Fate of Mucilage Cell Wall Polysaccharides during Coffee Fermentation

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Effects of a 20-h fermentation on cell wall polysaccharides from the mucilage of pulped coffee beans were examined and compared to those of unfermented beans, on alcohol insoluble residues (AIRs), their hot-water-soluble crude pectic substances (PECTs), and their hot-water-insoluble residues (RESs). Yields and compositions were very similar: AIRs, which consisted of ∼30% highly methylated pectic substances, ∼9% cellulose, and ∼15% neutral noncellulosic polysaccharides, exhibited no apparent degradation. However, PECTs from fermented beans were shown to have undergone a slight reduction of their intrinsic viscosity and weight-average molecular weight by capillary viscosimetry and high-performance size-exclusion chromatography. After fermentation, hot-water-insoluble pectic substances of RES exhibited partial de-esterification. Removal of coffee bean mucilage by natural fermentation seems to result from a restricted pectolysis, the mechanism of which remains to be elucidated.

Keywords: *Coffee; Coffea arabica L. var. typica Cramer; mucilage; fermentation; cell wall polysaccharides*

INTRODUCTION

Coffee cherry is a drupe consisting of an exocarp (skin), an outer mesocarp (pulp), an inner mesocarp (mucilage), and a fibrous endocarp (parchment) surrounding a seed (Figure 1)(*1*, *2*). In coffee wet processing, pulping of the coffee cherries removes their skin and pulp, leaving a viscous mucilage adhering to the parchment. This highly hydrated tissue, rich in pectic substances (*2*−*7*), is an obstacle to further drying of the beans. Thus, mucilage is usually degraded by natural fermentation in order to facilitate its elimination by washing; then the beans are dried and stored (*8*).

During the fermentation step, mucilage texture changes from viscous and slippery to watery and fluid. A common assertion to explain this observation is that mucilage pectic substances are extensively degraded by the natural microflora and/or endogenous coffee enzymes (*8*, *9*); however, that has never been proven. Conversely, a study on the coffee fermentation microflora (*10*) showed that the number of pectolytic microorganisms did not increase during this step. Furthermore, the isolated pectolytic bacterial strains produced only pectate lyase activities that are unable to degrade highly methylesterified coffee pectic substances (*11*, *12*). Finally, a histological examination of mucilage cells showed that, after fermentation, their cell walls still contained pectic substances (*2*).

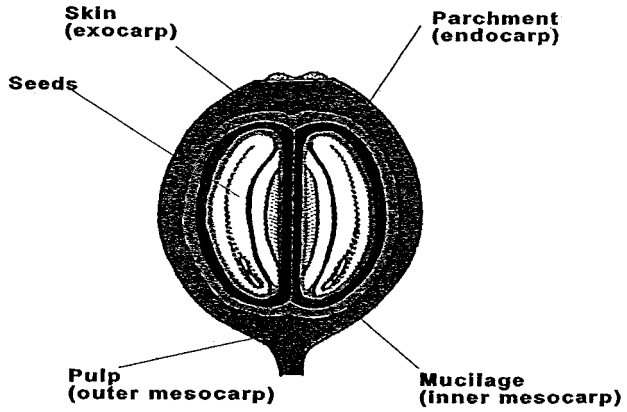

Figure 1. Longitudinal section of a coffee cherry.

Incomplete understanding of the mechanisms involved in the degradation of mucilage sometimes leads to underfermentation or overfermentation of the coffee beans, which is ultimately responsible for beverage defects (*13*−*15*). It is thus necessary, for better control of the fermentation process, to gain a deeper knowledge of the biochemical alterations of mucilage during fermentation. Until now, to our knowledge, no fine characterization of mucilage cell wall polysaccharides after fermentation has been published. The aim of the present work was, thus, to analyze cell walls of unfermented and fermented mucilage to determine whether the textural changes in the tissue are due to polysaccharide degradation.

MATERIALS AND METHODS

Plant Material. The coffee variety used in the present study was *Coffea arabica* L. var. *typica* Cramer. It was grown

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in the Coatepec area (Xalapa, Veracruz, Mexico) during the 1998 season. Coffee cherries were harvested at the mature stage and immediately pulped using a DV-255C PENAGOS pulper.

