Enzymatic Degradation of Cell Wall Polysaccharides from Mango (*Mangifera indica* L.) Puree

Didier Ollé,*,† Alain Baron,‡ Yves F. Lozano,† and Jean-Marc Brillouet†

Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Départment FLHOR, Montpellier, F-34032, France, and Institut National de la Recherche Agronomique (INRA), Station de Recherches Cidricoles et de Biotransformation des Fruits et Légumes, Le Rheu F-35650, France

Ripe mango puree (Smith cultivar) was treated with fungal polysaccharidases containing pectinolytic, hemicellulolytic, and cellulolytic activities for 2 h at 50 °C. A loss of 30% of the cell wall material (CWM) was measured. CWM polysaccharides were hydrolyzed to varying degrees: 88, 65, and 65% of, respectively, galacturonic acid-, arabinose-, and rhamnose-containing polymers were hydrolyzed, whereas 50% of cellulose was degraded. After 30 min of treatment, the ethanol precipitation test on the serum was negative, indicating that pectic substances were rapidly hydrolyzed. Oligogalacturonic acids (degree of polymerization, 1−12) were observed in the serum. A viscosity drop of 90% was measured after 2 h, confirming the dominant role of pectic substances in puree viscosity.

Keywords: *Mango; Mangifera indica L.; cell wall polysaccharides; enzymatic treatment; viscosity*

INTRODUCTION

Mango (*Mangifera indica* L.), a highly prized tropical fruit, is fragile, and significant postharvest losses occur in producing countries due to inadequate handling, transportation, storage, and ripening practices (Medlicott et al., 1986). Most of the world production of mangoes is consumed raw as a dessert fruit, and only a small proportion is processed into nectars, juice powder, fruit bars, canned mango slices in syrup, chutneys, and pickles (Sreenath et al., 1995). A reduction of postharvest wastage could be achieved by greater use of processing, in particular to produce intermediate food ingredients such as purees suitable for reuse by the fruit and vegetable industry.

Mango puree is highly viscous and consistent and therefore requires a preliminary enzymatic treatment to break down cell wall polysaccharides (mainly pectic substances) to reduce viscosity before further processing. Commercial enzyme mixtures, including pectinases and cellulases, have been successfully applied to mango pulp (Sreenath et al., 1995; Bhattacharya and Rastogi, 1998). However, even if pectic substances are thought to play a major role in mango puree consistency, to our knowledge, there has been no detailed study on the enzymatic degradation of mango cell wall polysaccharides.

The aim of our study was (i) to characterize cell wall polysaccharides from a puree of a commercial mango cultivar and (ii) to study how fungal polysaccharidases affect these constituents, and thus puree viscosity, during an enzymatic treatment.

* Author to whom correspondence should be addressed [telephone (33) 04 67 61 58 00, ext. 52 30; fax (33) 04 67 61 44 33; e-mail olle@cirad.fr].

† CIRAD-FLHOR.

‡ INRA.

MATERIALS AND METHODS

Preparation of the Puree. Fruits from the commercial monoembryonic Smith cultivar were harvested at the preclimacteric green mature stage in the IDEFOR experimental orchard at Korhogo (Ivory Coast) and rapidly air-freighted to our laboratory. Full ripening was obtained under ethylene as described by Ollé et al. (1996). A puree was prepared by removing the skin and the kernel of ripe fruits from a homogeneous batch (200 kg) with a rotating pulper (H. P. Auriol S.A., Marmande, France) equipped with a 6 mm screen and then further refined through a 1 mm screen (yield ∼ 60%/fruit weight). The puree was stored (12 months) in polyethylene bags at −20 °C before subsequent processing.

Enzymatic Treatment of the Mango Puree. A sample of frozen refined mango puree (30 kg; 18.3 °Brix) was gently thawed in a cold room (4 °C, 72 h), brought to room temperature, and then gradually heated to 50 °C under constant stirring in a jacketted closed tank (total duration ∼ 1 h). To the warm puree was then added 38 mL of a mixture (1:0.3:0.075, v/v/v) of three commercial enzyme preparations, Rapidase CPE (Gist Brocades France S.A., Seclin, France) and Celluclast 1.5 L, and Pectinex Ultra SP-L (Novo Ferment Nordisk Ltd., Basel, Switzerland), and the slurry was incubated at 50 °C for another 2 h under constant stirring. Aliquots were taken at each step of the process [starting puree (A) and puree after 30 min (B) and 2 h (C) of enzymatic treatment], immediately frozen at −50 °C, and kept at −20 °C until they were analyzed.

