Some physical aspects of Brazilian green coffee beans and the quality of the beverage*1/

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RESUMO

Amostras de café verde classificadas como Mole e Rio por provadores profissionais, foram estudadas quanto a alguns aspectos físicos, utilizando microscópio eletrônico de varredura e microscópio comum. Os resultados revelaram que os cafés Rio possuem paredes celulares mais finas, menor densidade, e essas paredes celulares ocupam um menor volume em relação aos cafés Mole. Os resultados parecem evidenciar que nos cafés Rio houve degradação das paredes celulares na colheita ou na armazenagem. Os compostos de degradação das paredes celulares podem afetar sensivelmente a qualidade da bebida do café, embora não se possa afirmar pelo presente trabalho, que tais compostos sejam os únicos responsáveis pelo gosto e aroma Rio. — Os autores.

Introduction

It is well known that defective beans (black beans, yellow, etc.) can depreciate the quality of the coffee beverage (6, 7, 13). However, few papers have been published which relate physical aspects, other than color and bean shape, with the quality of the beverage (4, 11).

Menchu and Ortega (11) did observe a significant positive correlation between quality of the beverage and apparent density of green coffee, and apparent density of roasted Guatemalan coffees.

Burgin (4) studied cellular structures and localized caffeine and chlorogenic acid in different varieties of coffee, using light microscopy, although he did not compare different qualities of the beverage from the same variety.

The aim of this work is to compare cellular characteristics of the outer endosperm (hard) of commercial green coffee beans of the same variety but which differ in the quality of the beverage. Electron scanning and light microscopy techniques were applied.

Materials and methods

Coffee bean samples were classified by the Brazilian Institute of Coffee and Cia. Cacique do Café Soluvel S. A., Londrina, Parana, Brazil, with respect to the quality of the beverage, as Soft (S) (mild flavor) and Rio (R) (phenolic like flavor). S-1, S-2 and R-1 samples were 3 years old and R-2, 7 years old. All coffee samples were of the same variety, *Coffea arabica* L., cultivar 'Mundo Novo'.

Electron scanning microscopy

Coffee beans from Soft-2 and Rio-2 samples were fractured with a scalpel and the hard endocarp (5) fractions were fixed with 2.5 per cent osmium tetroxide at pH 6.5 for 7 hours, washed with buffer and then dehydrated with an ethanol/water series of 30, 45, 70, 90, 100, and 100 per cent (1 hour each), then twice in amyl acetate for 20 min. and critical point dried with CO_2 in a SAMDRI, PVT-3, critical point drying apparatus.

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Samples were rotary shadowed with gold-palladium in a high vacuum evaporator and the micrographs were taken with a Mini-Sem scanning electron microscope using Type PN55 Polaroid film.

Some treatments prior to osmium tetroxide fixation were carried out to remove lipids (diethyl ether), proteins (trypsin, 1N NaCl, 1N NaOH) and polysaccharides (1N NaOH). However, all treatments, with the exception of 1N NaCl apparently removed all the structures of the fractured surfaces. A second fracture in a perpendicular direction from the treated surface revealed that the ether and NaOH treatments affected cellular components but no conclusions could be made, probably because of the thickness of cell wall which impedes the complete removal of the solubilized components.

Light microscopy

The outer hard endosperm samples (5) were fixed in a solution of glutaraldehyde (4 per cent final conc.), 1.5 per cent sucrose, 0.01 per cent $CaCl_2$, and 500 ppm ruthenium red buffered with 0.05 M cocodylate buffer (pH 7.4) for 7 hours.

Residual glutaraldehyde was washed from samples with cocodylate buffer in sucrose and $CaCl_2$, the fractured beans were fixed with osmium tetroxide (2.5 per cent final conc.), sucrose, $CaCl_2$ and ruthenium red (same conc. as for glutaraldehyde fixation) for 9 hours.

Unreduced osmium tetroxide was washed with cocodylate buffer with sucrose, $CaCl_2$, and ruthenium red.

Using a series of aqueous ethanol solutions (30, 40, 50, 60, 70,80, 90, 95, 100 and 100 per cent) for 30 min. each, fixed samples were dehydrated for embedment in the Epon 812 medium according to Luft (9). Modified impregnation times of 20 min. in 100 per cent propylene oxide, 6 hours in each step of a propylene oxide/Epon series (3:1, 1:1, 1:3, v/v), and in 100 per cent Epon (12 hr. at 37°C, 12 hr. at 45°C, and 24 hr. at 60°C.