Coffee Fermentation. Pulped coffee beans (500 kg) were put in a concrete tank and left to ferment. Samples were taken from the tank before (T_0) and after complete fermentation at time 20 h (T_{20}). Mucilage was obtained by carefully scraping the surface of the coffee beans with a scalpel blade.

Preparation of Alcohol Insoluble Residues. Mucilage was treated with boiling 80% ethanol (v/v) to remove low-molecular-weight components (*16*). The mixture was thoroughly homogenized with a blender and boiled for 15 min. After the mixture was centrifuged, the pellet was successively washed with ethanol, acetone, and ether, and left to dry overnight at ambient temperature. Alcohol insoluble residues (AIRs) were then dehydrated in a vacuum oven (55 °C; 9 kPa; 16 h) and weighed. AIRs were starch-free as checked by light microscopy (iodine staining).

Extraction of Crude Pectins. AIRs were treated with 50 parts of slightly acidified distilled water (pH 4.0) for 45 min at 90 °C under constant stirring. After the mixture was centrifuged, the opalescent supernatant was kept aside. The pellet was resuspended in 25 parts of distilled water (pH 4.0) and centrifuged. Both supernatants were mixed, and the hot-water-soluble crude pectic substances (PECTs) were freeze-dried, then weighed. Hot-water-insoluble residues (RESs) left after extraction were also freeze-dried, then weighed.

Analytical Procedures. Analyses were conducted in triplicate, and composition data are given on a moisture-free basis. All reagents were of analytical grade. Uronic acids were measured as galacturonic acid without preliminary de-esterification by the *m*-phenylphenol procedure (*17*) with, in the case of AIRs and RESs, preliminary dissolution in concentrated sulfuric acid (*18*). Estimation of methanol was carried out using the alcohol oxidase method (*19*). Acetyl groups were determined by an enzymatic UV method (*20*). Neutral monosaccharides were released from AIRs, PECTs, and RESs (5 mg) by hydrolysis with 2 M trifluoroacetic acid (TFA) for 2 h at 120 °C (*21*) or with 1 M sulfuric acid for 2 h at 100 °C. Cellulose-containing AIRs and RESs were also submitted to Saeman hydrolysis (*22*), (i.e., 72% (w/w) sulfuric acid, 1 h, 25 °C, then 1 M sulfuric acid, 2 h, 100 °C). Sugars were then derivatized into their alditol acetates (*23*) and analyzed by GC (*22*). Inositol was used as internal standard. Proteins (N × 6.25) were determined by a micro Kjeldahl procedure (*24*).

Intrinsic viscosity [η] of crude pectins was determined in 0.155 M NaCl at 25 °C using an Ubbelohde capillary viscosimeter, and viscosity-average molecular weight [M_v] was calculated (*25*). The molecular size distribution of pectins was also examined by high-performance size-exclusion chromatography (HPSEC) using 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, CA) with on-line refractive index detection (Shodex RI-71 detector thermostated at 25 °C). Calibration was performed with narrow pullulan molecular weight standards (P-5, $M_w = 5800$; P-10, $M_w = 12200$; P-20, $M_w = 23700$; P-50, $M_w = 48000$; P-100, $M_w = 100000$; P-200, $M_w = 186000$; P-400, $M_w = 380000$; P-800, $M_w = 853000$; Showa Denko, Shoko Co. Ltd., Japan). Weight-average molecular weights [M_w] were calculated using the following universal calibration equation: $\ln [M_w \times \eta] = 34.743 - (1.205 \times t_r)$, where t_r = column elution time at peak maximum) (*26*).

RESULTS AND DISCUSSION

The fresh mucilage contents of unfermented and fermented beans were not significantly different: $T_0 = 9.7 \pm 1.9\%$, $T_{20} = 9.2 \pm 1.8\%$ (fresh weight/fresh weight basis; $P > 0.9$). Similarly, percents of AIRs in the fresh mucilages were not significantly different before and after fermentation: AIR(T_0) = 3.5 ± 0.7%, AIR(T_{20}) = 3.1 ± 0.6% (dry weight/fresh weight; $P > 0.9$). These values were lower than the 5% previously reported (*27*).

