Isolation and Characterization of Soluble Polysaccharides and Insoluble Cell Wall Material of the Pulp from Four Mango (*Mangifera indica* L.) Cultivars

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Mature green fruits from monoembryonic (Amélie and Palmer) and polyembryonic (M'Bingue and Tête de Chat) mango cultivars were initiated to ripen with ethylene (10 ppm, 24 h) and then left to reach full ripeness (6 days). After elimination of skin and kernel, pulp was added with HEPES (1/5, w/w) and centrifuged. Soluble polysaccharides were obtained from the supernatant by precipitation with ethanol and freeze-drying. Cell wall material (CWM) was isolated from the pellet by the buffered phenol procedure and further enzymatically destarched. Soluble polysaccharides (∼0.5−0.8%/pulp fresh weight) were essentially highly esterified pectic substances (uronic acids content ∼50−60%; degree of methyl esterification ∼89−97%) and their molecular weights were higher in the polyembryonic cvs. CWM, ∼1%/pulp fresh weight, was mainly built of cellulose (∼20%) and highly esterified pectic substances (uronic acids ∼13−24%; degree of esterification ∼63−73%). Hemicellulosic glucans were more abundant in the monoembryonic (∼9%) than in the polyembryonic (∼4%) cultivars.

Keywords: *Mango; Mangifera indica L.; cultivars; pulp; soluble polysaccharides; cell wall material*

INTRODUCTION

Mango (*Mangifera indica* L.), described as the most favored and valuable fruit throughout the tropics (Purseglove, 1974; Sreenath *et al.*, 1995), is of major economic concern as reflected by its position on the fruit world market: fifth in terms of production, all temperate and tropical fruits considered, and second among tropical fruits beyond banana, India being by far the largest producer (∼10 million metric tons/year, *i.e.*, nearly 60% of world production) (Goguey-Muethon, 1995). Mango flavor and taste are highly prized in tropical areas, and, due to breeding of hundreds of cultivars around the world, texture and flavor vary from soft, fiber-free, sweet, and juicy to stringy and turpentine-flavored (Purseglove, 1974). The developing taste of consumers from temperate countries turns to nonfibrous cultivars exhaling a mild aroma.

Despite all its potentialities, mango fruit is fragile, and significant postharvest wastage occurs in producing countries due to insufficiently established practices of handling, convoying, storage, and ripening (Medlicott *et al.*, 1986).

Most of mango world production is consumed raw as a dessert fruit, the rest of it being processed into diverse products, such as nectar, juice powder, canned mango slices in syrup, chutneys, pickles, etc. (Subramanian *et al.*, 1976; Ammu *et al.*, 1976; Sahni and Khurdiya, 1989). Enzyme technology, nowadays widely spread in the fruit and vegetable industry of temperate countries, is increasingly considered for processing tropical fruits, *e.g.*, mango (Sreenath *et al.*, 1987, 1995), thus as an additional alternative to reduce postharvest losses and increase the added value of finished products. However, the use of exogenous fungal enzymes (mainly pectinases and cellulases) as processing adjuvants for, *e.g.*, reduction of pulp viscosity, improvement of juice extraction yields, or clarification of juices is not yet fully optimized on mango. These commercial enzymes mixtures degrade the soluble viscous pectic substances occurring in the liquid phase of the pulp (viscosity drop) (Sreenath *et al.*, 1987) and the insoluble highly hydrated cell wall framework (maceration or liquefaction) (Sreenath *et al.*, 1995).

Results from a recent study (Sreenath *et al.*, 1995) suggest that reduction of viscosity and increase of juice yield upon enzymatic treatment of pulp from ripe mangoes might vary according to the considered cultivars. Although data have been reported on soluble polysaccharides and cell walls of mango during ripening and storage (Roe and Bruemmer, 1981; Voragen *et al.*, 1983; Brinson *et al.*, 1988; Tucker and Seymour, 1991), to our knowledge, no comparative study on these components from various cultivars at ripe stage has been released. As a preliminary work in a wider research program on enzyme treatment of mango pulp, we determined the content and composition of cell wall material (CWM) and soluble polysaccharides, including their molecular weight distribution, from the pulp of four mango cultivars at the ripe stage, and the results are presented thereafter.

