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Characterization of 'Sabará' Jabuticabas at different maturation stages

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ABSTRACT. This work aimed to evaluate the physicochemical characteristics and enzymatic activity of "Sabará" jabuticaba fruits, which were cultivated in Lavras, Minas Gerais State, Brazil, at different maturation stages. The fruits were harvested and separated into five different stages: totally green fruit, fruit with incipient pigmentation, fruit with a light purple predominance, totally purple fruit and totally dark purple fruit. These fruits were physicochemically analyzed and evaluated for polygalacturonase enzymatic activity. Chlorophyll degradation and the synthesis of anthocyanin on fruit rinds were observed, as well as the degradation of the cell wall and its consequent softening. In the pulp, increased soluble solids, pH and sugars were observed, while acidity decreased as maturation progressed.

Keywords: Myrciaria jaboticaba, pectin, polygalacturonase, chlorophyll, anthocyanin.

Caracterização de Jabuticaba 'Sabará' em diferentes estádios de maturação

RESUMO. O presente trabalho teve como objetivo avaliar as características físico-químicas e de atividade enzimática de jabuticabas 'Sabará' cultivadas em Lavras, Estado de Minas Gerias, Brasil, em diferentes estádios de maturação. Os frutos foram colhidos e separados em cinco diferentes estádios: frutos totalmente verdes; frutos com início de pigmentação; frutos com predominância roxo claro; frutos totalmente roxos e frutos totalmente roxo-escuro. Em seguida, os frutos foram submetidos às análises físico-químicas e a atividade da enzima poligalacturonase. Foi possível observar a degradação da clorofila e a síntese de antocianinas na casca dos frutos, bem como a degradação da parede celular e o conseqüente amaciamento dos mesmos. Na polpa, observou-se um aumento de sólidos solúveis, pH e açúcares, bem como uma diminuição da acidez à medida que o grau de maturação foi aumentando.

Palavras-chave: Myrciaria jaboticaba, pectina, poligalacturonase, clorofila, antocianinas.

Introduction

Most species of jabuticaba trees are included in the genus *Myrciaria*, family Myrtaceae. Jabuticabasabará or jabuticaba-murta (*Myrciaria jaboticaba* (Vell.) O. Berg) comes from a native tree of Mata Atlântica. It is found in Honduras and in Brazil, in the states of Minas Gerais, São Paulo and Rio de Janeiro. It is the most cultivated species of jabuticaba tree in Brazil. Jabuticabas do not have high commercial value because the fruits are very perishable. Despite the high productivity of each tree, after harvest, the fruits only have three days of shelf life, making it difficult to market them. Jabuticabas are dark purple, spherical berries with juicy pulp; they are slightly acidic and very sweet (ANDERSEN; ANDERSEN, 1988; CORRÊA et al., 2007; LIMA et al., 2008).

Jabuticaba trees may flourish from two to three times in the same year, depending on the agricultural treatments (MATTOS, 1983). The

development cycle varies from 45 to 65 days, depending on the region of the crop (BARROS et al, 1996). The ideal maturity point for consumption may be determined visually by the color alteration of the rind, as well as increased tenderness, which is determined by finger compression.

In natura consumption of the fruit is very common, and this fruit is also used in the preparation of several processed products of higher aggregated value, such as sweets, jams, fermented beverages and liqueurs. Regarding the manufacturing of jabuticaba jams, the rinds and seeds, which together represent 50% of the fruit, are normally discarded. Identifying a use for these components would increase the value of this fruit (LIMA et al., 2008).

Physical, chemical and biochemical transformations occur in the fruit during maturation. Characteristics related to texture (such

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as degradation of the cell wall compounds) and flavor (such as alteration of pH, acidity and the formation of sugars) are important in defining the harvest point and the appropriate application of technologies for industrial processing of this fruit (CHITARRA; CHITARRA, 2005).

Previous studies have been conducted related to the main changes that occur during the maturation of jabuticabas. The focus of these studies has been the adoption of new technologies that improve both the *in natura* post-harvest conservation of the fruit and the processes related to industrialization.

The current study aimed to evaluate the physicochemical characteristics and enzymatic activity of "Sabará" jabuticabas cultivated in Lavras, Minas Gerais State, Brazil, at different maturation stages.

