JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Yellow Passion Fruit Rind—A Potential Source of Low-Methoxyl Pectin

BEDA MARCEL YAPO*,† AND KOUASSI LEONARD KOFFI‡

Unité de Formation et de Recherches en Sciences et Technologie des Aliments, Université d'Abobo-Adjamé, 02 BP 801 Abidjan 02, Côte d'Ivoire, and Institut National Polytechnique Felix Houphouet Boigny (INPHB), BP V 79 Abidjan, Côte d'Ivoire, France

Yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) rind pectic substances were fractionated with water, ammonium oxalate, and dilute acid solutions. The extracted pectins were rich in anhydrogalacturonic acid and had a low degree of methyl esterification. Moreover, their acetyl groups and neutral sugar contents were relatively low. Furthermore, a low amount of proteinaceous material was also found within them. Their gelling ability and viscoelastic properties as evaluated by the SAG and small amplitude oscillatory shear tests, respectively, were comparable to those of a commercial citrus low-methoxyl pectin. Hence, yellow passion fruit rind occurs as a potentially good source of naturally low-methoxyl pectin.

KEYWORDS: Yellow passion fruit; fractional extraction; low-methoxyl pectin

INTRODUCTION

Pectic substances, commonly known as pectins, are complex polysaccharides from higher plants, consisting of D-galacturonic acid and neutral sugar residues such as L-rhamnose (Rha), L-arabinose (Ara), and D-galactose (Gal). They are organized in a chainlike combination wherein the D-galacturonic acid residues are covalently α(1→4) linked to form a linear backbone. This main chain is interrupted in places by single residues of 2-O-linked-α-L-Rha (*1*), which sometimes bear neutral sugar side chains, mainly arabinans and/or arabinogalactans (*2*). Moreover, the galacturonic acid residues could be partly methyl-esterified at C-6 (*3*), and the hydroxyl groups at position O-2 and/or O-3 could be partly acetyl-esterified (*4*). Pectins extracted from several plant byproducts are widely used in many foodstuffs as gelling agents (*5*, *6*). Depending on the degree of methyl esterification (DM), which is defined as the percentage of anhydrogalacturonic acid (GalA) residues esterified with methanol (MeOH), pectins are referred to as high-methoxyl pectins (HMP) (DM ≥ 50) or low-methoxyl pectins (LMP) (DM < 50). A HMP forms a gel in an acidic medium (pH 2.0−3.5), if sucrose is present at a concentration ≥55wt %. In contrast, a LMP generally forms a gel in the presence of Ca^{2+} within a larger pH range (2.0−7.0) whether sugar is present or not. Commercial LMPs are manufactured from HMPs by acid, alkali, ammonia, or enzymatic deesterification. The main raw materials used to produce commercial LM and HM pectins are apple pomace and citrus peels (*5*). These industrial byproducts are also sources of dietary fibers (*7*, *8*). However, other dietary fiber-rich sources such as passion fruit (*Passiflora edulis*) seeds have also been reported (*9*). The dietary fiber from passion fruit seed has been found to consist mainly of cellulose, pectic substances, and hemicellulose (*9*). However, very little is known about the passion fruit pectic substances in general. Passion fruit is well-spread in tropical regions throughout the world. About 530 species have been identified in the Passifloraceae family (*10*). However, the two main edible species cultivated for a commercial purpose are purple passion fruit (*P. edulis* Sims), typically consumed fresh due to its sweeter taste, and yellow passion fruit (YPF) or maracuja (*P. edulis* f. *flavicarpa* Degener), commonly used for commercial pure or sweetened (due to its slight acidic taste) juice production. In the Ivory Coast, the juice industry generates hundreds of thousands of tons (3×10^5 tons/year) of YPF rinds, pulps, and seeds as agricultural byproducts (*11*), the bulk of which is merely incinerated after being sun-dried. The scope of this work is to examine the chemical and physicochemical features of pectin fractions isolated from YPF rinds.

MATERIAL AND METHODS

Chemicals. D-Galacturonic acid monohydrate, L-Rha monohydrate, L-fucose (Fuc), *myo*-inositol, L-Ara, D-xylose (Xyl), D-mannose (Man), D-Gal, D-glucose (Glu), and sodium borohydride were from Fluka (Buchs, Switzerland). Sodium hydroxide pellets, acetic acid (AcOH), citric acid, hydrochloric acid, nitric acid, sulfuric acid, acetic anhydride, dimethyl sulfoxide, dichloromethane, ethyl alcohol, methyl alcohol, and isopropyl alcohol were from Merck (Darmstadt, Germany). 3,5-Dimethylphenol, phenol, sodium chloride, and sodium fluoride were from Merck (Schuchardt OHG, Germany).

Pectins. A standardized commercial citrus LMP, Genupectin LM12CG (DM = 34%) (Hercules, Copenhagen, Denmark), and its purified form

* To whom correspondence should be addressed. Present address: Unité Biopolymères, Interactions et Assemblages, Institut National de la Recherche Agronomique, Rue de la Géraudière, BP 71627, F-44316 Nantes Cedex 03, France. Tel: +33 668 323 783. Fax: +33 240 675 066. E-mail: bedamarcel@yahoo.fr.

† Université d'Abobo-Adjamé.

‡ Institut National Polytechnique Felix Houphouet Boigny (INPHB).

10.1021/jf052605q CCC: $33.50
Published on Web 03/14/2006

(PLM12CG) in our laboratory were used as references. A sample of the Genupectin LM12CG was treated so as to remove primarily sugars (sucrose) according to the Food Chemicals Codex (FCC) method (*12*) with a slight modification. A 2% w/v aqueous pectin solution prepared at 40 °C was allowed to cool to room temperature, and 4 volumes of 95% ethanol were added and left for 1 h under gentle stirring and then stored at 4 °C overnight. The mixture was centrifuged at 30000*g* for 20 min, at 20 °C to recover pectin, which was washed with acidified 60% aqueous ethanol (5 mL of concentrated hydrochloric acid/L) until it became "sugar free", that is to say, when a blend of 1 volume of waste 60% aqueous ethanol filtrate with 1 volume of 5% w/v phenol and 5 volumes of concentrated sulfuric acid became colorless (*13*). Gelatinous pectin was then dried by solvent exchange with 95% ethanol and acetone, stored inside a ventilator (or fume hood) overnight, and finally dried in an air-circulated oven at 35 °C for 24 h. The dried flakes were finely ground using an IKA-A10 mill (Janke & Kunkel, IKA, GmbH & Co. KG, Labortechnik, Germany) to pass through a 60 mesh (0.25 mm) size screen sieve. This product was referred to as purified Genupectin LM12CG (PLM12CG) and packaged under airless conditions at room temperature until used.

