Polysaccharide Constituents of Coffee-Bean Mucilage

S. AVALLONE, J.-P. GUIRAUD, B. GUYOT, E. OLGUIN, AND J.-M. BRILLOUET

ABSTRACT: Alcohol-insoluble residues (AIRs) were isolated from hand-dissected and commercial mucilages of depulped coffee beans. Both AIRs had similar polysaccharide composition: pectic substances (about 30%), cellulose (about 8%), and neutral noncellulosic polysaccharides (about 18%). Crude pectins were extracted from AIRs (dry-matter yield: about 23% to 35%) with dilute nitric acid (pH 1.5, 90 °C). Both pectins contained about 60% uronic acids with high degree of methyl esterification (about 62%) and moderate degree of acetylation (about 5%). Their molecular weights were low (about12 to 29 kDa). They did not gel in the presence of sucrose at acidic pH.

Key Words: coffee, *Coffea arabica* L. var. *typica* Cramer, mucilage, cell-wall polysaccharides, mechanical demucilagination

Introduction

COFFEE CHERRY, A DRUPE, CONSISTS OF SMOOTH, TOUGH OUTER-skin or exocarp; soft yellowish pulp or outer mesocarp; and greyish-green fibrous endocarp (parchment) surrounding seeds (Purseglove 1974) (Figure 1). Pulping removes the exocarp and the fleshy outer mesocarp leaving a thin viscous, highly hydrated layer, the inner mesocarp, also called mucilage. In order to facilitate drying and hulling, beans must be fermented naturally in tanks for the mucilage to be removed. There are still conflicting views as assumptions to whether the mucilage is degraded by the natural microflora and/or by endogenous enzymes (Carbonell and Vilanova 1952; Wilbaux 1956; Rolz and others 1971). Conversely, there is general agreement that cell-wall pectic polysaccharides are degraded during the fermentation. In order to better control the wet fermentation process, a thorough knowledge of potential substrates for microbial and/or plant enzymes, in particular the polysaccharides, is required.

Mucilage is also occasionally removed mechanically (Purseglove 1974) yielding a wasted by-product that could be used as a source of industrial pectins. Several authors have reported on the presence of pectins in coffee mucilage (Coleman and others 1955; Wilbaux 1956; Calle 1958; Sudhakara Rao 1975; Garcia and others 1991). However, some of the reported data are somewhat controversial since the pectins were said not to form a firm gel with calcium addition, on the one hand (Garcia and others 1991), or to gel in the presence of calcium gluconate, on the other hand (Calle 1958). Furthermore, the reported degrees of methyl esterification of mucilage pectins seem to be very low (Garcia and others 1991).

In preliminary work, as part of an extensive research program on the coffee wet fermentation process, mucilage was prepared by careful hand-dissection, in contrast to obtaining commercial mucilage produced by mechanical demucilagination. The polysaccharide composition of these substrates was then analyzed.

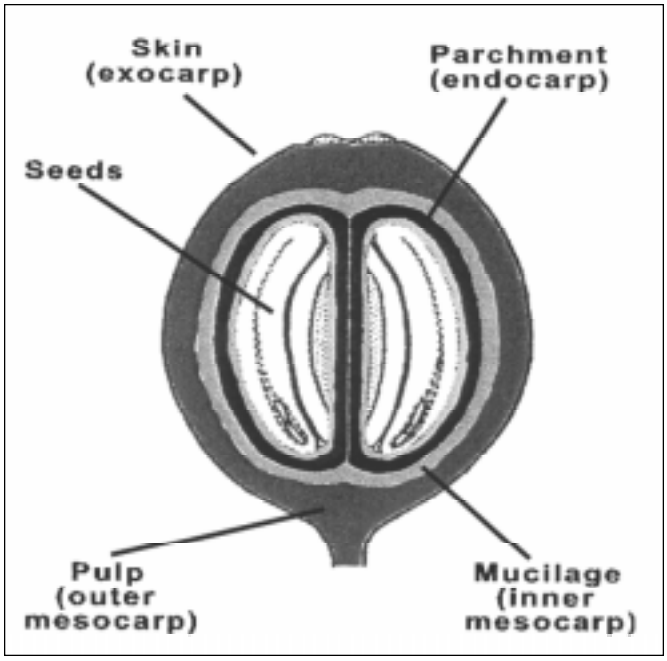

Figure 1—Longitudinal section of a coffee cherry

Materials and Methods

Plant material

The coffee variety used was *Coffea arabica* L. var. *typica* Cramer. It was grown in the Coatepec area (Xalapa, Veracruz, Mexico) during the 1998 season. Coffee cherries were harvested at the mature stage and immediately depulped using a DV-255C Penagos depulper. One-half of the batch of the depulped coffee beans was immediately frozen at −20 °C (sample A), while the other half was conveyed in a water stream to a Penagos prototype demucilaginator producing commercial mucilage (sample B); this was also frozen. Both samples were airfreighted at once and delivered to our laboratory. Sample B was then freeze-dried.

