



Changes in pectinases, dietary fibers, and physicochemical indices related to the flavor of cubiu fruits during ripening

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ABSTRACT. The objective of this study was to evaluate the changes in the activity of pectinases (pectinesterase and polygalacturonase), dietary fiber content (alcohol-insoluble solids, pectin, and total fiber), and physicochemical indices related to the flavor (pH, titratable acidity, soluble solids, Brix/acid ratio, and reducing and nonreducing sugars) of cubiu fruits (*Solanum sessiliflorum* Dunal) at different stages of ripening (green, turning, ripe, and fully ripe). Alcohol-insoluble solids and pectin were very similar, with the highest levels detected at the green and turning stages, and the lowest levels occurring at the fully ripe stage. The amount of total fiber was consistent at the green, turning, and ripe stages, but declined at the fully ripe stage. These changes correlated with the pectinase activities, the profiles of which resembled those of other species of the Solanaceae family during fruit ripening. However, cubiu fruits were significant sources of dietary fiber at all stages. The reducing sugar content reached the highest level at the turning stage, with glucose as the major sugar. The content of nonreducing sugars, such as sucrose, remained low at all stages. The other physicochemical indices displayed increases during ripening, characterizing cubiu as a very acidic fruit with a small degree of sweetness.

Keywords: *Solanum sessiliflorum* Dunal, fruit peel color, pectin concentration, medicinal plants.

Mudanças nas pectinases, fibras dietéticas e índices físico-químicos relacionados ao sabor de frutos de cubiu durante a maturação

RESUMO. O objetivo deste estudo foi avaliar as mudanças na atividade de pectinases (pectinesterase e poligalacturonase), teor de fibras dietéticas (sólidos insolúveis em álcool, pectina e fibra total) e índices físico-químicos relacionados ao sabor (pH, acidez titulável, sólidos solúveis, relação Brix/acidez, açúcares redutores e não-redutores) de frutos de cubiu (*Solanum sessiliflorum* Dunal) em diferentes estádios de maturação (verde, de vez, maduro e muito maduro). Os sólidos insolúveis em álcool e pectina apresentaram perfis muito similares, com teores maiores nos estádios verde e de vez, e menores no estádio muito maduro. Teores de fibra total mostraram-se uniformes nos estádios verde, de vez e maduro, diminuindo no estádio muito maduro. Essas mudanças correlacionaram-se com a atividade das pectinases, cujos perfis assemelharam-se àqueles de outras espécies da família Solanaceae durante a maturação dos frutos. No entanto, os frutos de cubiu apresentaram teores significativos de fibras dietéticas em todos os estádios. O conteúdo de açúcares redutores atingiu teores maiores no estádio de vez, sendo a glicose o açúcar principal. O conteúdo de açúcares não-redutores, como a sacarose, permaneceu baixo em todos os estádios. Os demais índices físico-químicos apresentaram aumento durante a maturação, caracterizando o cubiu como um fruto muito ácido com pequeno grau de doçura.

Palavras-chave: *Solanum sessiliflorum* Dunal, coloração da casca de frutos, concentração de pectina, plantas medicinais.

Introduction

The population of the Amazonian region has long used fruits from a species of the Solanaceae family commonly referred to as cubiu (*Solanum sessiliflorum* Dunal) in Brazil, cocona in Colombia and Peru, and tupiru in Venezuela (WHALEN et al., 1981). Cubiu is native to western Amazonia and was domesticated by the pre-Columbian Amerindians (LOPES; PEREIRA, 2005). Other historical records indicate that in 1760, a Spanish surveyor, Apolinar Diaz de la Fuente, found cubiu growing with maize and beans in an Indian garden located between

Guaharibos Falls and the juncture of the Casiquiare and Orinoco rivers (VOLPATO et al., 2004).