YPF Rinds. Mature "green" YPFs (*P. edulis* f. *flavicarpa* Degener) were a gift from a cooperative of local producers, in a city named Bacon, located in the southeastern region of the Ivory Coast. The fruits were washed with tap water, rinsed with distilled water, and cut into two pieces to remove the juice sacs, which comprised the soft orange pulps and seeds. The remaining rinds were immediately heated in boiling water in a stainless steel reactor flask under magnetic stirring for 3 min, to inactivate the cell wall endogenous enzymes, and then sun-dried to approximately 8% final moisture content. The dried rinds were roughly ground in a hammer mill (Winona Attrition Mill Co., United States) to pass through a 12 mm mean diameter size screen sieve. The ground rinds were used to prepare cell wall material in the form of alcohol insoluble solids (AISs) used as the starting material for pectin fraction isolation.

AISs Preparation. Ground rinds were heated in boiling 80% ethanol (solid−liquid ratio 1:4, w/v) for 45 min and filtered through a G3 sintered glass. The residue was extensively washed with 60% aqueous ethanol to remove impurities, pigments, and free sugars until the filtrate was colorless and then dried by solvent exchange with 95% ethanol and then acetone, and finally dried in an air-circulated oven at 35 °C for 24 h. The dried AISs were packaged in sealed polyethylene bags and stored under airless conditions at room temperature until used. Preparation of AIS was carried out three times.

Fractional Extraction of Pectins. Dried AISs were suspended in distilled water (solid−liquid ratio 1:25, w/v) under gentle magnetic stirring, at 250 rpm, for 1 h at 30 °C. The resulting slurry was rapidly cooled to a temperature below 20 °C and centrifuged at 30000*g* for 20 min. The supernatant was filtered through a G4 sintered glass and clarified syrup adjusted to pH 4.0 with a 0.1 M nitric acid (HNO_3) solution. The residue was resuspended in distilled water (solid−liquid ratio 1:25, w/v), extracted for one more hour, and managed as before. The supernatant from the second extraction was added to the first and adjusted to pH 4.0. The combined extracts were vacuum-concentrated at 35 °C, under variable pressure (min, 600 mbar; max, 950 mbar), and extensively dialyzed for 72 h against distilled water in 12000 molecular weight cut off (MWCO) tubing (Fisher Scientific, United States). The material remaining in the dialysis tubing was poured into 4 volumes of 95% ethanol (final ethanol concentration > 70%) and allowed to stand for 1 h at room temperature (*14*) and then stored at 4 °C overnight for pectin precipitation. The mixture was centrifuged as above, and gelatinous pectin material was dispersed into distilled water under gentle stirring and then vacuum-evaporated at 35 °C to remove ethanol remnant for a better dissolution. The dispersion was freeze-dried and finally dried in an air-circulated oven at 35 °C for 24 h and weighed. This product was referred to as a water-extracted pectin (WEP). The pellets from the water extraction were extracted with 0.5% w/v ammonium oxalate solution (solid−liquid ratio 1:25, w/v) at 30 °C, twice for 1 h each (2 × 1 h), and treated as the WEP to yield a chelating agent-extracted pectin (ChEP). The residue left from ammonium oxalate extraction was first washed twice with distilled water to remove oxalate remnant and extracted with a dilute nitric acid solution (solid−liquid ratio 1:25, w/v), at pH 1.3, for 2 × 1 h at 80 °C. The clarified supernatants were adjusted to pH 4.0 using a 0.1 M sodium hydroxide (NaOH) solution and processed as above. The final product was referred to as an acid-extracted pectin (HEP). Fractional extractions so performed were conducted in three replications.

Chemical Characterization of AIS and Pectin Fractions. Analyses were performed in triplicate for AIS and each pectin type unless otherwise specified. Before analysis, AISs were finely ground and sieved to pass through a 60 mesh size screen.

Proximate Analysis. The moisture content of AIS and pectins was determined by drying samples at 120 °C in an air-circulated oven for 2 h (*15*). All data were then calculated on a dry matter basis. The protein content ($N \times 6.25$) of samples was determined by the Kjeldahl procedure (*16*), using a Foss Tecator Kjeltec 2300 Analyzer, with automatic distilling and titration unit (Foss Tecator, Sweden). The calcium content of pectins was determined by flame atomic absorption spectrometry using an Aanalyst 300 spectrophotometer (Perkin-Elmer Corp., United States). The absorbance was read at 422.7 nm.

Acid Hydrolysis of AIS and Pectins for Neutral Sugars and Galacturonic Acid Analysis. To determine the neutral sugar and GalA contents of AIS and pectins, dried samples of AIS and pectins were treated with 12 M aqueous sulfuric acid for 1 h at room temperature (*17*) for solvolysis, after which they were diluted to 1 M H_2SO_4 and incubated under gentle stirring in a water bath at 100 °C for 3 h in order to obtain a complete hydrolysis of nonstarchy polysaccharides to monomers.

Gas Chromatography (GC) Analysis. Individual neutral sugars were quantified by GC method after their reduction and conversion to alditol acetates (*18*). The alditol acetates were analyzed using an HP-5890 GC system equipped with a high-performance HP-5 capillary column (30 m L × 0.32 mm i.d., 0.25 μm film thickness, cross-linked 5% phenylmethyl silicone) (Hewlett-Packard Co., United States). The injector port set in splitless mode and the flame ionization detector were heated to 250 and 300 °C, respectively. The oven initial temperature was 110 °C and was programmed to rise, at the rate of 4 °C/min, to 220 °C where it was kept for 4 min, and then to 300 °C, at the rate of 15 °C/min, and kept for 8 min. Helium was used as the carrier gas.