Preparation of mucilage A

Fresh inner mesocarp (mucilage) was obtained from sample A by carefully scraping with a scalpel blade so that the hard parchment fragments (endocarp) would not be removed from the surface of the frozen coffee beans (4 batches of 100 beans).

Preparation of alcohol-insoluble residues

Hand-dissected (A) and commercial (B) mucilages were then treated with boiling ethanol to remove low-molecular-weight components (Selvendran 1975). Mucilage (containing 1 part wa-

ter) was combined with boiling ethanol (4 parts), and the mixture was thoroughly homogenized in a blender and boiled for another 15 min. After centrifugation (18500 rpm, 10 min), the pellet was extensively washed with ethanol, acetone, and ether, 1 after the other, and allowed to dry overnight at ambient temperature. Alcohol-insoluble residues (AIRs) were further dehydrated in a vacuum oven (55 °C, 9 kPa, 16 h) and then weighed. All AIR samples were checked under a light microscope for the presence of lignified elements after staining with phloroglucinol-HCl (Marcelin and others 1993).

Extraction of crude pectins

AIRs (samples A or B, 1 part) were treated with 50 parts of water acidified with 1 M nitric acid (pH 1.5) for 45 min at 90 °C under constant stirring (Rouse and Crandall 1976). After centrifugation, the opalescent supernatant was set aside. The pellet was resuspended in 25 parts of acidified water (pH 1.5) and centrifuged at 18500 rpm for 10 min. The washing liquor was added to the above supernatant. The pH was adjusted to 4.0 with 1 M NaOH, and the solubilized polysaccharides were precipitated with acetone (1.5:1, v/v). The gelatinous mass was recovered by filtration over a Buchner funnel, washed extensively with 60% acetone, and freeze-dried after resuspending in distilled water. Crude pectins were further dehydrated in a vacuum oven (55 °C, 9 kPa, 16 h) and then weighed.

Analytical procedures

All composition data are given on a moisture-free basis. All reagents were of analytical grade. Uronic acids were measured as galacturonic acid without preliminary deesterification by the *m*-phenylphenol procedure (Blumenkrantz and Asboe-Hansen 1973; Ahmed and Labavitch 1977). Estimation of methanol was carried out using the alcohol oxidase method of Klavons and Bennett (1986). Acetyl groups were determined by an enzymatic UV method (Boehringer 1989). Neutral monosaccharides were released from AIRs and the crude pectins (5 mg) by hydrolysis with 2 M trifluoroacetic acid (TFA) for 2 h at 120 °C (Albersheim and others 1967) or with 1 M sulfuric acid for 2 h at 100 °C. AIRs were also submitted to Saeman hydrolysis as described by Hoebler and others (1989) using 72% (w/w) sulfuric acid for 1 h at 25 °C, then 1 M sulfuric acid for 2 h at 100 °C. Sugars were then derivatized into their alditol acetates (Blakeney and others 1983) and analyzed by GC according to Hoebler and others (1989) with inositol as the internal standard. Proteins (N x 6.25) were determined by a micro-Kjeldahl procedure (Bietz 1974).

Intrinsic viscosities [η] of crude pectins were determined in 0.155 M NaCl at 25 °C using an Ubellohde capillary viscosimeter, and viscosity-average molecular weights [*M*v] were calculated according to Owens and others (1946): $\eta = 1.4 \times 10^{-4} \times M_v^{1.34}$.

