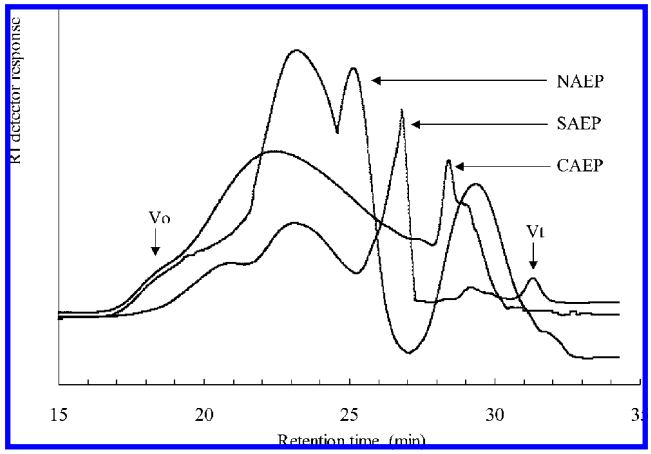

Figure 1. High-pressure size-exclusion chromatography elution patterns of pectins extracted from yellow passion fruit rind by the three types of acid at pH 1.8. CAEP, citric acid-extracted pectin; NAEP, nitric acid-extracted pectin; SAEP, sulfuric acid-extracted pectin.

acid-extracted pectin at pH 1.8 being significantly ($p < 0.05$) higher. In contrast, both their rhamnose and total neutral sugar contents were significantly ($p < 0.05$) different. In either case (concentration or pH factor), citric acid-extracted pectin contained higher rhamnose and total neutral sugar. Sodium citrate containing-nitric acid-extracted pectin got poorer in galacturonic acid as pH increased from 1.4 to 1.8. All these pectin samples contained ash and protein in relatively low amounts. Their galacturonic acid to rhamnose (GalA/Rha) molar ratio, as an indicator of copolymer block proportions, ranged from 14 to 35 (**Table 3**). Citric acid-extracted pectin had a lower GalA/Rha ratio than either nitric or sulfuric acid-extracted pectins. The GalA/Rha ratio of sodium citrate-containing nitric acid-extracted pectin decreased as pH increased from 1.4 to 1.8. The galacturonic acid and total neutral sugar contents and GalA/Rha ratio of lemon pectin were 76.9%, 7.5% (w/w), and 41, respectively. In terms of sugar composition and GalA/Rha ratio, sulfuric acid-extracted pectin at pH 1.8 was closer to the isolated lemon pectin.

Molecular Weight Features. **Figure 1** illustrates the MWD of pectins extracted from YPF rind by the three acid types at pH 1.8. Each exhibited a polydisperse and multimodal elution pattern with a broad range of polymeric and oligomeric materials. However, citric acid-extracted pectin was less heterogeneous in terms of polymer size distribution. Indeed, the elution profile of the latter showed only two distinguishable material peaks, including one major higher-molecular weight (HMW) material peak (>90% of the whole fraction) of wider range and one lower-molecular weight (LMW) material of narrower range, both eluting between the columns void (*Vo*) and total volumes (*Vt*), whereas the elution patterns of nitric- and sulfuric-extracted pectins showed the presence of three and four material peaks, respectively. The MWD of citric acid-extracted pectin globally suggests the presence of one population of pectin polymers together with free neutral sugar chains of relatively high sizes. In contrast, the MWDs of the two other pectins suggest the presence of at least two pectin populations accompanied with separate neutral sugar chains of relatively high and/or low sizes. In nitric acid-extracted pectin, HMW and medium (or intermediate)-molecular weight (MMW) materials were predominant (>85% of the whole fraction) and in sulfuric acid-extracted pectin, only MMW materials were major (>90% of the whole fraction). The *Mw* of pectin was estimated on the basis of noncomprehensive peak integration (retention time range of 19−28.5 min), which corresponds to polymeric materials. The *Mw* of YPF pectins varied from 104 to 246 kDa (**Table 3**). Pectin extracted with 0.03 M citric acid had a significantly ($p < 0.05$) higher *Mw* than pectin extracted with 0.01 M nitric acid. At pH 1.8, citric acid-extracted pectin had the highest *Mw* and sulfuric acid-extracted had the lowest *Mw*. The *Mw* of sodium citrate-containing nitric acid-extracted pectin significantly ($p < 0.05$) increased from 130 to 192 kDa as pH increased from 1.4 to 1.8. At pH 1.8, the *Mw* of pectin was higher when extracted in the presence than in the absence of sodium citrate. The *Mw* of isolated lemon pectin was 287 kDa and hence is the highest of the characterized pectins. In terms of *Mw*, pectin extracted with 0.03 M citric acid was closer to the lemon pectin.