Colorimetric Analysis. The anydrogalacturonic acid (GalA) content of AIS and pectins was determined by the 3,5-dimethylphenol colorimetric method (*19*), using a Perkin-Elmer Lambda 3 Double Beam Spectrophotometer (Perkin-Elmer Corp.). Briefly, to 300 μL aliquots of hydrolysate as described above (conveniently diluted with 1 M aqueous H_2SO_4 to contain uronic acids no more than 150 μg/mL), blank solution (1 M aqueous H_2SO_4) or standard solution placed in test tubes and 300 μL of sodium chloride−boric acid solution (2.0 g of NaCl and 3.0 g of H_3BO_3 dissolved in 100 mL of water) were added and mixed. Five milliliters (5 mL) of concentrated sulfuric acid was added and immediately vortex mixed and placed at 70 °C for 40 min. The test tubes were cooled to room temperature, and 200 μL of 3,5-dimethylphenol solution (0.1% w/v) (100.0 mg of 3,5-dimethylphenol dissolved in 100 mL of glacial AcOH) was added and thoroughly vortex mixed several times for 5 min. The absorbance of sample was measured at 400 and 450 nm against the blank solution 10 min later from the addition of 3,5-dimethylphenol. The reading at 400 nm was subtracted from that at 450 nm to correct for interference from hexoses. Galacturonic acid solutions (25, 50, 75, 100, and 150 μg/mL) prepared from D-galacturonic acid monohydrate, taking into account a conversion factor of 0.915 for weighing, with 1 M aqueous H_2SO_4 were used to construct the calibration curve. A factor of 0.907 was used to convert galacturonic acid to GalA, the form in pectin polysaccharides. Total carbohydrates (or total sugars) of AISs and pectins were determined using the colorimetric phenol−sulfuric acid method (*13*).

High-Performance Liquid Chromatography (HPLC) Analysis. MeOH and AcOH were released from AIS and pectin samples by saponification with 0.2 M NaOH for 2 h at 4 °C and then separated and quantified by HPLC method (*20*) on an Aminex HPX-87H ion exchange column (300 mm L × 7.8 mm i.d.; BioRad, United States), mounted on a Spectra Physics SP-8800 HPLC system (Spectra Physics, United States). Samples (25 μL) were injected onto the column, and elution was carried out with 5 mM H_2SO_4 at a constant temperature of

25 °C and at a flow rate of 0.6 mL/min with refractometric detection. The DM and degree of acetyl esterification (DA) were calculated as the molar ratio of MeOH or AcOH, respectively, to GalA quantified by the 3,5-dimethylphenol colorimetric method.

Physicochemical Properties. *Intrinsic Viscosity.* To determine the intrinsic viscosities of extracted pectins, viscosities (η) of pectin solutions at different concentrations were first determined by means of capillary viscosity experiments using an Ubbelohde capillary viscometer (capillary no. I; i.d., 0.63 mm; Schott-Geräte GmbH, Germany) with a constant K of 0.01 and immersed in a water bath thermostated at 25 °C. Pectin solutions were prepared at seven different concentrations (C) (0.01, 0.03, 0.05, 0.08, 0.10, 0.15, and 0.20 g/100 mL) by dissolving dried pectins at room temperature in an aqueous solution containing 90 mM sodium chloride, 10 mM sodium fluoride, and 1 mM Na_2EDTA at pH 6.5 (*21*). The pectin solutions and solvent were filtered using 0.45 μm membrane filters (Millipore Corp., United States) before viscosity measurements. Pectin solutions (15 mL) were pipetted into the capillary viscometer. Flow times were recorded with a stopwatch with a precision of ±0.1 s. Densities of solutions were measured using a 25 mL Gay−Lussac pycnometer [Boeco, Boeckel & Co. (GmbH & Co.), Germany]. The pectin solution viscosities (η) and solvent viscosity (η_s) were calculated from the measured solution flow times and densities and then the specific viscosities (η_{sp}) using eq 1. The intrinsic viscosities ([η]) of pectins were finally determined by plotting the reduced viscosities (η_{sp}/C) vs concentrations (C) and extrapolating to zero polymer concentration (*22*). Experiments were carried out three times, and average values were taken for plotting.

$$\eta_{sp} = (\eta - \eta_s)/\eta_s \quad (1)$$

Viscosity Average Molecular Weight (M_w). The viscosity average molecular weight of pectin was calculated, whenever possible, by applying the established Mark−Houwink−Sakurada relation for pectin irrespective of its degree of esterification and its source (*21*) and is defined in eq 2 as

$$[\eta] = 9.55 \times 10^{-4} M_w^{0.73} \quad (2)$$

where [η] is the intrinsic viscosity (dL/g) and M_w is the viscosity average molecular weight (g/mol).

This relation can be used to determine the intrinsic viscosity of a pectin polymer if its average molecular weight (M_w) is known and is lower than 100000 g/mol or the viscosity average molecular weight for pectin whose intrinsic viscosity is lower than 4.27 dL/g as calculated from eq 2.

Gelling Capacity. The gelling capacity of pectin was determined using the SAG method. For gel strength measurements, the standard FCC method for LMP as described by the National Research Council (*23*) was used. Although the SAG method does not evaluate the internal gel strength (inner firmness or breaking strength) given that jellies should not be destroyed during measurements, it is, however, the most commonly used method because of its simple handling and good reproducibility, apart from a relatively long standing time (about 24 h) that molded gels should stand before starting measurements with the ridgelimeter. Standard pectin gels were prepared according to the suggested method of the National Research Council (*23*) with a proposed modification (*24*). A pectin gel final composition was 1% for extracted pectins or PLM12CG and 1.4% for LM12CG in order to get a similar GalA content (*24*), 30% soluble solids (sucrose), and 25.8−27.9 mg Ca^{2+}/g pectin, at pH 3.0. The soluble solid content was measured by an AO Reichert Abbe Mark II Refractometer (Reichert, Inc., Scientific Instrument Division, United States). The pH of the jelly was adjusted to 3.0 using diluted citric acid/sodium citrate solutions, whenever needed, and a Cole-Parmer Digital pH meter (Cole-Parmer Instrument Co., United States). The prepared hot pectin gels were molded as fully described (*24*) and allowed to cool to room temperature and then stored at 4 °C for 24 h before starting measurements with a ridgelimeter (Bulmer Food Co., United Kingdom). Pectin gels were prepared in double replications for each pectin sample.