The molecular size distribution of pectins was examined by high-performance size-exclusion chromatography (HPSEC) with an OHpak SB-804 HQ Shodex column (8 × 300 mm), eluted with 0.1 M $LiNO_3$ at 0.45 mL/min from a SpectraSystem P1000XR pump (Thermo Separation Products, San Jose, Calif.), with online refractive index detection (Shodex RI-71 detector thermostated at 25 °C). Calibration was performed with narrow pullulan molecular-weight standards (P-5, M_w = 5 800; P-10, M_w = 12 200; P-20, M_w = 23 700; P-50, M_w = 48 000; P-100, M_w = 100 000; P-200, M_w = 186 000; P-400, M_w = 380 000; P-800, M_w = 853 000; Showa Denko, Shoko. Co. Ltd., Tokyo, Japan). Weight-average molecular weights [*M*w] were obtained by the following universal calibration equation: $\ln [M_w \times \eta] = b - a \times t_r$ (t_r = column retention time at peak maximum).

Gelation was tested according to the recommendations of the IFT Committee for high-methoxyl pectins (IFT 1959): Dry pectin from commercial mucilage (0.1 g) was thoroughly mixed with sucrose (0.6 g), and the mixture was added with distilled water (9.5 mL). The mixture was boiled for 1 min under constant stirring, and then sucrose (14.4 g) was added. After boiling for 5 min, 47 μL tartaric acid (48.8%) was added. The solution was poured into a beaker after complete dissolution. The weight of the solution was readjusted to 24.6 g with distilled water, mixed, and left to stand overnight at ambient temperature.

Results and Discussion

SEVERAL BATCHES OF 100 FROZEN DEPULPED BEANS (42.7 ± 3.2 g, n = 4) were carefully hand-dissected with a scalpel blade, and the mucilage-A was kept in the cold (4 °C) to prevent the action of endogenous enzymes. The average wet mucilage content of beans was 9.4 ± 1.0% (w/w, wet basis). The value is not in agreement with the levels (about 20% to 30%) determined by Menchù and Rolz (1973) by total enzymatic degradation of the mucilage. This discrepancy could probably be explained by different depulping conditions leaving variable proportions of pulp, in addition to mucilage, adhering to the beans, or to different moisture contents of the wet mucilage.

AIRs were prepared from both viscous and highly hydrated hand-dissected mucilage (AIR-A) and from deep-brown freeze-dried commercial mucilage (AIR-B). Light microscope examination showed that mucilage-B contained traces of phloroglucinol/HCl-positive lignified elements, whereas mucilage-A was apparently free of such lignified elements. Both AIRs were obtained as greyish-white fine powders, and their yields were 3.4 ± 0.2%/wet mucilage (n = 4) for AIR-A and 15.8%/freeze-dried mucilage for AIR-B. Our yield in AIR-A was slightly lower than the 5% given by Rolz and others (1971). Both AIRs were then analyzed for their polysaccharide composition. The results are shown in Table 1.

As usually observed in purified plant cell walls (Voragen and others 1983), polysaccharides and their methyl and acetyl ester groups were found to be the major constituents in AIR-A (62.9%) and AIR-B (56.0%). The lower percent observed in AIR-B could be explained by its higher level of proteins with regard to AIR-A. Due to exposure of beans to air during mechanical demucilagination, oxidation of phenolic compounds in mucilage-B, as evidenced by its deep brown color, seems to insolubilize some soluble nonwall proteins.

Uronic acids, which were presumed to be essentially of the galacturonic type (since it is associated with the pectic neutral sugars rhamnose and arabinose), were important constituents of both AIRs (about 30%); their degree of methylation (DM) was high. Their amounts are similar to those found in lemon albedo cell walls (about 20% to 30%) (de Vries and others 1984; Ralet and Thibault 1994). They are a major raw material from the citrus-juice industry for producing commercial pectins. Again, our data are not in agreement with previously reported pectin contents (expressed as anhydrogalacturonic acid) of dry mucilage containing its soluble sugars and organic acids (about 30%) (Sudhakara Rao 1975) or of alcohol-insoluble residues (about 50%) (Rolz and others 1971). Contamination by pulp could again be the reason of such discrepancies since coffee pulp is richer in pectins than mucilage (Garcia and others 1991).

In both cases, arabinose was the major neutral noncellulosic monosaccharide followed by galactose, xylose, rhamnose, glucose, and minor amounts of mannose and fucose. The (uronic acids/arabinose/rhamnose) average molar ratios were almost identical in both AIRs (2.8/1/0.14), which means that these monosaccharides must be linked in similar pectic macromolecular structures. The (galactose/xylose) average molar ratios were also similar (1/0.86). Rare sugars, 2-*O*-methyl-fucose, 2-*O*-methyl-xylose, and apiose, were also detected indicating the presence, as in most plant cell walls (Matoh and others 1996), of rhamnogalacturonan II (RG-II).