Isolation of Cell Wall Material (CWM). Puree aliquots (A−C; 10 g each) were thawed in 50 mL of cold HEPES buffer (40 mM, pH 7.0, 4 °C) (Huber, 1991), thoroughly homogenized in a Potter Elvehjem homogenizer, and centrifuged (13500*g*, 4 °C, 30 min), and the pellet was thoroughly washed with HEPES buffer with intermittent centrifugations. CWM was obtained by treatment of the pellets with buffered phenol (Huber, 1991; Ollé et al., 1996) and further destarched (Brillouet et al., 1988). CWM was dried by solvent exchange, stored overnight in a vacuum oven (50 °C, 9 kPa) under P_2O_5, weighed (0.01 mg accuracy), and cryomilled in liquid nitrogen (−196 °C) with a Spex 6700 freezer mill (Spex Industries Inc., Edison, NJ) for 5 min at top impact frequency.

Analytical Procedures. Neutral monosaccharides were released from CWM (5 mg) by hydrolysis with 2 M trifluoro-

10.1021/jf990924+ CCC: $19.00
Published on Web 06/21/2000

Table 1. Characteristics of Commercial Enzymatic Preparations

	type of preparation		
	Rapidase CPE[a]	Celluclast 1.5 L[b]	Pectinex Ultra SP-L[a]
proteins (mg/mL)	0.70	70	11.3
activities (nkat/mL)[c]			
pectin lyase			21515 (44536)
polygalacturonase	13 (359)[d]	350 (2901)	24659 (51044)
pectin methyl esterase	1517 (41930)		6000 (12420)
endoglucanase[e]	63 (1741)	4270 (35398)	721 (1492)
cellobiohydrolase			10 (21)
β-glucosidase		140 (1161)	172 (356)

[a] Marcelin (1992). [b] Massiot et al. (1989). [c] 1 nkat = 1 nmol of substrate degraded/s. [d] Values in parentheses are nkat for 30 kg of puree. [e] Carboxymethylcellulose as substrate.

acetic acid (TFA) for 75 min at 120 °C (Albersheim et al., 1967). They were also submitted to Saeman hydrolysis as described by Hoebler et al. (1989), using 72% (w/w) sulfuric acid for 3 h at 25 °C and then 1 M sulfuric acid for 2 h at 100 °C. Sugars were then derivatized into their alditol acetates (Blakeney et al., 1983) and analyzed by GC according to the method of Hoebler et al. (1989) with inositol as internal standard. Uronic acids were measured without de-esterification after preliminary dissolution in concentrated sulfuric acid according to the *m*-phenylphenol procedure (Blumenkrantz and Asboe-Hansen, 1973; Ahmed and Labavitch, 1977) using galacturonic acid as a standard. Proteins (N × 6.25) were determined according to a micro-Kjeldahl procedure (Bietz, 1974). Oligogalacturonic acids were analyzed in the serum (13500*g*, 4 °C, 30 min) of samples A−C by high-performance anion-exchange chromatography (HPAEC) as follows: 25 μL of 5-fold-diluted serum was injected onto a Carbopac PA-1 column (0.4 × 25 cm; Dionex) fitted with a Carbopac PA-1 guard column (0.4 × 5 cm) and eluted at 1 mL/min on a Dionex 300 chromatographic system (PAD detector) using 100 mM NaOH/1 M sodium acetate. The conditions were as follows: 0 → 15 min, isocratic elution at 90:10 (v/v) and injection at 5 min; 15 → 35 min, linear gradient to 50:50 (v/v); 35 → 60 min, linear gradient to 30:70 (v/v); 60 → 70 min, linear gradient to 100% 1 M sodium acetate. Identification of oligogalacturonic acids was achieved by using two methods: injection or co-injection of diluted serum aliquots with either a mixture of commercial pure mono-, di-, and trigalacturonic acids (Aldrich-Sigma Chimie, St. Quentin Fallavier, France) or a mixture of higher oligomers [up to degree of polymerization (DP) of 20] obtained by limited hydrolysis of polygalacturonic acid with a purified endopolygalacturonase (endo-PG) (Pellerin et al., 1996). 1,4-Linked α-D-oligogalacturonides were also located on the chromatograms and differentiated from visible unknown peaks and artifacts by extensive digestion of a serum aliquot with the above purified endo-PG. Concentrations of mono-, di-, and trigalacturonic acids were determined with titrated solutions of commercial standards. Having determined the PAD detector response factors of these three standards, concentrations of higher oligomers were tentatively estimated by attributing to them the response factor of the trimer. Apparent viscosity of aliquots A−C was measured at various shear rates using a Haake VT 550 (Berlin, Germany) viscosimeter (25 °C) fitted with a coaxial cylinder sensor system NV.