Rheological Measurements. A dynamic small amplitude oscillatory shear (SAOS) test was performed in a Bohlin CVO controlled-stress rheometer (Bohlin Instruments Ltd., United Kingdom) using a cone-plate geometry (40 mm plate diameter, 4° cone angle, and 150 μm gap). A frequency sweep performed on selected samples showed nonsignificant frequency dependence within the selected interval of frequency (0.1−10 Hz). Therefore, a single frequency (1 Hz) experiment was performed throughout testing. The viscoelastic behaviors of pectin dispersion were studied on a temperature sweep during a cooling scan from 95 to 5 °C at the rate of 3 °C/min, a holding time or not at 5 °C, and a subsequent heating from 5 to 95 °C at the rate of 3 °C/min. Dynamic measurements were performed for a 1% strain amplitude. First of all, an amplitude sweep was performed to ensure that the selected strain amplitude matches the linear viscoelastic region of pectin gels. Pectin solutions (1.0%, w/w) containing calcium ions at different concentrations (0, 1.7, 3.4, 6.8, 13.6, and 20.4 mM) and 30% sucrose were prepared as previously described (*24*) with modification. Briefly, mixtures of pectin powders and a half amount of sugar were dissolved in 100 mM sodium chloride solution at pH 3 under gentle stirring at room temperature. The mixtures were then heated to boiling point under stirring, and an appropriate amount of a preheated dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) solution prepared in 100 mM sodium chloride was added slowly under vigorous stirring to give different calcium concentrations (1.7, 3.4, 6.8, 13.6, and 20.4 mM). To prevent pregelation when adding calcium to mixtures containing both sucrose and high calcium concentration, the other half amount of sucrose was dissolved in the amount of dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$) solution to add. The pH was controlled and kept constant by adding if necessary either dilute citric acid or sodium citrate solutions. After the desired weight was reached, the hot pectin dispersion was directly applied to a preheated rheometer at 95 °C. A thin layer of low-viscosity paraffin oil was used to cover the exposed surface of the sample in order to minimize evaporation.

Rheological properties of molded gels were isothermally investigated at 5 °C over a period of 24 h, at a frequency of 1 Hz, and for a 1% strain amplitude using a plate−plate (parallel plate) geometry (40 mm, 1000 μm gap). The gels were cut into thick slices with the help of a sharpened thin cutter. Samples were then carefully placed on the lower plate and constantly compressed by lowering the upper plate. An isothermal equilibrium time of 3 min was observed before testing began. Each experiment was performed in three replications.

RESULTS AND DISCUSSION

Yields. The yield of AIS preparation was 82.3% of the starting dried rind. The extraction yields of WEP, ChEP, and HEP were 2.9, 2.1, and 11.3%, respectively, of dried AIS (**Table 1**). Hence, WEP, ChEP, and HEP accounted for 2.4, 1.7, and 9.3%, respectively, of the dried rind, which made up to a total pectin extract yield of 13.4% of dried rind. The HEP yield was the highest and accounted for about 70.0% of the overall pectin extract yield.

Chemical Features. The chemical composition of AIS is shown also in **Table 1**. The GalA content of AIS was 23.2% (on a dry weight basis). Hence, about 19.1% of dried YPF rind consists of pectic substances (on anydrogalacturonic acid basis). In our literature review, we have not found previous works reporting on the pectin content of passion fruit rind. Therefore, we compared our data with those available for commonly known sources of pectins. The amount of pectin found in the YPF rind was within the range of that reported for citrus peel (15−30%) (*25*), sugar beet pulp (15−30%) (*6*), or sunflower head residues (15−25%) (*26*). The main neutral sugars found in AIS were Glu (30.8%) and Xyl (12.3%). The amounts of the other neutral sugars, namely, Gal, Man, Ara, Rha, and Fuc, were relatively low. The AIS contents of total neutral sugars (NSs) (sum of the individual neutral sugars) and in total carbohydrates were 56.7 and 81.2%, respectively, showing the predominance of polysaccharides in YPF rind cell wall material. The remainder may consist of proteins, organic acids, lipids, ash, lignin, and/

Table 1. Yields (g/100 g of AIS), Chemical Composition (% w/w), DM (Mol %), and DA (Mol %) of Pectin Samples[a]

	AIS	WEP	ChEP	HEP	LM12CG	LM12CG
yield	100	2.9 (0.2)	2.1 (0.3)	11.3 (0.7)		
GalA	23.2 (0.9)	76.3 (2.2)	78.1 (1.7)	75.2 (1.8)	53.9 (1.1)	75.8 (1.5)
Rha	1.9 (0.1)	1.7 (0.3)	1.2 (0.1)	2.3 (0.1)	0.7 (0.1)	1.1 (0.1)
Fuc	0.7 (0.1)					
Ara	3.2 (0.4)	1.5 (0.1)	1.3 (0.1)	2.9 (0.3)	0.4 (0.1)	0.4 (0.1)
Xyl	12.3 (0.5)	0.6 (0.1)	0.5 (0.1)	0.7 (0.1)		
Man	3.5 (0.1)	0.7 (0.1)		0.3 (0.1)		
Gal	4.3 (0.2)	2.3 (0.1)	1.7 (0.1)	4.1 (0.2)	1.9 (0.2)	3.0 (0.2)
Glu	30.8 (1.2)	0.8 (0.1)	0.7 (0.1)	0.8 (0.1)	0.5 (0.1)	0.7 (0.1)
NS	56.7 (1.5)	7.6 (0.3)	5.4 (0.2)	11.1 (0.5)	3.5 (0.2)	5.2 (0.1)
TS	81.2 (2.8)	85.9 (2.7)	84.2 (1.6)	88.1 (1.2)	ND	ND
MeOH	1.3 (0.1)	4.5 (0.1)	3.9 (0.2)	3.6 (0.1)	3.2 (0.1)	4.3 (0.1)
DM	31.8 (0.6)	32.4 (1.7)	27.5(1.3)	26.3 (2.2)	33.7 (1.2)	31.2 (0.9)
AcOH	1.1 (0.3)	0.5 (0.2)	0.4 (0.1)	0.3 (0.2)		
DA	13.9 (0.8)	1.9 (0.2)	1.5 (0.1)	1.2 (0.1)		
protein	ND	1.4 (0.3)	1.4 (0.2)	5.1 (0.3)	0.9 (0.1)	ND
calcium (μmol/g)	ND	45.6 (1.3)	70.8 (2.1)	42.9 (1.6)	32.2 (0.9)	20.1 (1.1)