Table 1. Composition[a,b] of Alcohol-Insoluble Residues Before and After Fermentation

	AIR(T_0)	AIR(T_{20})
uronic acids[c]	33.0 ± 2.2	30.4 ± 1.8
neutral noncellulosic polysaccharides[d]	16.1 ± 0.8	15.2 ± 0.8
cellulose[e]	9.0 ± 0.4	8.8 ± 0.5
methanol	3.6 ± 0.1 (60.0)[f]	3.4 ± 0.1 (61.3)
calcium	1.10 ± 0.08	0.93 ± 0.09
proteins (N × 6.25)	13.2 ± 1.0	15.3 ± 1.4
rhamnose[g]	5.4	4.9
fucose[g]	0.9	0.5
2-*O*-CH_3-xylose[g]	0.1	0.4
arabinose[g]	27.9	26.3
xylose[g]	10.3	13.8
mannose[g]	1.6	2.1
galactose[g]	14.3	11.5
glucose (noncellulosic)[g]	4.3	4.4
glucose (cellulosic)[g]	35.2	36.1

[a] Reported as %, dry matter basis. [b] Significance of differences was defined at $P > 0.95$. [c] Expressed as anhydrogalacturonic acid. [d] Neutral polysaccharides obtained by hydrolysis with dilute acid (TFA or sulfuric acid) and GC of the alditol acetates, and expressed as sum of anhydrosugars. [e] Glucose obtained by difference between Saeman and dilute acid hydrolyses. [f] Values in parentheses are the degrees of methylation calculated as the molar ratio of the average methanol vs anhydrogalacturonic acid contents × 100. [g] Mole % of constituent monosaccharides.

The overall compositions of AIRs isolated from unfermented and fermented beans were similar (Table 1). Cell wall polysaccharides were the major constituents, representing 58.1% and 54.4% of AIR(T_0) and AIR(T_{20}), respectively. Pectic substances, accounting for one-third of both AIRs as anhydrogalacturonic acid, were found to be highly methylated and not significantly different. The calcium contents of both AIRs were similar. The cellulose and neutral noncellulosic polysaccharides contents of both AIRs were also close. The monosaccharide compositions of cell wall polysaccharides were similar, showing a dominant proportion of arabinose, followed by galactose, xylose, rhamnose, glucose, and minor amounts of mannose and fucose.

After having prepared both AIRs, we examined the 80% ethanol supernatants for the presence of oligogalacturonic acids by thin-layer chromatography: they were not detected before and after fermentation. Thus, it seems that fermenting microflora did not degrade the mucilage pectic substances into oligomeric products, or if so, these degradation products would have been metabolized by the fermenting microflora.

So, contrary to the general agreement about a degradation of mucilage pectic substances by fermenting microorganisms (*14*, *28*−*30*), we did not observe on alcohol insoluble residues significant alteration of these polymers after fermentation. Cellulose and neutral noncellulosic polysaccharides also were not degraded.

With the aim to detect more discrete degradations, we submitted AIRs to extraction by slightly acidified boiling water. Hot-water-soluble crude pectins were obtained in non-significantly different yields: PECT-(T_0) = 44.1 ± 2.5%, PECT(T_{20}) = 42.7 ± 3.1% (dry matter basis; $P > 0.95$). It must be mentioned that, in the case of the native mucilage AIR(T_0), the high solubility of pectins in hot water reflects the loosened character of the coffee mucilage cell walls network as observed by light microscopy (*2*); indeed, water solubility of pectic substances from native plant materials is usually far lower (e.g. 4% for lemon albedo cell walls; (*31*)). Both crude pectic substances had high and not significantly different contents of uronic acids (Table 2)