Material and methods

The experiment was conducted in the Laboratory of Post-Harvest Fruits and Vegetables in the Food Sciences Department at the Federal University of Lavras, using jabuticabas (*Myrciaria jaboticaba* Berg cv. Sabará) fruits obtained from an orchard containing 10 trees in Lavras, Minas Gerais State. The fruits were manually harvested at five maturation stages (approximately 30 days after the flowering peak).

After discarding damaged jabuticabas, the remaining fruits were standardized by size and classified according to their maturation degree. A visual inspection was then conducted to determine the rind color, taking into account that fruits at stage 1 are already mature once they have reached a certain size and once the matte green color has changed to bright green. Jabuticabas were then separated according to the description presented in Table 1 and Figure 1.

Table 1. Classification of the maturation stages of jabuticabas according to the degree of fruit rind coloration.

Stage	Characteristics
1 (TG)	Totally green
2 (IP)	Incipient pigmentation
3 (PLP)	Predominance of light purple
4 (TP)	Totally purple
5 (TDP)	Totally dark purple



Figure 1. The degree of rind coloration of evaluated jabuticabas.

Twenty fruits were randomly selected for color and texture characterization, and the remaining fruits were frozen in liquid nitrogen and kept in a freezer at -18°C until the beginning of the physicochemical and enzymatic analyses.

Coloration was determined on an equatorial region at two equidistant points of the rind, using a CR-400 Minolta colorimeter, using determination mode CIE L*a*b*. The color parameters measured in relation to the standardplate were as follows: luminosity (L*), which varies from black (0) to white (100); a*, which varies from green (-60) to red (+60); and b*, which varies from blue (-60) to yellow (+60). The following parameters were used in the determination of color indexes: Hue (Hue angle), which consists of an indicative tone, and Chroma, which defines the color intensity.

To evaluate fruit firmness, a TA-XT2i texturometer was used with a penetration distance of 20 mm, a speed of 2.0 mm s⁻¹ and a probe point of SMP/N.

The total chlorophyll was determined from 1 g of ground rind in 10 mL of water, with the aid of a tissue homogenizer. The extract was transferred to a volumetric flask of 50 mL, to which the appropriate volume of acetone was added. Filtration was performed after a darkened rest period. The extract absorbance was read at 652 mm, and the results were expressed in mg 100 g⁻¹ of fresh rind. The calculations were performed according to the equation adopted by Engel and Poggiani (1991).

The chemical determination of total anthocyanin was performed only for the rind, using the pH difference method (GIUSTI; WROLSTAD, 2001), in which two buffer systems were dissolved: potassium chloride - hydrochloric acid at a pH of 1.0 (0.025 M) and sodium acetate at a pH of 4.5 (0.4 M). All other physicochemical and enzymatic analyses were performed with rind fractions and pulp.

The pH was measured using a potentiometer with a glass electrode (AOAC, 1992). The titratable acidity (TA) was determined by titration of the filtrate (dilution 1:5) with NaOH 0.1 N, standardized according to the technique established by the Institute Adolfo Lutz (IAL, 1985) and expressed in mg of citric acid/100 g of tissue. The soluble solids content (SS) was evaluated by means of an Atago PR-100 digital refractometer ("Palette" model) with automatic temperature compensation and according to the AOAC (1992) methodology. The results were expressed in "Brix. In addition, the relationship between SS/TA was determined.

The total soluble sugars were determined by the anthrone method, as described by Dische (1962), and the results were expressed in g per 100 g of rind or pulp.

Polygalacturonase activity (PG) (EC 3.2.1.15) was determined by measuring the reducer groups released from polygalacturonic acid, according to the methodology of Pressey and Avants (1973). This method involves the incubation of the enzymatic extract with solution polygalacturonic acid at 0.25% in buffer sodium acetate (37.5 mM, pH 5) at 30°C for 3h. The reaction was interrupted with a boiling water bath. The reducer groups were determined according to the technique described by Somogy modified by Nelson (1944),galacturonic acid as standard. The unit of enzymatic activity (UEA) was determined as the capacity of the enzyme to catalyze the formation of one nanomole of reducing sugar.

Analyses were conducted according to a simple, entirely randomized design (ERD) with

five maturation stages and 3 repetitions per analysis. The statistical analyses were performed using the SISVAR software (FERREIRA, 2011). The responses of evaluated variables were submitted to an analysis of variance, which was applied to the ERD. A regression analysis was performed for the interactions between variables; the results are illustrated graphically by the trend line and the coefficient of determination.