[a] Numbers in parentheses are standard deviations ($n = 3$); ND, not determined.

or residual water. The chemical composition of pectin fraction is shown also in **Table 1**. The GalA contents of WEP, ChEP, HEP, LM12CG, and PLM12CG were 76.3, 78.1, 75.2, 53.9, and 75.8%, respectively (**Table 1**). From these results, it could be calculated that the amount of standardizing sugar (sucrose) removed from the LM12CG accounted for 28.9% of its initial weight, and 9.5, 7.1, and 36.6% of the initial amount of GalA of AIS were recovered in WEP, ChEP, and HEP, respectively, which made up to a total of 53.2% recovery. The amount of GalA of HEP accounted for nearly 69.0% of the total GalA recovered, indicating that under the extraction conditions used, the dilute nitric acid extraction of pectin from YPF rind was the most effective. The GalA content of the three extracted pectin fractions was slightly higher than 65%, which is the lower limit of the FCC (*12*) purity specification for pectin. Therefore, the pectin extracts obtained from YPF rind could be considered of a high purity. The NS contents of WEP, ChEP, and HEP were 7.6, 5.4, and 11.1%, respectively (**Table 1**). Those of LM12CG and PLM12CG were 3.5 and 5.2% (**Table 1**). Hence, the extracted pectins from YPF rind were slightly richer in neutral sugars than commercial citrus LMP. The HEP had the highest neutral sugar content. Gal, Ara, and Rha were the main individual neutral sugars present in the three pectin fractions, suggesting the presence of arabinan, galactan, and/or arabinogalactan side chains. In contrast, in LM12CG, only Gal and Rha were present as the major individual neutral sugars, suggesting, therefore, the predominance of galactan side chains. Other neutral sugars such as Xyl, Man, and Glu were also present within pectin fractions (except for Man in the ChEP) but in relatively low amounts. Fuc was not found in any of the pectin fractions. On the other hand, the values of the molar ratio of Rha to GalA, as it could be inferred from **Table 1**, suggested that the pectin molecules of the three fractions could consist mainly of polygalacturonic acid-rich "smooth" regions. Furthermore, if the molar ratio of Rha to (Ara and Gal) taken altogether is indicative of the degree of side chain branching, then the HEP seemed the most branched product, given that it had the highest value of Rha/GalA along with the lowest value of Rha/(Ara + Gal) molar ratios, as it might also be calculated from **Table 1**. Their content in total carbohydrates ranged from 84.2 to 88.1% (**Table 1**). The methoxyl groups contents of WEP, ChEP, and HEP were 4.5, 3.9, and 3.6%, respectively, and corresponding DM values of 32.4, 27.5, and 26.3 (**Table 1**). Hence, all of the three extracted pectins had low DMs. DM values of extracted pectins were slightly lower than that of LM12CG. A DM of 33.7% (**Table 1**) was found for the commercial citrus LMP when analyzed by the HPLC method, consistent with that provided (34%) by the manufacturer, which is generally determined by titrimetry. Furthermore, the acid/ethanol washing had not highly affected the DM of the commercial citrus LMP, since 31.2% DM was found for the PLM12CG. The DM of WEP was slightly higher than those of ChEP and HEP. Their contents in acetyl groups ranged from 0.3 to 0.5%, and corresponding DAs ranged from 1.2 to 1.9 (**Table 1**). No acetyl groups were found in the commercial citrus LMP. The WEP was more esterified and richer in neutral sugars than the ChEP. Similar observations have previously been made for an acetate buffer and a strong chelating agent (CDTA) pectin fractions isolated from tomato cell wall material (*27*). The three pectin fractions contained protein residues in the order of 1.4−5.1%, which is higher than that found (0.9%) in the commercial citrus LMP (**Table 1**). Hence, acid extraction of pectin from YPF rind was likely to "cosolubilize" more protein residues than those of water or ammonium oxalate. The calcium content ranged from 20.1 to 70.8 μmol/g (**Table 1**); the ChEP had the highest calcium content.

Table 2. Intrinsic Viscosity ([η]), Viscosity Average Molecular Weight (M_w), and Gel Strength of Pectin Samples[a]

	WEP	ChEP	HEP	LM12CG	PLM12CG
[η] (dL/g)	3.07	2.91	2.62	ND	ND
M_w (g/mol)	63723	59218	51286	ND	ND
gel strength	110.9	115.7	101.5	104.8	104.1

[a] Gel strength data are averages of double replications; ND, not determined.

Physicochemical Properties. *Intrinsic Viscosity.* Magnitudes of the intrinsic viscosities of WEP, ChEP, and HEP determined by extrapolating to zero concentration the plots of reduced viscosities vs pectin concentrations were 3.07, 2.91, and 2.62 dL/g, respectively (**Table 2**). Hence, the HEP had the lowest intrinsic viscosity, probably because it occurred as the most branched polymer (*28*) and therefore might have possibly exhibited a smaller hydrodynamic size (*29*, *30*). The intrinsic viscosity values of extracted pectins are within the limit to be fitted, for viscosity average molecular weight assessment using eq 2. As a result, the viscosity average molecular weights of WEP, ChEP, and HEP were calculated, and values are shown also in **Table 2**. These values are within the range of values reported (*25*) for citrus LMPs.

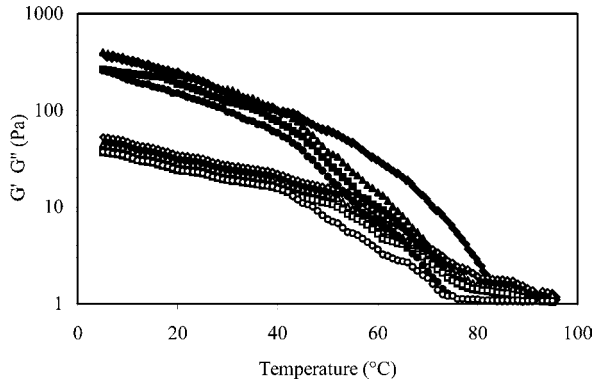

Figure 1. Evolution of storage modulus G' (filled symbols) and loss modulus G'' (open symbols) with temperature of LMP dispersions (1% w/w pectin, 30% sucrose, 6.8 mM calcium concentration, pH 3). LM12CG, ♦; WEP, ■; ChEP, ▲; and HEP, ●.