Table 2. Composition[a,b] of Crude Pectins Before and After Fermentation

	PECT(T_0)	PECT(T_{20})
uronic acids[c]	53.3 ± 2.6	51.7 ± 2.4
neutral noncellulosic polysaccharides[d]	19.3 ± 0.9	18.1 ± 0.7
methanol	6.4 ± 0.3 (66.0)[e]	6.0 ± 0.2 (63.8)
acetic acid	0.72 ± 0.01 (3.9)[e]	1.54 ± 0.03 (8.6)
rhamnose[f]	11.5	10.9
fucose[f]	1.8	0.4
2-*O*-CH_3-xylose[f]	0.2	0.1
arabinose[f]	61.1	55.9
xylose[f]	3.4	7.3
mannose[f]	0.1	2.7
galactose[f]	21.5	21.6
glucose[f]	0.4	1.1
η (mL/g)	155	103
M_v (Da)	32400	23900
M_w (Da)	23000	17200

[a] Reported as %, dry matter basis. [b] Significance of differences was defined at $P > 0.95$. [c] Expressed as anhydrogalacturonic acid. [d] Neutral polysaccharides obtained by hydrolysis with dilute acid (TFA or sulfuric acid) and GC of the alditol acetates, and expressed as sum of anhydrosugars. [e] Values in parentheses are the degrees of methylation and acetylation calculated as the molar ratio of the average methanol and acetic acid vs anhydrogalacturonic acid contents × 100. [f] Mole % of constituent monosaccharides.

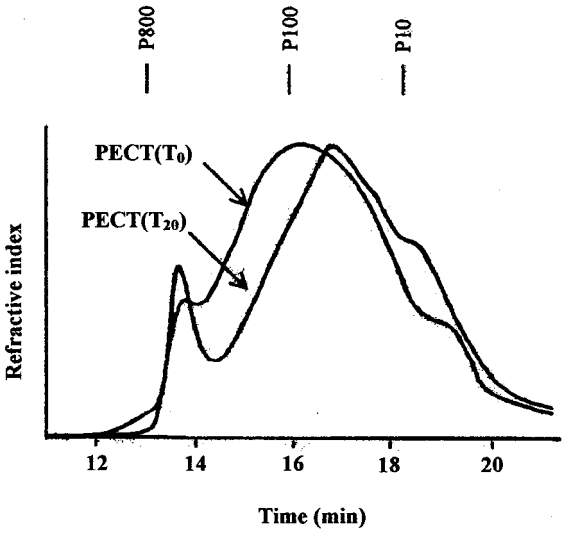

Figure 2. High-performance size-exclusion chromatography profiles of crude pectins before [PECT(T_0)] and after [PECT(T_{20})] fermentation. Elution times of narrow pullulan molecular-weight standards, P-10 (M_w = 12200), P-100 (M_w = 100000), and P-800 (M_w = 853000), are also shown.

as observed on pectic acid extracted from coffee mucilage by dilute alkali (*3*). They also exhibited high and close degrees of methyl esterification (DM); thus, contrary to previously reported low DMs (*6*), coffee mucilage pectic substances are high-methoxyl pectins (HM). Furthermore, fermentation did not lead to de-esterification. Both crude pectins had similar contents of neutral noncellulosic polysaccharides with dominant proportions of arabinose, galactose, and rhamnose. A noticeable difference was observed between the two pectic substances: PECT(T_{20}) had a degree of acetylation ~2× that of PECT(T_0).

The intrinsic viscosity [η] and viscosity-average molecular weight [M_v] of unfermented mucilage crude pectins [PECT(T_0)] were low, which is in agreement with previously reported data (*6*, *11*). After fermentation, PECT(T_{20}) exhibited lower [η] and [M_v] than PECT(T_0) which could be an indication of a limited degradation.

This was confirmed by HPSEC (Figure 2): the elution profile of PECT(T_{20}) was slightly shifted toward lower molecular weights as compared to that of PECT(T_0). The weight-average molecular weight [M_w] also decreased slightly after fermentation. Thus, contrary to what was observed on AIRs, it seems that the hot-water-soluble pectins would have undergone a rather limited degradation. These data are in agreement with comparative light microscopy observations of unfermented and fermented mucilages showing that cell walls were still stained by ruthenium red after fermentation as they were before fermentation (*2*). Similarly, a major coffee fermenting pectolytic microorganism, *Erwinia dissolvens*, produces only an exo-pectate-lyase having a very restricted action, due to methyl esterification, on high-methoxyl pectins (*11*). Finally, coffee-fermenting pectolytic microflora was shown to be stable during fermentation producing only pectate−lyase activities (*10*, *12*). As we showed that highly methylated mucilage pectins were not de-esterified during fermentation, it is not surprising that the observed degradation was very limited.