MATERIALS AND METHODS

Fruits and Preparation of Pulp. Monoembryonic (Amélie and Palmer) and polyembryonic (M'Bingue and Tête de Chat) cultivars were obtained from the CIRAD-IDEFOR experimental orchard of Korhogo (Ivory Coast). Fruits were harvested at the preclimacteric mature green stage (Medlicott

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Table 1. Chemical Composition of Pulps

cultivar	pH	titratable acidity, mEq (100 g)$^{-1}$	soluble solids[a]	glucose[a]	fructose[a]	sucrose[a]	sugar/acid
Amélie	4.70	2.74	13.1	1.45	3.85	3.55	3.2
Palmer	4.83	3.02	17.2	0.60	2.35	8.10	3.7
M'Bingue	4.24	5.12	16.6	1.00	3.05	7.65	2.3
Tête de Chat	4.48	3.59	16.3	1.10	3.40	6.00	2.9

[a] Percent of pulp fresh weight.

et al., 1992) and batches (~200 kg for each cultivar) were constituted on a fruit grade basis (average fruit weight, n = 30; Amélie, 321.4 ± 30.6 g; Palmer, 480.4 ± 42.5 g; M'Bingue, 159.9 ± 16.4 g; Tête de Chat, 213.3 ± 20.6 g). Fruits were rapidly air-freighted and delivered to our laboratory. Immediately on arrival, fruits were brought to the fully ripe stage by storing for 24 h in a temperature controlled room (21 ± 1 °C) under ethylene atmosphere (10 ppm) then for 6 days under normal atmosphere (Tucker and Seymour, 1991). Skin and kernel were removed without preliminary treatment by passing the entire ripe mangoes through a prototype pulper (H. P. Auriol S. A., Marmande, France) equipped with a 6-mm screen and the pulp was further refined through a 1-mm screen and added with sulfur dioxide (10 ppm) to prevent oxidation upon storage. After sampling, pulp was immediately frozen at −20 °C.

Isolation of Soluble Polysaccharides and Cell Wall Material. Pulp (10 g) was thawed in 50 mL of cold HEPES buffer (40 mM, pH 7.0; Huber, 1991), thoroughly homogenized in a Potter Elvehjem homogenizer, and after centrifugation (13500*g*, 4 °C, 30 min), supernatant was kept aside. The pellet was extensively washed with HEPES buffer with intermittent centrifugation, and the washing liquors were added to the above supernatant. Soluble polysaccharides were then precipitated by adding 96% ethanol to the HEPES supernatant (5:1 v/v) and the medium was stored overnight at 4 °C. After centrifugation, the pellet was extensively washed with 80% ethanol, freeze-dried, stored overnight in a vacuum oven (50 °C, 9 kPa) in the presence of P_2O_5 and then weighed (0.01 mg accuracy). CWM was purified by treating the pellet from HEPES extraction with buffered phenol (Huber, 1991). After extensive washing with Milli-Q water with intermittent centrifugation, the pellet was further enzymatically destarched according to Brillouet *et al.* (1988). At the end of the destarching step the reaction medium was added with 96% ethanol (1:5 v/v) to precipitate onto the CWM the pectic substances which could have been solubilized from the cell walls by boiling water. Residue was recovered by filtration through a weighed sintered glass crucible (porosity no. 4), washed with 80% ethanol, dried with acetone (100 mL) and diethyl ether (10 mL), stored overnight in a vacuum oven in the presence of P_2O_5, and weighed. Prior to analysis, CWM was cryomilled in liquid nitrogen (−196 °C) with a Spex 6700 Freezer-mill for 5 min (top impact frequency). Determinations were run in duplicate.

Analytical Procedures. All reagents were of analytical grade.

Characterization of Pulp. pH was measured by a Hanna LCD pH meter, acidity by titration with 0.1 M NaOH, and soluble solids by a hand refractometer. Glucose, fructose, and sucrose were measured, after homogenization of the pulp in a Potter Elvehjem homogenizer and centrifugation, by high performance anion-exchange chromatography (HPAEC) on a CarboPac PA-1 column (0.4 × 25 cm, Dionex, USA) with a CarboPac PA-1 guard column (0.4 × 5 cm) eluted at 1 mL/min on a Dionex 300 chromatography system (PAD detector) using the following gradient: 50 mM NaOH from 0 to 15 min (injection after 5-min elution) then linear gradient up to 120 mM NaOH in 10 min. Arabinose was used as internal standard.