Thick sections (0.7 μm) were made with glass knives using an LKB Ultratome III and stained with the Huber (6) procedure. Using a Zeiss calibration standard and Kodak panchromatic negative film, photographic records were made of each of the 4 samples.

Physical analysis

Cell wall thicknesses were recorded with the aid of a Zeiss Particle Analyzer (Model TG23), measuring only those wall areas forming linear boundaries between adjacent sides (examples shown by arrows in Fig. 1). Relative volumes of cell wall and cytosol components were calculated on the basis of weighing cut out cell wall and cytosol regions of light photomicrographs.

Bean density was calculated by dividing the weight of 100 beans by their aggregate volume. Five replications of each coffee sample were made using a volumetric cylinder (0.2 ml accuracy) filled with 100 coffee beans of the respective types. Fine sand was added to each cylinder, which filled spaces between the beans, a process promoted by vibrating the cylinder. The cumulative volume of the beans was simply determined as that fraction of the total bean-sand volume not represented by the sand. The percentage of water (10.7-11.2 per cent) was not taken into consideration in these density measurements.

Results and discussion

Table 1 shows data obtained from the light photomicrographs and other physical measurements. There is a positive correlation between increasing density of the bean, increasing cell wall thickness, and increasing percentage volume of cell wall. Soft coffees showed a higher density and a higher cell wall volume than the Rio coffees. The weight of 500 beans did not show a good relationship with the quality of the beverage, probably because of differences in the size of the beans.

Table 1.—Physical measurements of the whole bean and of the hard (outer) endosperm of *Arabica* coffee beans which differ in quality of the beverage. (The simbols in parenthesis represent number of counts).

Coffee sample	Wt. 500 beans g	Density	Cell wall thickness (mean) μm	Volume of cell walls %
Soft — 1	71.03	1.085	6.2 (972)	50.7
Soft — 2	57.69	1.014	5.6 (822)	49.7
Rio — 1	58.21	0.967	5.0 (731)	37.5
Rio — 2	49.24	0.781	4.3 (475)	35.6

Figure 1 shows the type of light micrographs from which the wall thickness data of Table 1 was obtained. Figure 2 shows the distribution pattern of cell wall thickness which gives more valuable information than a simple average. The distributions did not follow the Gausian curve, but the peaks of maxima are clearly different and corroborate with the data of Table 1.

Figures 3 and 4 show two scanning electron micrographs of a fracture of Soft-2 and Rio-2 coffee samples. The thinner cell walls of Rio-2 coffee are evident.

These results show that the samples which have the Rio flavor passed through a process which induced the degradation of the cell walls (possibly other cell components too), during harvesting, processing and/or storage. An interesting point to note is that between the two Rio coffees the 7-year old (R-2) showed the lower density, cell wall thickness and per cent volume cell wall. It is well known that coffee loses dry weight upon storage (12) and the volatiles liberated might come also from cell wall components. On the other hand, compounds liberated from cell walls might depreciate the quality of the beverage. In this respect, Amorim *et al* (2, 3) and Melo and Amorim (10) observed that proteins and phenolic components might

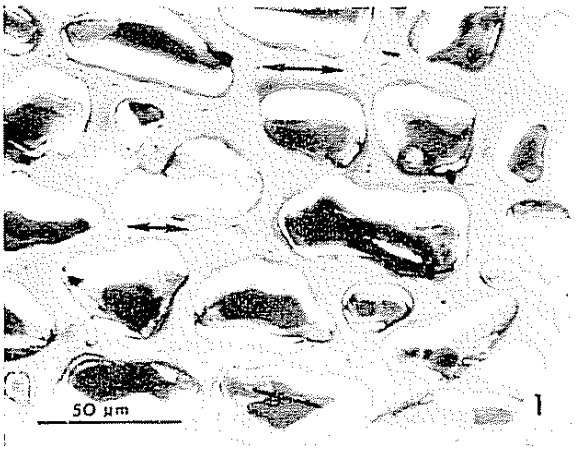

Fig. 1 —*Light micrograph of a thick section (0.7 µm) of an Epon embedded* Coffea arabica *L. Soft-1 sample, illustrating the areas from which wall thickness measurements were taken. Arrows indicate breadth of regions which are relatively common between two cells.*

be involved in the reactions which lead to the Rio flavor, because fewer NaOH soluble proteins (3) and hydrolizable phenols (2) were found in these coffees. In addition, if the higher absorption at 400 nm of TCA solubles (10) found in Rio coffees really represents a higher content of oxidized phenolics, (that could be lignin degradation products), this also corroborates and