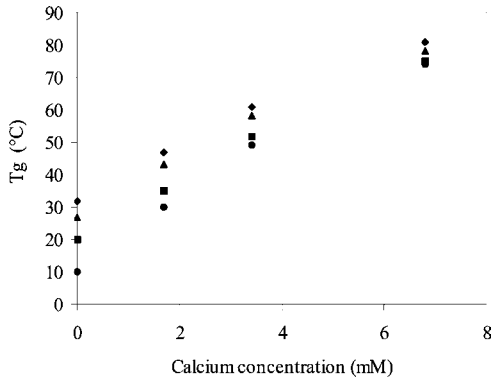

Figure 2. Calcium dependence of gel point temperature (T_g) of LMP gels (1% w/w pectin, 30% sucrose, pH 3). LM12CG, ♦; WEP, ■; ChEP, ▲; and HEP, ●.

Gelling Capacity. The gel strength of pectin fractions as evaluated by the standard SAG method is shown in **Table 2**. The gel strengths of WEP (110.9) and ChEP (115.7) were slightly higher than those of LM12CG (104.8) or PLM12CG (104.1). The ChEP displayed an apparent greater gel strength, probably due to its slightly higher calcium content (*24*) than the other pectin fractions. Moreover, the inner pectin conformation may also be implicated, as its intrinsic viscosity was the highest of all. The gel strength of LM12CG was in good agreement with a value of 109.0 previously reported (*24*) under similar conditions. The gel strength of citrus LMP was not significantly affected by the removal of standardizing sucrose before preparation of pectin gel.

Rheological Properties. The viscoelastic properties of pectin dispersions and gels were studied by following the evolution of storage (or elastic) modulus (G') and loss (or viscous) modulus (G'') during testing.

Temperature Dependence of the Viscoelastic Moduli. Figure 1 shows the trends of storage modulus (G') and loss modulus (G'') traces on a temperature sweep at the frequency of 1 Hz during a cooling scan of hot LM pectin dispersions (1% w/w pectin, 30% sucrose, and 6.8 mM Ca^{2+}, pH 3). Both storage (G') and loss (G'') moduli increased with decreasing temperature. Whatever the pectin fraction, a crossover of storage and loss modulus traces occurred at a characteristic temperature, indicating the "gel point" temperature (T_g). The gel point temperature increased with increasing calcium concentration (**Figure 2**). At a low calcium content, gel point temperatures of pectin fractions were different from one another, but after a certain calcium concentration, the T_g values of pectin fractions

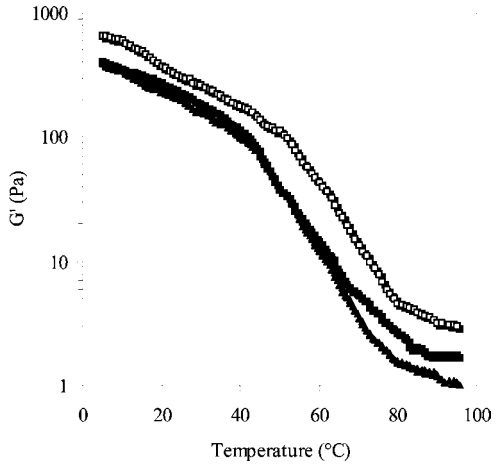

Figure 3. Temperature dependence of storage modulus (G') of ChEP dispersion (1% w/w pectin, 30% sucrose, 6.8 mM calcium concentration, pH 3). Cooling scan, ▲; heating scan after cooling to 5 °C, ■; and heating scan after cooling to 5 °C for 24 h, □.

nearly coincided. At a very high calcium concentration, the determination of T_g was not possible as pectin dispersions formed gels almost at the starting point of cooling. At a temperature higher than T_g, the magnitude of G' was slightly lower than that of G'' and pectin dispersions were liquidlike. In contrast, below T_g, the magnitude of G' was distinctly greater than that of G'' and pectin dispersions were gellike, indicating the formation of a three-dimensional cross-link network by complexation of calcium ions with segments of two pectin chains (*31*). Below T_g, a rapid increase in magnitudes of G' and G'' occurred when the temperature decreased until about 25 °C after which a somewhat slower subsequent increase took place. When the temperature was near 5 °C, the storage modulus (G') of a pectin gel was much greater than the corresponding loss modulus (G'') ($6 \leq G'/G'' \leq 9$), indicating a reinforcement of gel structure. At 5 °C, the highest value of G'/G'' ratio was shown by ChEP gel, confirming its apparent slightly higher gelling ability at 4 °C as aforementioned. Because the magnitude of G' was much greater than that of G'' below T_g, then much concern was given to the behavior of storage modulus (G') for the rest of testing.

When reheating a pectin gel precooled to 5 °C, the magnitude of G' decreased with increasing temperature (**Figure 3**). The magnitude of G'' also decreased with increasing temperature, and traces of G' and G'' crossed again at a characteristic temperature, indicating a "melting point" temperature (not shown). For a given pectin gel, it was observed that the melting point temperature was lower, as compared to the corresponding gel point temperature. Furthermore, when reheating almost immediately a pectin gel cooled to 5 °C, the heating trace of G' nearly coincided with that of the corresponding cooling trace (**Figure 3**). In contrast, after 24 h of aging at 5 °C, the heating trace of G' clearly strayed away from that of the cooling (**Figure 3**), showing a "true" thermal hysteresis, possibly due to structural changes during aging as previously reported for an olive LMP (*32*). However, as it could be seen, the general trends of heating and cooling traces of G' of pectin gel were rather similar, probably because the same types of interactions were responsible for stabilizing the gel (*32*) of LMP from YPF rind.