Analysis of Soluble Polysaccharides and CWM. Neutral monosaccharides were released from soluble polysaccharides and CWM (5 mg) by hydrolysis with 2 M trifluoroacetic acid (TFA) for 75 min at 120 °C (Albersheim *et al.*, 1967). CWM was also submitted to Saeman hydrolysis as described by Hoebler *et al.* (1989), *i.e.*, 72% (w/w) sulfuric acid, 3 h, 25 °C, then 1 M sulfuric acid, 2 h, 100 °C. Sugars were then derivatized into their alditol acetates (Blakeney *et al.*, 1983) and analyzed by GC according to Hoebler *et al.* (1989) with inositol as internal standard. Uronic acids were measured without preliminary desterification by the *m*-phenylphenol procedure (Blumenkrantz and Asboe-Hansen, 1973) with, for CWM, preliminary dissolution in concentrated sulfuric acid (Ahmed and Labavitch, 1977). Estimation of methanol was carried out using the alcohol oxidase method of Klavons and Bennett (1986). Proteins (N × 6.25) were determined by a micro-Kjeldahl procedure (Bietz, 1974).

Table 2. Pulp Content[a] in Soluble Polysaccharides and Cell Wall Material

cv.	soluble polysaccharides	CWM
Amélie	0.79	0.75
Palmer	0.74	1.05
M'Bingue	0.47	1.05
Tête de Chat	0.62	0.96

[a] Percent of pulp fresh weight.

Molecular weight distribution of soluble polysaccharides was determined by high performance size-exclusion chromatography (HPSEC) as reported (Pellerin and Brillouet, 1992) on two serial (0.8 × 30 cm) Shodex OHpak KB-803 and KB-805 columns (Showa Denkko, Japan) with a (0.6 × 5 cm) OHpak KB-800P guard column, equilibrated at 1 mL/min with 0.1 M $LiNO_3$ (total volume of the system 23 mL). The elution profile was monitored with an Erma-ERC 7512 (Erma, Japan) refractive index detector thermostated at 40 °C.

RESULTS AND DISCUSSION

Medlicott *et al.* (1990) stated that ripening of mangoes of various physiological ages (except immature fruits) can be initiated and more or less synchronized by ethylene until full ripeness is achieved. Thus the ethylene-induced procedure described by Tucker and Seymour (1991) was applied to the fruits from four mango cultivars harvested at the preclimacteric mature green stage. Firmness, as estimated by hand, decreased over the 7-day ripening period and the skin color turned from green to different patterns of yellow. After seven days the fruits were considered fully ripe. Full ripeness was attested by the pulp content in sucrose and soluble solids, titratable acidity, and the sugar/acid ratio (Table 1) (Medlicott *et al.*, 1986; Wu *et al.*, 1993). Although the Amélie cultivar exhibited lower figures, its sugar/acid ratio was similar to others, low sucrose content being counterbalanced by its low acidity.

Fruits were then pulped and the yields in refined pulp obtained were Amélie 60%/fruit weight and Palmer 62% in agreement with average yields reported by Wu *et al.* (1993), M'Bingue 34%, and Tête de Chat 39%. Differences between both pairs of cultivars were to be attributed essentially to the (fruit/stone volume) ratio which is higher in the two former cvs.

Amélie and Palmer had a slightly higher content in soluble polysaccharides (~0.7−0.8%/pulp fresh weight) than M'Bingue and Tête de Chat (~0.5−0.6%/fw) (Table 2). Tandon and Kalra (1984) found a similar level (~0.8%/fw) of water-soluble pectic substances in the pulp of ripe mangoes of the Dashehari cv. These soluble polysaccharides were essentially pectic in nature ac-

Table 3. Composition[a] of Soluble Polysaccharides

	cv.			
	Amélie	Palmer	M'Bingue	Tête de Chat
uronic acids	51.9 (90.8)[b]	50.0 (96.7)	63.8 (91.3)	56.5 (88.7)
rhamnose[c]	0.5	0.7	0.5	0.3
fucose[c]	0.2	0.3	0.4	0.1
arabinose[c]	2.7	4.2	4.6	2.1
xylose[c]	0.2	0.6	0.4	0.1
mannose[c]	0.3	0.2	1.9	0.1
galactose[c]	9.6	9.9	2.6	1.3
glucose[c]	1.2	3.0	0.4	0.3
proteins	6.7	6.0	3.9	5.8

[a] Percent of dry matter. [b] Values in parentheses are the degrees of methylation calculated on the basis of uronic acid contents. [c] Values obtained by TFA hydrolysis.