RESULTS AND DISCUSSION

The mango puree was treated for 2 h at 50 °C with a mixture of commercial enzymatic preparations, various activities of which are shown in Table 1. The pectin methylesterase/polygalacturonase ratio was adjusted to ∼1 for maximum hydrolysis of the pectic backbone (Le Quéré et al., 1986). The level of β-glucosidase was sufficient to prevent inhibition of endoglucanase activity by cellobiose liberated by cellobiohydrolase activity. Nonetheless, partial inhibition of the β-glucosidase activity was expected due to the presence of glucose (0.5%/fw) in the puree. Other side activities (α-arabinofuranosidases, β-galactosidases, etc.) were also present.

Table 2. Composition[a] of CWM from the Starting Puree (A) and from Puree Samples after 30 min (B) and 2 h (C) of Enzymatic Treatment

	starting puree (A)	puree after 30 min (B)	puree after 2 h (C)
yield (%)	1.0	0.8	0.7
rhamnose[b]	0.2	0.1	0.1
fucose[b]	0.7	0.6	0.6
arabinose[b]	1.4	0.7	0.7
xylose[c]	4.1	3.4	3.1
mannose[c]	3.2	2.6	1.7
galactose[b]	2.5	1.9	2.1
glucose (noncellulosic)[b]	7.4	8.0	10.0
glucose (cellulosic)[d]	23.6	17.8	16.8
uronic acids	16.5	5.1	2.8
proteins	18.9	31.5	33.4

[a] Percent of dry matter. [b] Values from TFA hydrolysis. [c] Values from Saeman hydrolysis. [d] Obtained by difference between Saeman and TFA hydrolyses.

Yield and composition of CWM were obtained for the starting puree (A) and the purees after 30 min (B) and 2 h (C) of enzymatic treatment, and the results are shown in Table 2. Sample A had a CWM content similar to those previously obtained for other mono- and polyembryonic cultivars (Ollé et al., 1996). However, its protein content was surprisingly high compared to the 9.4%/CWM found in CWM prepared 12 months earlier from Smith puree just after delivery of the fruits. This doubling of CWM protein content is entirely responsible for the increase of puree CWM content observed after 12 months storage at −20 °C (1.0%/fw versus 0.84%). Additional nonwall proteins were found in samples B and C, total proteins representing up to one-third of CWM. This might be due to the denaturation of soluble nonwall proteins during heat treatment (total duration of ∼3 h at 50 °C) and these proteins becoming resistant to the CWM purification procedure.

The polysaccharide composition of CWM from cv. Smith (sample A, Table 2) was very similar to those from two other monoembryonic mangoes, Amélie and Palmer cultivars, particularly with regard to their uronic acids content (∼13−17%) and the presence of noncellulosic glucose (∼8%) (Ollé et al., 1996). Degradation of insoluble CWM was measured after 30 min (sample B) and 2 h (sample C) of enzymatic treatment (Table 2). Compared to the starting puree (A), samples B and C contained lower proportions of CWM, and because the weight of the puree used in the experiment (30 kg) remained unchanged (no evaporation), the hydrolysis/solubilization of CWM could be calculated as ∼20 and ∼30% after 30 min and 2 h, respectively.

Sugar analysis of CWM in samples A−C (Table 2) allowed calculation of the percentage of hydrolysis/solubilization of each monosaccharide, which could reflect to some extent the cell wall polysaccharides having undergone depolymerization. Taking into account the CWM percent yield of the different samples (A−C), 76 and 88% of uronic acids (i.e., acidic pectic substances) were eliminated from the walls after 30 min and 2 h, respectively. Rhamnose and arabinose, two monosaccharides mainly associated with acidic (rhamnogalacturonans) and neutral (arabinans and arabinogalactans) pectic substances (Aspinall, 1980), were also extensively degraded after 30 min and 2 h (65% in both cases). All other neutral sugars disappeared from