Table 3. Composition[a,b] of Residues Before and After Fermentation

	RES(T_0)	RES(T_{20})
uronic acids[c]	10.9 ± 0.6	9.9 ± 0.5
neutral noncellulosic polysaccharides[d]	15.9 ± 0.8	14.5 ± 0.7
cellulose[e]	17.6 ± 0.9	19.7 ± 1.2
methanol	0.64 ± 0.02 (30.2)[f]	0.30 ± 0.01 (16.7)
calcium	1.30 ± 0.10	1.12 ± 0.09
proteins (N × 6.25)	22.1 ± 1.9	25.1 ± 2.3
rhamnose[g]	1.8	1.4
fucose[g]	0.3	0.4
arabinose[g]	13.1	12.1
xylose[g]	15.5	13.2
mannose[g]	2.0	1.5
galactose[g]	6.3	5.6
glucose (noncellulosic)[g]	5.9	6.5
glucose (cellulosic)[g]	55.1	59.3

[a] Reported as %, dry matter basis. [b] Significance of differences was defined at $P > 0.95$. [c] Expressed as anhydrogalacturonic acid. [d] Neutral polysaccharides obtained by hydrolysis with dilute acid (TFA or sulfuric acid) and GC of the alditol acetates, and expressed as sum of anhydrosugars. [e] Glucose obtained by difference between Saeman and dilute acid hydrolyses. [f] Values in parentheses are the degrees of methylation calculated as the molar ratio of the average methanol vs anhydrogalacturonic acid contents × 100. [g] Mole % of constituent monosaccharides.

With the aim of going further into the comprehension of events occurring during fermentation, we analyzed the cellulose-enriched residues (RESs) left after hot-water extraction of crude pectins (Table 3). Yields of residues were not significantly different: RES(T_0) = 41.8 ± 2.9%, RES(T_{20}) = 40.3 ± 2.2% (dry matter basis; $P > 0.95$). The amounts of uronic acid-containing polysaccharides, cellulose, neutral noncellulosic polysaccharides, and calcium in both residues were not significantly different. The monosaccharide relative distributions were also similar. The only significant difference was in the methanol contents (thus the degrees of methyl esterification) of hot-water-insoluble pectins: this fraction which represents only ~13% of total AIR pectins (expressed as anhydrogalacturonic acid) seemed to have undergone a de-esterification during fermentation. This discrete alteration could be due to the action of coffee mucilage pectinmethylesterase (*32*), as the fermenting pectolytic microflora was shown not to produce this activity (*12*).

CONCLUSION

Having examined in detail the cell wall polysaccharides of coffee mucilage before and after fermentation,

we conclude that they exhibit very little differences in their amounts and compositions. Cellulose was not hydrolyzed. Hot-water-soluble pectins seemed to have been slightly depolymerized during fermentation which would hardly be attributable, as already mentioned, to a bacterial exo-pectate-lyase (*11*). The mechanism of this discrete depolymerization remains to be clarified. De-esterification of hot-water-insoluble pectins after fermentation also remains unexplained.

Thus, if microorganisms participate to the liquefaction of coffee mucilage, their main intervening must not be at the level of their enzymatic activities. Microscopic examinations showed that, after fermentation, clusters of mucilage cells having apparently intact walls are separated from the parchment by a breaking of walls of the first layer of mucilage cells at their basal sites of attachment to the underlying parchment sclerenchymatous cells (*2*). A possible concerted action between a limited pectolysis and physicochemical changes occurring in the mucilage during fermentation, i.e., consumption of sugars and production of organic acids by the fermenting microflora (*10*), remains to be elucidated.

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