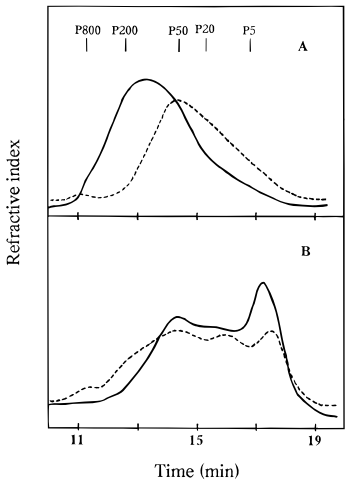

Figure 1. HPSEC profiles of soluble polysaccharides from mango pulp. (A) Cultivars M'Bingue (—) and Tête de Chat (- - -); (B) cultivars Amélie (—) and Palmer (- - -). Elution times of pullulan molecular weight standards (P5−P800) are also shown.

cording to their high uronic acid content (∼50−60%) and the predominance of arabinose and galactose as neutral sugars (Table 3). A high degree of methyl esterification was observed for the four cultivars. The soluble polysaccharides from Amélie and Palmer cvs. contained a far higher level of galactose than those from M'Bingue and Tête de Chat cvs, a slighty lower level of uronic acids, and some glucose, which might reflect the occurrence in both monoembryonic cultivars of pectic−hemicellulosic (xyloglucans) complexes (Stevens and Selvendran, 1984).

The molecular weight distribution of these polysaccharides (Figure 1) showed major differences between both pairs of cultivars: Amélie and Palmer exhibited similar profiles with three visible populations eluting at ∼14, 16, and 17 min. M'Bingue and Tête de Chat showed broad distributions eluting at ∼13−14 min with no material of low hydrodynamic volume as in the case of Amélie and Palmer. Soluble pectic substances from pulp of the two polyembryonic cultivars having higher hydrodynamic volumes (related to molecular weight via intrinsic viscosity; Grubisic *et al.*, 1967) than in the two monoembryonic mangoes, one can expect a higher viscosity for pulps from the former cultivars. The differential influence of hydrodynamic volume of water-soluble pectic substances on pulp viscosity was also mentioned in the case of mesocarp and endocarp of guava fruit (Marcelin *et al.*, 1993a). Although the molecular weight distribution of soluble polysaccharides from mango pulp (mesocarp) is highly dependent on maturity stage (Tucker and Seymour, 1991; Muda *et al.*, 1995), the fully ripe status of examined cultivars points out an actual major difference between the two mono- and polyembryonic cultivars with regard to their soluble pectic substances molecular weights.

Table 4. Composition[a] of Cell Wall Material

	cv.			
	Amélie	Palmer	M'Bingue	Tête de Chat
uronic acids	17.0 (66.2)[b]	13.0 (72.4)	24.0 (72.8)	13.4 (63.1)
rhamnose[c]	0.2	0.2	0.3	0.2
fucose[c]	0.9	1.0	0.8	0.8
arabinose[c]	2.2	2.4	3.0	2.0
xylose[d]	5.0	4.9	4.4	4.1
mannose[d]	2.5	1.8	1.9	1.7
galactose[c]	5.0	4.1	4.2	4.5
glucose[c] (noncellulosic)	8.6	8.7	3.8	4.1
glucose[e] (cellulosic)	19.1	18.4	19.6	18.1
proteins	4.9	8.9	6.0	10.3

[a] Percent of dry matter. [b] Values in parentheses are the degree of methylation calculated on the basis of uronic acid contents. [c] Values obtained by TFA hydrolysis. [d] Values obtained by Saeman hydrolysis. [e] Obtained by difference between Saeman and TFA hydrolyses.