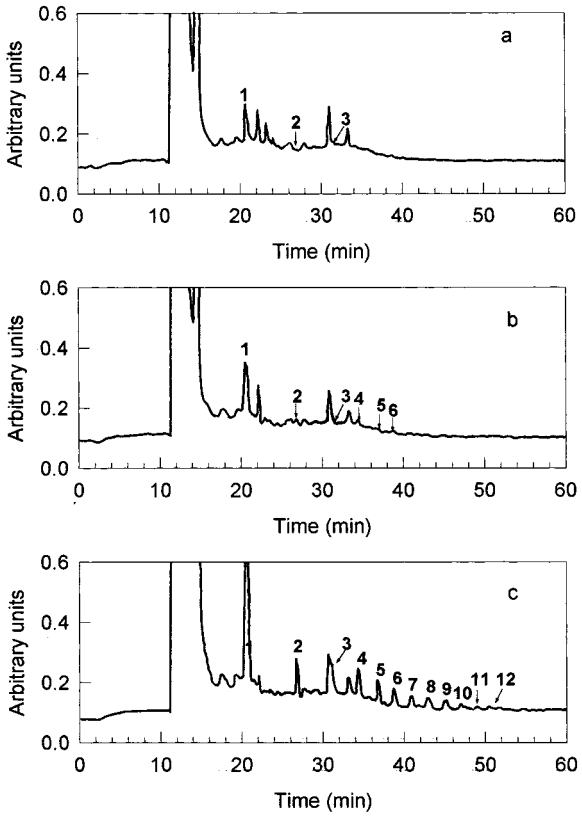

Figure 1. HPAEC analysis of 1,4-linked α-D-oligogalacturonic acids (DP 1−12) generated by the enzymatic mixture at times 0 (a), 30 min (b), and 2 h (c).

the CWM to various degrees, except for noncellulosic glucose, which probably belongs to dicotyledonous hemicellulosic xyloglucans (Aspinall, 1980) and which remained unchanged. Only 40 and 50% of the cell wall cellulose were degraded after 30 min and 2 h, respectively. Mango flesh mainly consists of parenchymatous cells with thin walls but also contains some secondary lignified elements (Ollé et al., 1996), the weight of which, as a proportion of total CWM, is unknown. It is therefore likely that lignin-encrusted cellulose of these cell types was resistant to cellulolysis. Furthermore, the glucose content of the puree (0.5%) was high enough to partially inhibit the β-glucosidases in the enzymatic mixture.

Acidic degradation products (oligogalacturonic acids) resulting from the action of pectinases on cell wall pectic substances were also analyzed by HPAEC (Figure 1). The serum of the starting puree (A) (Figure 1a) contained some galacturonic acid and other unknown compounds but virtually no oligogalacturonic acids. After 30 min (sample B, Figure 1b), galacturonic acid had slightly increased and higher oligomers of DP 2−6 became visible. After 2 h (sample C, Figure 1c), 1,4-linked α-D-oligogalacturonic acids up to DP 12 were apparent, and the level of galacturonic acid had greatly increased. Tentative quantification of mono- and oligogalacturonic acids after 2 h gave a total of 0.6%/puree fresh weight. This should be compared to a maximum of 0.53%/fw present in the puree of the Smith cultivar (soluble and insoluble pectic substances expressed as anhydrogalacturonic acid; Ollé, 1997). This confirms that soluble and insoluble acidic pectic substances were entirely hydrolyzed after 2 h of treatment to low DP fragments, as is also indicated by the negative ethanol precipitation test on the serum.

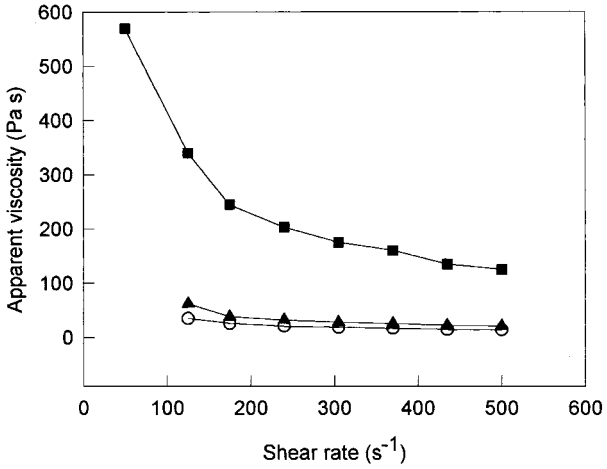

Figure 2. Rheogram of the puree at time 0 (■) and after 30 min (▲) or 2 h (○) of enzymatic treatment.

Viscosity fell by 84 and 90% (measured at a shear rate of 250 s^{-1}) after 30 min and 2 h of treatment, respectively (Figure 2). Similar figures (70−90%) have been reported by Sreenath et al. (1995) and by Bhattacharya and Rastogi (1998) after treatment of purees from various mango cultivars with commercial pectinases alone or in association with cellulases. Our results show that the treatment of mango puree with industrial amounts of commercial pectinases and cellulases allowed almost complete hydrolysis of pectic substances and a very large fall in viscosity after only 30 min of treatment, whereas cellulose was only partly hydrolyzed.