Results and discussion

The analysis of variance showed interactions among all variables (data not shown), as presented in dispersion graphics with the quadratic curve and the coefficient of determination. The changes in coloration (from green to dark purple) in different maturation stages, as presented in Figure 2, were associated with a decrease of chloroplast pigments, which occurred due to chlorophyll degradation on the fruit rind as well as anthocyanin synthesis throughout maturation.

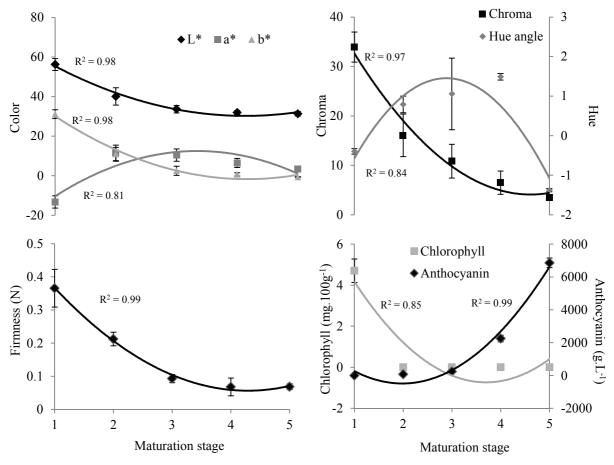


Figure 2. Color, chroma, Hue angle, firmness, anthocyanin and chlorophyll content of jabuticaba rind (epicarp) at different maturation stages.

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A decrease in L* and b* values was observed, indicating that the fruits became darker, as well as an increase in a* values, indicating that the fruits became less green as maturation advanced. Oliveira et al. (2003) noted that for jabuticaba 'Sabara', intense dark purple staining was preferred during an assessment performed by panelists. A change in color is one of the most important criteria used by consumers to judge the degree of maturity and fruit quality. For the hue index, fruits at stage 5 (totally dark purple) presented the lowest values. According to the Cielab system, the greater the angle, the yellower the fruit, and the smaller the angle, the redder the fruit. According to McGuire (1992), the chroma determines the color intensity; a higher chroma indicates greater yellow color intensity. The chroma reached a maximum value in the first stage of maturation and decreased throughout maturation.

Figure 2 shows that the fruits' firmness decreased as the maturation degree advanced, and in stages 4 and 5, the values were below 0.1 N. Fruit firmness is an important quality attribute because fruits with a higher firmness are more resistant to mechanical injuries during transport and commercialization.

The maturation of most fresh fruit is frequently characterized by the loss of firmness through modifications to and degradations of the cell wall components. The soluble solids content, titratable acidity, pH and ratio SS/TA of rind and pulp are presented in Figure 3.

The soluble solids content increased as a function of the maturation stage, achieving maximum values of 10.27 °Brix in the rind and 14,67 °Brix in the pulp at stage 5. These values are similar to those found by Lima et al. (2008), who obtained contents of 9.3 °Brix for the rind and 14.13 °Brix for the pulp of cv. Sabará in an evaluation of mature jabuticabas and the rind, pulp and seed fractions.

The titratable acidity content expressed in % of citric acid was higher in stage 1 fruits. These values decreased as maturation progressed, with significant variations mainly in the pulp as a function of the maturation stage; for stage 5 fruits, the values were 0.55 and 0.30% of citric acid in the rind and pulp, respectively. According to Chitarra and Chitarra (2005), the acidity is reduced as fruit maturation progresses because acidity is consumed during fruit metabolism and by the release of sugars. This trend was observed in the present experiment.

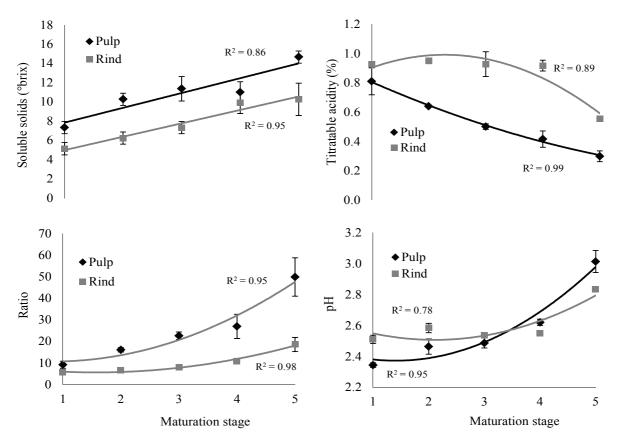


Figure 3. The soluble solids, titratable acidity, pH and ratio (SS/TA) of jabuticaba-sabará rind (epicarp) and pulp (mesocarp) during different maturation stages.