Gelling Properties. Results of gelling power and velocity measurements of pectins are shown in **Table 3**. All of the selected pectins were capable of forming acid gels of high soluble solids (sucrose) content. The gelling power of pectins from YPF rind ranged from 127 to 179 depending on pectin type. Pectin extracted with 0.03 M citric acid displayed a significantly ($p < 0.05$) higher gelling power than pectin extracted with 0.01 M nitric acid. At pH 1.8, citric acid-extracted pectin exhibited higher gelling power than either nitric or sulfuric acid-extracted pectins. Sodium citrate-containing nitric acid-extracted pectin showed a significant ($p < 0.05$) increase in gelling power from 148 to 159 as pH increased from 1.4 to 1.8. At the latter pH, sodium citrate-containing nitric acid-extracted pectin had a significantly ($p < 0.05$) higher gelling capacity than pure nitric acid-extracted pectin. The gel strength of lemon pectin was 205 and hence is the highest of all the pectins submitted to jelly tests. As regards the gelling capacity, pectin extracted with 0.03 M citric acid appeared closer to the extracted lemon pectin.

The setting time of YPF pectin jelly preparations varied from 841 to 1236 s (**Table 3**). Pectin extracted with 0.03 M citric acid displayed a significantly ($p < 0.05$) lower setting

time than pectin extracted with 0.01 M nitric acid. At pH 1.8, sulfuric acid-extracted pectin show a higher setting time. Sodium-citrate containing nitric acid-extracted pectin showed a decrease in setting time from 1078 to 894 s as pH increased from 1.4 to 1.8. At the latter pH, the setting time of sodium citrate-containing nitric acid-extracted pectin was lower than pure nitric acid-extracted pectin. Lemon pectin exhibited a lower setting time. In terms of gelling properties, pectins extracted with 0.03 M citric acid and with sodium citrate-containing nitric acid at pH 1.8, to a lesser extent, were closer to the lemon pectin.

DISCUSSION

In my previous studies which aimed at adding value to the YPF rind, an unexploited byproduct from the juice industry in most tropical and subtropical areas, hot dilute nitric acid and nitric acid-containing oxalate extractants had been used to extract pectins from YPF rind under optimized conditions (*20, 22*). Even though satisfactory yields in the range of 11 to 15% (w/w) had been obtained from these extraction procedures, pectins extracted were characterized by DE considerably less than 50% and therefore were restricted (limited) to the preparation of calcium-mediated LMP gels (*20*). In this study, different acidic conditions achieved with three acid types were experimented in order to establish the best extraction conditions to solubilize high methoxy-esterified pectins along with good yields from YPF rind. The variation of extracting acid concentration showed that not only acid type but also its concentration can influence the extracted pectin yield. Generally, increasing the acid extractant concentration from 0.01 to 0.03 M significantly increases the pectin yield, with the exception of sulfuric acid. The latter case could be explained by degradation of some isolated pectin polymers to small oligomeric materials in higher concentrations of sulfuric acid and consequently their loss during dialysis and/or alcohol-precipitation of pectin extracts. By extracting soy hull pectins with hydrochloric acid at four different concentrations (0.05, 0.1, 0.2, and 0.3 N), significant effects of the acid extractant concentration on the yield of pectin have also been reported (*16*). However, in their case, lower concentrations (0.05 and 0.1 N) have been found to lead to the highest yields in contrast to the trend observed here. This is probably due to differing acid types and/or concentrations of acids, the latter having been used here under much lower concentrations and milder extraction temperature (75 vs 90 °C). At the same extractant concentration, citric acid recorded the lowest pectin yield. Surprisingly, the yield of pectin extracted with 0.01 M sulfuric acid is higher than the yield of pectin extracted with 0.03 M citric acid. These observations corroborate previous reports on pectin extraction from buttercup squash flesh (*19*) wherein 0.2 M citric acid extractant led to considerably lower total recovery of pectin isolate compared with either 0.1 M hydrochloric acid or 0.2 M nitric acid extractants.