Table 1—Composition of alcohol-insoluble residues

	Hand-dissected (mucilage-A)	Commercial (mucilage-B)
Uronic acids [a]	32.2	25.3
Neutral noncellulosic polysaccharides [b]	16.7	17.8
Cellulose [c]	9.1	8.9
Proteins (N x 6.25)	14.2	17.1
Methanol	3.5 (59.8) [d]	2.8 (60.8) [d]
Acetic acid	1.4 (12.7) [d]	1.2 (14.4) [d]
Rhamnose [e]	4.9	4.2
2-*O*-CH_3-fucose [e]	0.3	—
Fucose [e]	0.6	0.4
2-*O*-CH_3-xylose [e]	0.1	0.1
Arabinose [e]	34.2	29.6
Apiose [e]	0.2	—
Xylose [e]	10.2	12.6
Mannose [e]	3.1	3.6
Galactose [e]	12.4	13.8
Glucose (noncellulosic) [e]	3.7	5.2
Glucose (cellulosic) [e]	30.3	30.5

[a] Expressed as anhydrogalacturonic acid.
[b] Neutral polysaccharides obtained by hydrolysis with dilute acid (TFA or sulfuric acid) and GC of the alditol acetates and expressed as sum of anhydrosugars.
[c] Glucose obtained by difference between Saeman and dilute acid hydrolyses.
[d] Values in parentheses are the degrees of methylation (DM) and degrees of acetylation (DA), respectively, calculated as the molar ratio of methanol and acetic acid against anhydrogalacturonic acid x 100.
[e] Mole % of constituent monosaccharides.

Table 2—Yield and composition of crude pectins

	Hand-dissected (mucilage-A)	Commercial (mucilage-B)
Yield[a]	34.5	22.9
Uronic acids[b]	59.6	60.4
Neutral polysaccharides[c]	13.4	11.4
Proteins (N x 6.25)	3.4	4.9
Methanol	6.7 (61.8)[d]	6.9 (62.8)[d]
Acetic acid	1.6 (7.9)[d]	1.2 (6.1)[d]
Rhamnose[e]	9.6	9.6
Fucose[e]	1.3	1.0
Arabinose[e]	52.5	14.7
Xylose[e]	8.9	12.2
Mannose[e]	0.8	1.4
Galactose[e]	19.7	41.7
Glucose[e]	7.8	19.3
Intrinsic viscosity η (mL/g)	135.6	96.8
Mv (Da)	29600	22800
Mw (Da)	12100	nd[f]

[a] Yield expressed as g of dry crude pectin/100 g of dry AIR.
[b] Expressed as anhydro-galacturonic acid.
[c] Neutral sugars determined by GC of the alditol acetates and expressed as anhydrosugars.
[d] Values in parentheses are the degrees of methylation (DM) and acetylation (DA), respectively, calculated as the molar ratio of methanol and acetic acid against anhydrogalacturonic acid x 100.
[e] Mole % of constituent monosaccharides.
[f] not determined.

Several authors (Carbonell and Vilanova 1952; Coleman and others 1955) have described the mucilage of freshly pulped mature coffee cherries as a hydrogel exhibiting no cellular organization. Conversely, cellulose was present in our case, indicating that coffee-bean mucilage has actually a cellular structure with cells surrounded by pectocellulosic cell walls. This was confirmed by light microscope examination (Avallone and others 1999).

Crude pectins were also extracted from both mucilages by nitric acid (Rouse and Crandall 1976). Yields and compositions are presented in Table 2. Their dry-matter extraction yields were roughly proportional to the amounts of uronic acids present in starting AIRs; 63.9% and 54.7% of uronic acids were extracted from AIR-A and AIR-B, respectively. Their uronic-acid contents were high and comparable to pectic acid extracted by dilute alkali from coffee mucilage (Coleman and others 1955) or to acid-extracted pectins from lemon-peel fibers (Ralet and Thibault 1994). Their degrees of methyl esterification (DM) were high, indicating that pectic substances from coffee-bean mucilage were high-methoxyl pectins (HM). Garcia and others (1991) reported average degrees of methylation at about 23%. It is possible that, in their case, partial demethoxylation occurred due to the strongly acidic precipitating medium (75% ethanol containing 0.6 M HCl) used for recovery of pectins. Degrees of acetylation (DA) were intermediate between citrus-peel (about 1% to 2%) (Ralet and Thibault 1994) and sugar-beet (about 23%) (Guillon and Thibault 1988) pectins and comparable to apricot pectins (about 5%) (Souty and others 1981). It must be mentioned that these DAs are lower than that of AIRs, probably because some acetyl groups are carried in AIRs by other nonpectic polysaccharides such as xylans (Marcelin and others 1993). Search for feruloyl ester groups according to Guillon and Thibault (1988) was negative. Both pectins contained similar amounts of associated neutral sugars with different mole percentages: Arabinose was predominant in pectin from AIR-A, while galactose was the major constituent in pectin from AIR-B. The (arabinose/rhamnose) ratio of pectin from AIR-A was similar to that observed by Coleman and others (1955) in pectic acid from coffee mucilage.