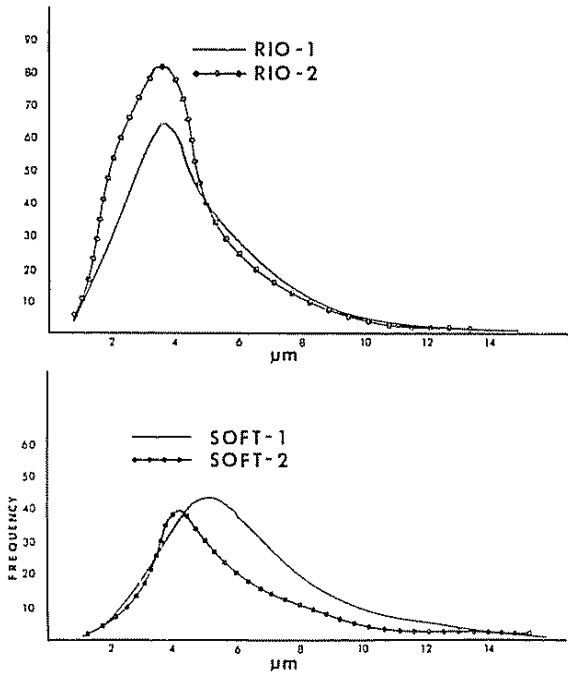

Fig. 2 —*Distributions of frequencies of cell wall thickness of four samples of green coffee* (Arabica) *beans.*

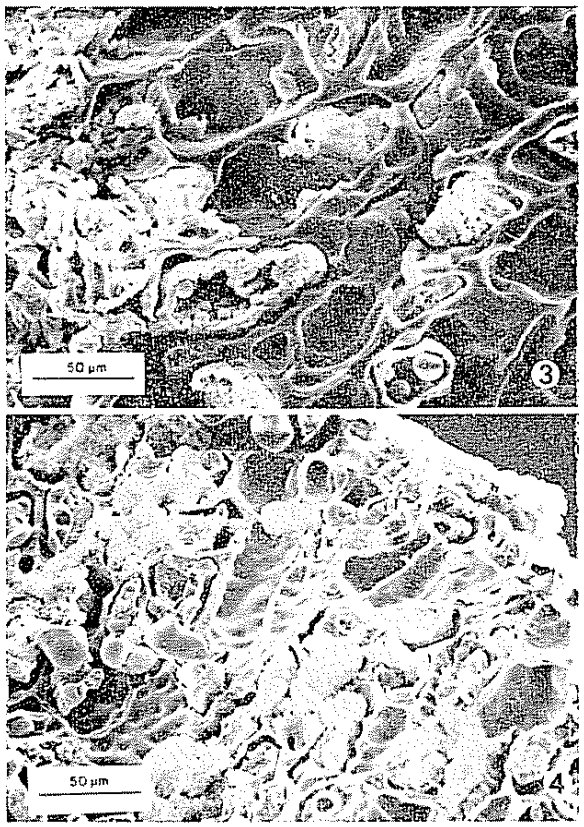

Fig. 3 *and 4. Scanning electron micrographs of Soft-2 and Rio-2 samples showing secondary fractures with many of the cytosol structures.*

links together the chemical analysis performed earlier (2, 3, 10) and the structural differences observed in this work.

The fact that no significant differences were found in free sugars and water soluble polysaccharides between Soft and Rio coffees (1) do not invalidate our present assumption, because there is a strong possibility that the sugars liberated from cell walls are metabolized giving rise to the production of CO_2 which may account for the loss in weight on storage and also in Rio coffees. The mechanism of such reactions and the production of volatiles in green coffee are at present under investigation in our laboratory.

Conclusions

By the results obtained in this work it is evident that the green coffee beans which give the Rio flavor after roast and infusion have thinner cell walls which account for a lower ratio on the volume of cell wall/cytosol and also showed low densities. These differences may be attributed to cell wall degradation on harvesting/processing and/or storage conditions. However, components of cell wall degradation cannot, at present, be

indicated as the sole source of Rio flavor, because other components of the cell might undergo degradative reactions too

Summary

Green coffee samples classified as Soft (mild taste) and Rio (phenolic or medicinal taste) with respect to the quality of the beverage were subjected to physical analysis. Electron scanning microscopy and light microscopy of sections were used to compare both types of coffee. The results showed that Rio coffee had lower densities, thinner cell walls and lower cell wall/cytosol volume ratio. These differences were attributed to cell wall degradation on bad conditions upon harvesting/processing and/or storage. These studies indicate the probable role of cell wall degradation products on coffee flavor, although at present it is not possible to conclude that cell wall components are the sole factors responsible for the Rio flavor.

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