Effect of Calcium Concentration on the Gelation of LMP Fraction. Figure 4 shows the effect of calcium concentration on storage modulus (G') of pectin gels at 5 °C. For figure fluidness, only ChEP was shown as its general trend is also valid for the other pectin fractions. In the pectin dispersion

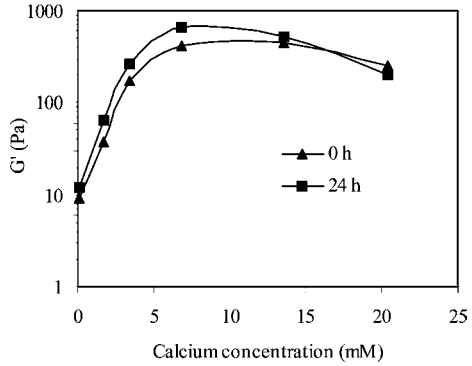

Figure 4. Evolution of storage modulus (G') with Ca^{2+} concentration of ChEP gels (1% w/w pectin, 30% sucrose, pH 3) at 5 °C and at two different aging times.

wherein calcium chloride solution was not added (≈0 mM calcium concentration), a very weak gel was formed in the presence of 30% sucrose, at pH 3, as the magnitude of G' (although higher than that of G'') was relatively low, about 9 Pa at the beginning of measurement and 12 Pa 24 h later. In this case, gel formation could be mediated by calcium initially present within pectin extract and an action which might have been strengthened by sucrose added as this latter reduced water activity, thereby favoring a "bringing together" of pectin chains. The magnitude of G' increased with increasing calcium concentration up to a "critical" calcium concentration, above which the magnitude of G' started to decrease, suggesting an appearance of "breaking point" in pectin gel structure and the formation of a nonhomogeneous network (the onset of collapse of the three-dimensional network). When the gelling properties of molded gels with different calcium concentrations were evaluated immediately after cooling them to 5 °C, a decrease in magnitude of G' was observed above 13.6 mM calcium concentration. In contrast, when gels were stored at 5 °C for at least 24 h of aging, a decrease in the magnitude of G' occurred at 13.6 mM calcium concentration, suggesting that a "critical" calcium concentration likely to lead to a destabilization of gel structure during aging was reached. This result also indicated that at a calcium concentration lower than 13.6 mM, the structural rearrangement occurring during aging, favored a reinforcement of gel structure, whereas at a concentration higher, a destabilization of gel structure was favored. Indeed, after 24 h of aging, a visual inspection of pectin gels with calcium concentration ≈13.6 mM showed turbid zones. In pectin gels containing calcium concentrations higher than 13.6 mM, a more severe destabilizing phenomenon occurred as syneresis (macroscopic serum separation) was observed, possibly due to a more preferential pectin−pectin interaction than pectin−water interaction, which resulted in a tight pectin aggregation. It has been reported that in the presence of excess ionic calcium, several primary units could form sheetlike aggregates (*33*). From these results, it could be inferred that 1% YPF rind LMP forms a coherent and clear gel with 30% sucrose, at pH 3, if the calcium concentration is <13.6 mM.

Time Dependence of Storage Modulus (G'). Figure 5 shows the behavior of storage modulus (G') on an isothermal time sweep over a period of 24 h for a precooled gel at 5 °C. The magnitude of G' increased rapidly during the first 8 h of aging, probably due to a slow formation and rearrangement of junction zones (*34*), and then started to flatten when an apparent "plateau" was reached, reflecting a state close to equilibrium, a typical behavior of LM and HM pectin gels previously reported (*32*, *34*).

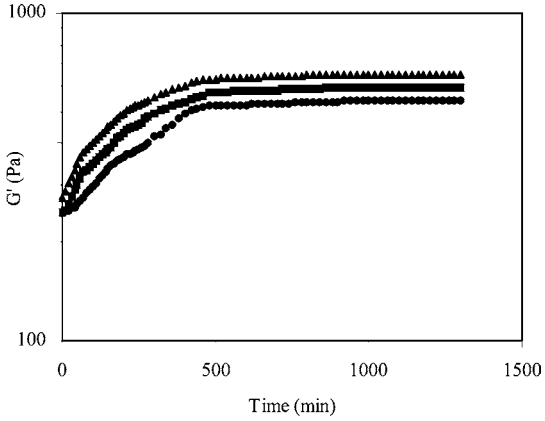

Figure 5. Time dependence of storage modulus (G') of LMP gels (1% w/w pectin, 30% sucrose, 6.8 mM calcium concentration, pH 3) at 5 °C. WEP, ■; ChEP, ▲; and HEP, ●.

YPF rind was found to consist of 19.1% pectic substances (on a dry weight basis). The fractionation of those pectic substances with water, ammonium oxalate, and dilute nitric acid solutions yielded pectin fractions with a high GalA content. All of the extracted pectins had a low DM and also contained a very low amount of acetyl groups. Their gelling ability in the presence of calcium ion, sucrose, and in an acid medium was comparable to that of a commercial citrus low-methoxl pectin. Their viscoelastic properties as evaluated by SAOS test were calcium-dependent. Indeed, at a very low calcium concentration, a weak gel was formed whereas above a "critical" calcium concentration, gel structure destabilization occurred, leading to an incoherent gel. In contrast, below and around the critical calcium concentration, a clear, coherent, and relatively strong gel was formed. The macroscopic structures of molded gels prepared from the extracted pectins were rather similar, although the ChEP gel showed an apparent stronger gel. Hence, on the basis of the relatively good physicochemical properties of extracted pectins, YPF rind occurs as a potentially new source of naturally LMP.

ABBREVIATIONS USED

AcOH, acetic acid; AISs, alcohol insoluble solids; Ara, arabinose; ChEP, chelating agent-extracted pectin; DA, degree of acetyl esterification; DM, degree of methyl esterification; Fuc, Fucose; Gal, galactose; GalA, anhydrogalacturonic acid; GC, gas chromatography; Glu, glucose; HEP, acid-extracted pectin; HMP, high-methoxyl pectin; HPLC, high-performance liquid chromatography; LMP, low-methoxyl pectin; Man, mannose; MeOH, methanol; MWCO, (nominal) molecular weight cut off; NS, total neutral sugar; Rha, rhamnose; TS, total carbohydrates or total sugars; WEP, water-extracted pectin; Xyl, xylose; YPF, yellow passion fruit.

ACKNOWLEDGMENT

We thank V. Besson and fellowship for calcium quantification and rheological analysis. We also express our gratitude to the Groupement à Vocation Cooperative de Bacon for kindly supplying mature "green" YPFs.

LITERATURE CITED

(1) Aspinall, G. O.; Craig, J. W. T.; Whyte, J. L. Lemon-peel pectin. Part I. Fractionation and partial hydrolysis of water-soluble pectin. *Carbohydr. Res.* **1968**, *7*, 442−452.