Intrinsic viscosities [η], viscosity-average molecular weights [*M*v], and weight-average molecular weights [*M*w] of pectins were low and of the same magnitude as those given by Castelein and Pilnik (1976) and Garcia and others (1991) for coffee-mucilage pectins.

In agreement with Garcia and others (1991), mucilage crude pectin did not gel when dissolved in the presence of sucrose (65% soluble solids) at acidic pH. Addition of Ca^{2+} (150 mg/g pectin) did not promote gelation either. This could be ascribed to their noticeable content of acetyl groups and to their very low molecular weight.

Conclusions

THE MUCILAGE LAYER FROM MATURE COFFEE CHERRIES EXHIBITS, contrary to previous assertions on a cell-free hydrogel nature, a conventional tissue organization with thin cell walls built of cellulose, pectic substances, and noncellulosic neutral polysaccharides. Mechanical demucilagination results in a by-product similar to mucilage obtained under laboratory conditions and relatively rich in polyuronides. Acid-extracted pectins were characterized, in disagreement to previously reported data, as high-methoxyl (HM) pectins. They do not form a gel in the presence of sucrose and at acidic pH. More work is needed to characterize the gelation properties of these pectins after partial demethoxylation (LM pectins). The value of the total mucilage (including cellulose) as a thickening agent or in other application remains to be examined.

References

Ahmed AER, Labavitch JM. 1977. A simplified method for accurate determination of cell wall uronide content. J Food Biochem 1:361-365.

Albersheim P, Nevins DJ, English PD, Karr A. 1967. A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. Carbohydr Res 5(3):340-345.

Avallone S, Guyot B, Michaux-Ferrière N, Guiraud JP, Olguin Palacios E, Brillouet JM. 1999. Cell wall polysaccharides of coffee bean mucilage. Histological characterization during fermentation. In: Association Scientifique Internationale du Café (ASIC), editor. 18th International Scientific Colloquium on Coffee; 1999 August 2-8; Helsinki (Finland). Paris (France): Association Scientifique Internationale du Café (ASIC), publisher. P 463-470.

Bietz JA. 1974. Micro-Kjeldahl analysis by an improved automated ammonia determination following manual digestion. Anal Chem 46:1617-1618.

Blakeney AB, Harris PJ, Henry RJ, Stone BA. 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohydr Res 113(2):291-299.

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

Boehringer. 1989. Acetic acid-UV method. Technical leaflet, 589.3.248.878. Germany: Boehringer Mannheim GmbH.

Calle VH. 1958. Tentativas para la preparacion de productos alimenticios del cafeto, distintos a la bebida. Cenicafe (Colombia) Boletin Informativo 9(10):222-227.

Carbonell RJ, Vilanova MT. 1952. Beneficiado ràpido y eficiente del café mediante el uso de sauda caùstica. Centro Nacional de Agronomia, Ministerio de Agricultura y Ganaderia: El Salvador. Boletin Técnico 13:65-66.

Castelein JM, Pilnik W. 1976. The properties of the pectate-lyase produced by *Erwinia dissolvens*, a coffee-fermenting organism. Lebensm-Wiss u-Technol 9(5):277-283.