The SS/TA ratio increased considerably among the fruit stages, both in the rind and pulp, as all fruits presented a higher ratio at the later degrees of maturation (Figure 3). The reduction of TA and the increase of both SS and the SS/TA ratio after complete fruit maturation demonstrate the balance between sweet and acidic flavors and indicate that jabuticabas may be harvested at the stage of complete maturation (TDP) for storage, processing and in natura consumption. The more resistant fruits at earlier stages of maturation may more suitable for transportation and processing, but without full ripening, the sugar content required for consumption is not yet Technological studies of other maturation stages should be conducted to identify the stages that are most appropriate for use in processing fruit jellies, sweets, beverages and other products.

The total soluble sugars, soluble pectin and polygalacturonase (PG) enzymatic activity of rind and pulp are presented in Figure 4. During fruit maturation, one of the main changes was related to sugar accumulation, which increased simultaneously with decreasing acidity. The sugar content achieves a maximum near the end of maturation, guaranteeing quality (excellence) of the product (CHITARRA; CHITARRA, 2005). Figure 4 shows the increase in total sugar content; at stage 5, the fruits had values of 8.16 and 4.86 g for 100 g of rind and 100 g of pulp, respectively.

The soluble pectin content increased during fruit maturation; the maximum was reached at stage 5 in both the rind and the pulp. The increase in the percentage of soluble pectin, mainly in the 4 and 5th stages, coincided with intense PG activity in these stages and a consequent increase in the solubilization of pectin. The polygalacturonase activity was significantly affected by the maturation degree of the fruits; it increased gradually until achieving its maximum value in stage 4 (TP). Pectin substances, which are derived from polygalacturonic acid and serve as a luting material, are mainly deposited on the cell wall. These substances are responsible for changes of the fruit texture (CHITARRA; CHITARRA, 2005). Polygalacturonase catalyzes the α -1,4 bonds between adjacent residues of galacturonic acid (SEYMOUR et al., 1987).

The high activity of PG in jabuticaba rind and pulp suggests that this enzyme may be mainly responsible for pectin solubility during the softening process of this fruit. According to Whitaker (1994), other enzymes can act on pectins, such as PME, which catalyzes the deesterification of pectic waste, thus hydrolyzing methyl-ester groups and producing pectins with a lower degree of methylation (which will serve as the substrate for PG). However, no PME activity was detected in any of the stages (data not shown). Magalhães et al. (1996) reported that the contents of pectin in jabuticaba 'Sabará' were much lower than those of other carbohydrate structures and further decreased during the later stages of maturation; thus, pectin may be associated with the ripening of this fruit.

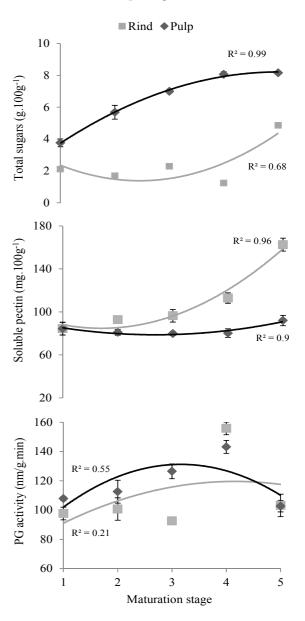


Figure 4. The total sugars (g 100^{-1}), soluble pectin (mg 100^{-1}) and PG activity (nm g⁻¹ min.) of the rind (epicarp) and pulp of "jabuticaba-sabará" at different maturation stages.

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Conclusion

The progressive maturation stages of jabuticaba-sabará are characterized by color changes, a decrease of firmness and an increase of soluble pectin and polygalacturonase activity. The rind showed increases in total soluble solid content, sugar, pH and the SS/TA ratio and a decrease in titratable acidity as maturation progressed. The pulp also showed increases in total soluble solid content, pH, sugar, the SS/TA ratio, pectin content and polygalacturonase enzymatic activity as well as a decrease in acidity as maturation progressed.

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