ACKNOWLEDGMENT

Thanks are due to O. Gibert (CIRAD-AMIS) for viscosimetric assays.

LITERATURE CITED

Ahmed, A. E. R.; Labavitch, J. M. A simplified method for accurate determination of cell wall uronide content. *J. Food Biochem*. **1977**, *1*, 361−365.

Albersheim, P.; Nevins, D. J.; English, P. D.; Karr, A. A method for the analysis of sugars in plant cell wall polysaccharides by gas−liquid chromatography. *Carbohydr. Res.* **1967**, *5*, 340−345.

Aspinall, G. O. Chemistry of cell wall polysaccharides. In *The Biochemistry of Plants*; Preiss, J., Ed.; Academic Press: New York, 1980; pp 473−500.

Bhattacharya, S.; Rastogi, N. K. Rheological properties of enzyme-treated mango pulp. *J. Food Eng.* **1998**, *36*, 249−262.

Bietz, J. A. Micro-Kjeldahl analysis by an improved automated ammonia determination following manual digestion. *Anal. Chem*. **1974**, *46*, 1617−1618.

Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291−299.

Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem*. **1973**, *54*, 484−489.

Brillouet, J.-M.; Rouau, X.; Hoebler, C.; Barry, J. L.; Carré, B.; Lorta, E. A new method for determination of insoluble cell walls and soluble nonstarchy polysaccharides from plant materials. *J. Agric. Food Chem*. **1988**, *36*, 969−979.

Hoebler, C.; Barry, J. L.; David, A.; Delort-Laval, J. Rapid acid hydrolyses of plant cell wall polysaccharides and simplified quantitative determination of their neutral monosaccharides by gas−liquid chromatography. *J. Agric. Food Chem*. **1989**, *37*, 360−367.

Huber, D. J. Acidified phenol alters tomato cell wall pectin solubility and calcium content. *Phytochemistry* **1991**, *30*, 2523−2527.

Le Quéré, J.-M.; Baron, A.; Drilleau, J.-F. La flottation appliquée à la clarification des jus de pomme. Essais d'optimisation au laboratoire. In: *Proceedings of the 19th IFU Symposium*, Den Haag (The Netherlands), 1986; Juris Druck and Verlag: Zurich, Switzerland, 1987; pp 199−217.

Marcelin, O. Caractérisation des polysaccharides pariétaux de la goyave *(Psidium guajava* L.). Application à l'obtention de nouveaux types de purées par voie enzymatique. Thesis, Université des Sciences et Techniques du Languedoc, Montpellier, France, 1992.

Massiot, P.; Thibault, J. F.; Rouau, X. Degradation of carrot (*Daucus carota* L.) fibres with cell wall polysaccharide-degrading enzymes. *J. Sci Food Agric.* **1989**, *49*, 45−57.

Medlicott, A. P.; Reynolds, S. B.; Thompson, A. K. Effects of temperature on the ripening of mango fruit (*Mangifera indica* L. var. Tommy Atkins). *J. Sci. Food Agric.* **1986**, *37*, 469−474.

Ollé, D. Caractérisation des polysaccharides et des composés aromatiques de différents cultivars de mangue (*Mangifera indica* L.). Devenir de ces constituants lors de la préparation de concentrés aromatiques pulpeux. Thesis, Ecole Nationale Supérieure des Industries Agricoles et Alimentaires, Massy, France, 1997.

Ollé, D.; Lozano, Y. F.; Brillouet, J.-M. Isolation and characterization of soluble polysaccharides and insoluble cell wall material of the pulp from four mango (*Mangifera indica* L.) cultivars. *J. Agric. Food Chem.* **1996**, *44*, 2658−2662.

Pellerin, P.; Doco, T.; Williams, P.; O'Neill, M. A. Structural characterization of red wine rhamnogalacturonan II. *Carbohydr. Res.* **1996**, *290*, 183−197.

Sreenath, H. K.; Sudarshana Krishna, K. R.; Santhanam, K. Enzymatic liquefaction of some varieties of mango pulp. *Lebensm.-Wiss. -Technol.* **1995**, *28*, 196−200.

Received for review August 19, 1999. Revised manuscript received March 10, 2000. Accepted April 25, 2000. This research work was supported by the European Union (Contract TS3* CT93-0209).

JF990924+