Coleman RJ, Lenney JF, Coscia AT, DiCarlo FJ. 1955. Pectic acid from the mucilage of coffee cherries. Arch Biochem Biophys 59:157-164.
Garcia R, Arriola D, de Arriola MC, de Porres E, Rolz C. 1991. Characterization of coffee pectin. Lebensm-Wiss u-Technol 24(2):125-129.
Guillon F, Thibault JF. 1988. Further characterization of acid- and alkali-soluble pectins from sugar beet pulp. Lebensm-Wiss u-Technol 21(4):198-205.
Hoebler C, Barry JL, David A, Delort-Laval J. 1989. Rapid acid hydrolysis of plant cell wall polysaccharides and simplified quantitative determination of their neutral monosaccharides by gas-liquid chromatography. J Agric Food Chem 37(2):360-367.
IFT (Institute of Food Technologists). 1959. Pectin standardization. Final Report of the HM Committee. Food Technol 496-500.
Klavons JA, Bennett RD. 1986. Determination of methanol using alcohol oxidase and its application to methyl ester content of pectins. J Agric Food Chem 34(4):597-599.
Marcelin O, Williams P, Brillouet JM. 1993. Isolation and characterization of the main cell-wall types from guava (*Psidium guajava* L.) pulp. Carbohydr Res 240:233-243.
Matoh T, Kawaguchi S, Kobayashi M. 1996. Ubiquity of a borate-rhamnogalacturonan II complex in the cell walls of higher plants. Plant Cell Physiol 37(5):636-640.
Menchù JF, Rolz C. 1973. Coffee fermentation technology. Café Cacao Thé 17(1):53-61.
Owens HS, Lotzkar H, Schultz TH, Maclay WD. 1946. Shape and size of pectinic acid molecules from viscosimetric measurements. J Am Chem Soc 68:1628-1632.
Purseglove JW. 1974. Rubiaceae. In: Purseglove JW, author. Tropical crops dicotyledons. London: The English Language Book Society and Longman Group. Ltd. p 451-492.
Ralet MC, Thibault JF. 1994. Extraction and characterization of very highly methylated pectins from lemon cell walls. Carbohydr Res 260(2):283-296.
Rolz C, Menchù JF, Espinosa R, Garcia-Prendes A. 1971. Coffee fermentation studies. In: Association Scientifique Internationale du Café (ASIC), editor. 5th International Colloqium on the Chemistry of Coffee; 1971 June 14-19; Lisbon (Portugal). Paris (France): Association Scientifique Internationale du Café (ASIC), publisher. P 259-268.
Rouse AH, Crandall PG. 1976. Nitric acid extraction from citrus peel. Proc Fla State Hort Soc 89:166-168.
Selvendran RR. 1975. Analysis of cell wall material from plant tissues: extraction and purification. Phytochemistry 14(4):1011-1017.
Souty M, Thibault JF, Navarro-Garcia G, Lopez-Roca JM, Breuils L. 1981. The pectic substances from apricot (*Prunus armeniaca* L.) c.v. Rouge du Roussillon. General characteristics and ion exchange chromatography study. Sci Alim 1(1):67-80.
Sudhakara Rao G. 1975. Pectins as potential by-products of coffee waste. J Coffee Res 5(1):29-35.
Voragen FGJ, Timmers JPJ, Linssen JPH, Schols HA, Pilnik W. 1983. Methods of analysis for cell-wall polysaccharides of fruit and vegetables. Z Lebensm Unter Forsch 177:251-256.
de Vries JA, Rombouts FM, Voragen AGJ, Pilnik W. 1984. Comparison of the structural features of apple and citrus pectic substances. Carbohydr Polym 4:89-101.
Wilbaux R. 1956. Les caféiers au Congo Belge. Technologie du café Arabica et Robusta. Bruxelles, Belgium, Direction de l'Agriculture, des Forêts et de l'Elevage. p 12.

The authors acknowledge the excellent technical assistance of Claudie Dhuique-Mayer (CIRAD-FLHOR, Montpellier, France).

Authors Avallone and Guyot are with the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Département CP, BP 5035, 34032 Montpellier Cedex 1, France. Authors Avallone and Brillouet are with the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Département FLHOR, BP 5035, 34032 Montpellier Cedex 1, France. Author Guiraud is with the Université des Sciences et Techniques du Languedoc, Laboratoire de Génie Biologique et de Sciences des Aliments (USTL-GBSA), Place Eugène Bataillon, 34095 Montpellier Cedex 5, France. Author Olguin is with the Instituto de Ecologia, km. 2.5 Ant. Carr. a Coatepec, C.P. 91000, Apdo. Postal 63, Xalapa, Veracruz, Mexico. Direct correspondence to J.-M. Brillouet (E-mail: brillouet@cirad.fr)