(2) De Vries, J. A.; Rombouts, F. M.; Voragen, A. G. J.; Pilnik, W. Comparison of the structural features of apple and citrus pectic substances. *Carbohydr. Polym.* **1984**, *4*, 89−101.
(3) Pilnik, W.; Voragen, A. G. J. Pectin substances and other uronides. In *The Biochemistry of Fruits and Their Products*; Hulme, A. C., Ed.; Academic Press: London, 1970; Vol. 1, pp 53−87.
(4) Rombouts, F. M.; Thibault, J.-F. Feruloylated pectic substances from sugar-beet pulp. *Carbohydr. Res.* **1986**, *154*, 177−187.
(5) May, C. D. Industrial pectins: Sources, production and applications. *Carbohydr. Polym.* **1990**, *12*, 79−99.
(6) Pilnik, W.; Voragen, A. G. J. Gelling agents (pectins) from plants for food industry. *Adv. Plant Cell Biochem. Biotechnol.* **1992**, *1*, 219−270.
(7) Larraurri, J. A.; Ruperez, P.; Bravo, L.; Saura-Calixto, F. High dietary fibre powders from orange and lime peels: Associated polyphenols and antioxidant capacity. *Food Res. Int.* **1996**, *29*, 757−762.
(8) Leontowicz, M.; Gorinstein, S.; Bartnikowska, E.; Leontowicz, H.; Kulasek, G.; Trakhtenberg, S. Sugar beet pulp and apple pomace dietary fibres improve lipid metabolism in rats fed cholesterol. *Food Chem.* **2001**, *72*, 73−78.
(9) Chau, C. F.; Huang, Y. L. Characterization of passion fruit seed fibres−A potential fibre source. *Food Chem*. **2004**, *85*, 189−194.
(10) Chassagne, D.; Boulanger, R.; Crouzet, J. Enzymatic hydrolysis of edible *Passiflora* fruit glycosides. *Food Chem.* **1999**, *66*, 281−288.
(11) BNETD. Problèmes écologiques des déchets agricoles: Fruits et légumes. *Les Annales Agronomiques*; Abidjan: Côte d'Ivoire, 2003.
(12) *Food Chemicals Codex*, 3rd ed.; National Academy of Sciences: Washington, DC, 1981.
(13) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350−356.
(14) Yapo, B. M.; Robert, C.; Etienne, I.; Wathelet, B.; Paquot, M. Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. *Food Chem*. **2006**, in press.
(15) AACC. *Approved Methods of the AACC*; American Association of Cereal Chemists: St. Paul, MN, 1983.
(16) AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984.
(17) Saeman, J. F.; Moore, W. E.; Mitchell, R. L.; Millet, M. A. Techniques for the determination of pulp constituents by quantitative paper chromatography. *Tappi* **1954**, *37*, 336−343.
(18) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccahride analysis. *Carbohydr. Res.* **1983**, *113*, 291−299.
(19) Scott, R. W. Colorimetric determination of hexuronic acids in plant materials. *Anal. Chem.* **1979**, *51*, 936−941.
(20) Voragen, A. G. J.; Schols, H. A.; Pilnik, W. Determination of the degree of methylation and acetylation of pectins by HPLC. *Food Hydrocolloids* **1986**, *1*, 65−70.
(21) Anger, H.; Berth, G. Gel permeation chromatography and the Mark−Houwink relation for pectins with different degrees of esterification. *Carbohydr. Polym.* **1986**, *6*, 193−202.
(22) Chou, T. D.; Kokini, J. L. Rheological properties and conformation of tomato paste pectins, citrus and apple pectins. *J. Food Sci.* **1987**, *52*, 1658−1664.
(23) National Research Council. Pectin. In *Food Chemicals Codex*, 2nd ed.; National Academy of Sciences: Washington, DC, 1972; pp 577−581.
(24) Chang, K. C.; Miyamoto, A. Gelling characteristics of pectin from sunflower head residues. *J. Food Sci.* **1992**, *57* (6), 1435−1438, 1443.
(25) Padival, R. A.; Ranganna, S.; Manjrekar, S. P. Low methoxyl pectins from lime peel. *J. Food Technol.* **1979**, *14*, 333−342.
(26) Shi, X. Q.; Chang, K. C.; Schwarz, J. G.; Wiesenborn, D. Effect of countercurrent ethanol washing on sunflower pectin quality. *Carbohydr. Polym.* **1995**, *27*, 171−175.
(27) Huber, D. J. Acidified phenol alters tomato cell wall pectin solubility and calcium content. *Phytochemistry* **1991**, *30*, 2523−2527.
(28) Hwang, J.; Kokini, J. L. Contribution of the side branches to rheological properties of pectins. *Carbohydr. Polym.* **1992**, *19*, 41−50.
(29) Graessley, W. W. Detection and measurement of branching in polymers. *Characterization of Macromolecular Structure*; National Academy of Sciences: Washington, DC, 1968; pp 371−379.
(30) Bates, T. W. The melt viscosity of branched polydisperse polymers. *Eur. Polym. J.* **1972**, *8*, 19−34.
(31) Grant, G. T.; Morris, E. R.; Rees, D. A.; Smith, P. J. C.; Thom, D. Biological interactions between polysaccharides and divalent cations: The egg-box model. *FEBS Lett.* **1973**, *32*, 195−198.
(32) Cardoso, S. M.; Coimbra, M. A.; Lopes da Silva, J. A. Temperature dependence of the formation and melting of pectin-Ca^{2+} networks: A rheological study. *Food Hydrocolloids* **2003**, *17*, 801−807.
(33) Thakur, B. R.; Singh, R. K.; Handa, A. K. Chemistry and uses of pectins−A review. *Crit. Rev. Food Sci. Nutr.* **1997**, *37*, 47−73.
(34) Lopes da Silva, J. A.; Gonçalves, M. P.; Rao, M. A. Kinetics and thermal behaviour of the structure formation process in HMP/sucrose gelation. *Int. J. Biol. Macromol.* **1995**, *17*, 25−32.

Received for review October 20, 2005. Revised manuscript received February 8, 2006. Accepted February 13, 2006. This research work was financially cosupported by the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique de Côte d'Ivoire and Fonds d'Aide à la Recherche aux Pays du Sud, Section Afrique-Francophone.

JF052605Q