The variation of the initial pH of extracting solvent showed that pH can also affect the yield of extracted pectin. Indeed, the amount of extracted pectin decreased as the pH of extractant increased from 1.8 to 2.5 irrespective of acid type. Citric acid solubilized a lower amount of pectin than either nitric or sulfuric acids. At pH 2.5, nitric acid has been reported to extract the highest amount of pectin from apple pomace flour compared with citric and sulfuric acids, but with larger variations between five replicates (*18*). As a result, the highest average yield was finally found for citric acid-extracted pectin in contrast to what has been observed here for pectins extracted from YPF rind. Since the pH of an acidic solvent has to do with the protic nature and much more with the degree of dissociation of acids dissolved in water, higher citric and nitric acid concentrations were needed to give the same initial pH as sulfuric acid solvents. At the same concentration or pH, the yield of sulfuric acid-extracted pectin was similar to the yield of nitric acid-extracted pectin, both yields being higher than the citric acid-extracted pectin yield. This suggests that discrepancies between citric acid-extracted and nitric (or sulfuric) acid-extracted pectin yields are not likely to result from their mono-, di-, or triprotic feature but most probably from their strong or weak nature.

The DE of pectin decreases when the acid extractant concentration increases from 0.01 to 0.03 M, sulfuric acid having a more pronounced effect. In contrast, only citric acid decreasingly influences the pectin DE when the pH varies from 2.5 to 1.8 to reach an esterification level as low as that yielded by sulfuric and nitric acids. This suggests that the pectin deesterifying action of citric acid solvent becomes similar to those of nitric and sulfuric acids only when its pH is around 1.8. At both concentrations and at pH 2.5, citric acid-extracted pectin has a higher DE than sulfuric or nitric acid-extracted pectin, indicating that the citric acid extractant possesses or exerts the least deesterifying effect on pectin solubilization from the cell wall. Hence, citric acid is likely to extract esterified pectins in a state closer to their (native) esterification level in the cell wall. This possibility is underpinned by a higher DE of pectin extracted using sodium citrate-containing nitric acid solvent at pH 1.8 compared to pectin extracted with pure (sodium citrate-free) nitric acid solvent. These differences in the deesterifying action between citric acid and the other acids may be caused by differences not only in their acid nature (weak or strong) but also in their stereochemistry, the former being molecularly bigger may have much difficulty accessing (the steric arrangement of) pectin methoxy groups. The DE of pectin is more than 50% under some extraction conditions, namely, using citric acid under all the conditions established, nitric acid at 0.01 M concentration, pH 1.8 and 2.5, sulfuric acid at pH 1.8 and 2.5, and sodium citrate-containing nitric acid at pH 1.4 and 1.8. This shows that high ester-methoxy pectins can be extracted from YPF rind (and other plant sources) either by weak or strong acids provided that sufficiently diluted solvents are used in the case of strong mineral acids. This is corroborated by the fact that a recent extraction of YPF rind pectin with dilute nitric acid at pH 1.3 (which is closer to 0.03 M nitric acid; theoretical pH ~1.5) has also yielded low methoxy-esterified pectins (*20*), whereas high methoxy-esterified pectins are obtained here using nitric acid extractant at pH 1.8 or 0.01 M concentration (theoretical pH ~2). Furthermore, hot dilute solvents of nitric acid (200 mM), hydrochloric acid (100 mM), and citric acid (200 mM) have been found to solubilize from buttercup squash flesh, pectins of 32, 42, and 50% of DE, respectively (*19*). Therefore, the possibility that lower concentrations of nitric acid would have been less damaging to ester groups has been put forward, which is evidenced in the present study. The boiling of rinds from a nonidentified passion fruit in dilute citric acid (7.5 g/3 L (~13.0 mM)) has yielded pectin fractions of 61% of DE (calculated from reported 46.17% of anhydrouronic acid and 4.96% of methoxy content) (*24*), and hot dilute citric acid (0.086% w/v (~4.5 mM)) treatment of YPF peel flour has been reported to be the satisfactory condition for obtaining high-ester pectin (78.59% of DE) (*30*); also, hot dilute citric acid (6.2 g/100 mL (~323.0 mM)) appeared to be the best condition to extract a high amount of high