Several procedures (Selvendran, 1975; Huber, 1991) were tested for extraction and purification of cell wall material (CWM) from mango pulp. The buffered phenol technique (Huber, 1991) was found the most appropriate to mango since it provided CWM retaining the maximum amount of uronic acids vs pulp fw (low degree of solubilization of cell wall pectic substances) and of the lowest nitrogen content. CWM contents (Table 2) averaged 1%/fw whatever the considered mango cultivar except Amélie. Extra determinations conducted on pulp of ripe fruits from monoembryonic Smith and Kent cvs also gave ∼1%/fw. Such data, suggesting some homogeneity among ripe mangoes of various origins with regards to pulp CWM content, were surprising since M'Bingue and Tête de Chat cvs., having a rather fibrous flesh, were expected to give higher figures than commercial Amélie and Palmer cvs., which are far less fibrous in texture. CWM content of mango pulp could be compared with those found in apple cortex (1.5%/fw; Stevens and Selvendran, 1984), runner-bean pod parenchyma (0.9%/fw; Ryden and Selvendran, 1990), or guava endocarp and mesocarp (0.9 and 1.1%/fw, respectively; Marcelin *et al.*, 1993b). No pertinent comparisons could be made with literature data on structural material from mango pulp, since several authors (Roe and Bruemmer, 1981; Voragen *et al.*, 1983; Brinson *et al.*, 1988; Askar *et al.*, 1990) reported a range of 2−3%/pulp fw of alcohol or acetone insoluble residues (AIR) which contain, in addition to CWM, soluble polysaccharides, coprecipitated soluble proteins, salts of organic acids, and other minor compounds (Selvendran, 1975).

Only ∼60−70% of the CWM matter could be determined as cell wall neutral and acidic polysaccharides and proteins (Table 4). Similar data were obtained with olive pulp (Coimbra *et al.*, 1994) and inner parenchyma of kiwi fruit (Redgwell *et al.*, 1988), and this was interpreted as the occurrence of polyphenolics either native to the walls (*e.g.*, lignin) or as intracellular compounds (*e.g.*, tanins), possibly oxidized during the purification procedure. Observation of mango CWM under light microscope using phloroglucinol-HCl (Marcelin *et al.*, 1993b) revealed pink-stained xylem elements and the Bate-Smith reaction (Bate-Smith, 1975), specific for polymeric tanins, was negative. Thus, missing matter could be accounted marginally for some lignin (not determined).

CWMs from the pulp of the four considered mango cultivars had virtually the same monosaccharide compositions except for noncellulosic glucose. Since CWMs were submitted, after the buffered phenol extraction, to a destarching treatment and subsequently checked for the absence of starch by light miscroscopy (iodine test), monoembryonic Amélie and Palmer cultivars differed definitely from polyembryonic M'Bingue and Tête de Chat cvs. by having a higher level of noncellulosic (xylo)-glucans in their CWMs. Cellulose, the main structural polymer (~30−40% of cell wall polysaccharides), was remarkably constant among the four CWMs. As frequently observed (Rinaudo and Chambat, 1976; Lecas and Brillouet, 1994) mannose and, to a lower extent, xylose were obtained in higher levels after Saeman hydrolysis than after TFA one, indicating some degree of crystallinity of mannose and xylose containing polymers. The 4 N NaOH soluble hemicellulosic fraction of mango pulp CWM was described, among other fruits studied, as the richest in mannose and, to a lower extent, in xylose (Voragen *et al.*, 1983), which might indicate a certain degree of secondarization of part of the tissues. However, contrary to guava flesh (Marcelin *et al.*, 1993b), it has not been possible to separate the different cellular types (mainly primary parenchyma, but also xylem) of mango pulp. Other sugars, arabinose, galactose, fucose, and rhamnose, were obtained in amounts comparable to those from TFA hydrolysis (Selvendran *et al.*, 1979). Acidic pectic polysaccharides were present in noticeable amounts showing, contrary to cellulose, some variations among the studied cultivars. Their degrees of methylation were high as in other parenchymatous tissues of fruits, *e.g.*, apple (Stevens and Selvendran, 1984) or kiwi fruit (Redgwell *et al.*, 1988). Although being reasonably low, the protein level found in purified mango CWMs might indicate the occurrence of phenolics of unknown nature (Coimbra *et al.*, 1994).

We characterized separately soluble polysaccharides (*i.e.*, mainly pectic substances) and cell walls occurring in the pulp (mesocarp) of ripe fruits from two monoembryonic and two polyembryonic mango cultivars. Pectic substances were almost fully methyl esterified and their molecular weight (hydrodynamic volume) was found higher in the two polyembryonic cvs. Reduction of pulp viscosity, *i.e.*, breakdown of soluble pectic substances, could thus be achieved with, in addition to polygalacturonase, a sufficient proportion of pectin methyl esterase in an enzymatic mixture. Insoluble cell walls were found in similar low percents and their total liquefaction for, *e.g.*, production of clear mango juice (Sreenath *et al.*, 1995) would require, in addition to pectinases, cellulolytic enzymes. Modelization of enzymatic hydrolysis of these two pulp components with varying proportions of pectinolytic and cellulolytic enzymes is now under way.

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