methoxy pectin (DE = 68.84%) from apple pomace flour (*18*). The GalA/Rha molar ratios of pectins are largely >1, indicating that homogalacturonans are the predominant building blocks of their macrostructure. However, the proportions of homogalacturonans to rhamnogalacturonans I appear lower in citric acid-extracted pectins than in the other pectins as a result of less degradation. Hence, higher concentrations of the strong acid enriched isolated pectins in homogalacturonic regions as a result of degradation of neutral sugar-containing rhamnogalacturonic regions, which are more acid-sensitive. The *Mw* of citric acid-extracted pectin is higher, which confirms that citric acid solubilizes pectins from the cell wall material with less degradation and therefore of longer chains. Upon treating buttercup squash flesh with hot dilute citric, hydrochloric, or nitric acids, it has been observed that citric acid-extracted pectin has a greater chain length (*19*). The lower *Mw* of sulfuric acid-extracted pectin suggests that splitting might have partly occurred in homogalacturonic regions as well. In the presence of high soluble solids content and acid, gelation occurred with all of the selected pectins. However, citric acid-extracted pectin gels are firmer than either nitric acid- or sulfuric acid-extracted pectin jellies and could be explained by the positive gel-forming capabilities that higher DE and *Mw* generate. The stronger gel-forming ability of citric acid-extracted pectin is supported by higher gelling velocity as shown by lower setting time and corresponding higher setting temperature. The gelling power of sulfuric acid-exracted pectin is rather low and therefore might be good for Ca^{2+}-mediated LMP jellies. In terms of gelling properties, pectin isolated using sodium citrate-containing nitric acid solvent at pH 1.8 is closer to citric acid-extracted pectin of comparable DE and/or *Mw*, the latter being rather closer to lemon pectin of similar DE but different *Mw*.

This study shows that pectins of various degrees of esterification are extracted from yellow passion fruit rind using different sorts of acids at different concentrations or pH. High methoxy pectins are solubilized by using either weak organic acid or strong mineral acid extractants on condition that the latter are sufficiently diluted or tempered with the help of a weak acid conjugate base such as sodium citrate, an added advantage of this method being the obtaining of satisfactory pectin yields.

ABBREVIATIONS USED

DE, degree of esterification; DM, degree of methoxy-esterification in the absence of acetyl-esterification; HMP and LMP, high and low methoxy pectins, respectively; HMW, MMW, and LMW, higher, medium (or intermediate), and lower molecular weights, respectively; MWD, molecular weight distribution; *Mw*, weight-average molecular weight; YPF, yellow passion fruit.

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