Mature and ripe fruit can be distinguished by the following description: mature fruit is ready for consumption, whereas ripe fruit is ready for picking (POTTER; HOTCHKISS, 1998). Fruit ripening is a complex, genetically programmed process resulting in remarkable changes in the color, texture, flavor, and aroma of the fruit (ALEXANDER; GRIERSON, 2002). In addition to aiding in the judgment of fruit maturation time, the fruit peel color is an important quality parameter and

marketing attribute that influences consumer acceptance (LARRIGAUDIÈRE et al., 1996; YONG-ZHONG et al., 2006). This and the other sensory attributes mentioned above are at prime quality in the fully ripe fruit. In contrast, overripe fruit is likely to become soft and mealy soon after harvest (WU et al., 2011). Soluble solids mainly consist of sugars (e.g., sucrose and fructose) and organic acids, which are good indicators of the ripening stage of fruits (PEDRO; FERREIRA, 2005; PRETEL et al., 2008; PURKAYASTHA; MAHANTA, 2011).

However, in the Amazonian region, the fleshy berries of the cubiu shrubs are picked regardless of the ripening stage. This timing of the fruit harvest is used in part because the ingredients necessary to prepare the traditional fish stew are not easily available throughout the Amazonian territory; thus, the native population uses whatever foods are available to add substance to the daily meal. When used as condiments, these fruits have a pleasing acidulous flavor reminiscent of citrus, which is suitable for direct consumption or use in salads, fish, and meat dishes as well as juices and other beverages (WHALEN et al., 1981). As food supplements, ripe and fully ripe cubiu fruits are relatively poor in macronutrients such as proteins and lipids, but rather rich in secondary metabolites such as total carotenoids (ANDRADE JÚNIOR; ANDRADE, 2012). These fruits are also rich in iron, niacin, and dietary fibers (ANDRADE et al., 2010; SCHUELTER et al., 2009). Dietary fibers are usually classified into two types according to their solubility in water. These fibers are designated as insoluble fibers (e.g., cellulose and lignin) or soluble fibers (e.g., pectin). The gelling properties of the pectin present in cubiu fruits are well known to both researchers in the Amazonian region and home jam makers (ANDRADE et al., 2010; WILLATS et al., 2006; YUYAMA et al., 2008). As a medicinal plant, cubiu has tested positively for hypoglycemic effects in rats and humans in short-term studies (PARDO, 2004; SILVA FILHO et al., 2003; YUYAMA et al., 2008). Mid-term and long-term studies should still be conducted for a more detailed assessment of cubiu efficacy in diabetes mellitus.

Other appearance factors (e.g., the consistency) of cubiu fruits are also important for the fresh market. Breakdown of the cell wall polysaccharides by pectinase activities during fruit ripening causes softening of the fruit that may make it unacceptable for consumption. Pectin is a major plant cell wall polysaccharide that is a complex polymer of

galacturonic acid linked by 1,4- α bonds and methoxylated to a varying extent at the carboxyl moieties (JOLIE et al., 2010). Among the pectinases, pectinesterase (EC 3.1.1.11) catalyzes the demethoxylation of esterified pectins (JOLIE et al., 2010). This enzymatic activity releases protons when the pectin methoxyl groups are converted to carboxyl groups, reducing the pH of the milieu and being thereby easily detectable in the laboratory (HENSEL et al., 2002). Polygalacturonase (EC 3.2.1.15), another pectinase, is considered a key enzyme involved in pectin depolymerization and solubilization (OBENLAND et al., 2003). Notably, not only is pectin disassembly important for the food industry, but its derived oligosaccharides have also been found to exert various positive effects on human health (BURANA-OSOT et al., 2010).

Thus, the first objective of this study was to evaluate the changes in the pectinase activities at different ripening stages, providing a basis for the food industry to preserve the textural quality and extend the shelf life of cubiu fruits. The second objective of this study was to evaluate the changes in dietary fibers, thereby providing pertinent data for researchers and consumers. The third objective of this study was to evaluate the changes in the basic physicochemical indices related to the flavor of these fruits at different ripening stages, e.g., pH, titratable acidity, soluble solids, Brix/acid ratio, reducing sugars (glucose and fructose), and nonreducing sugars (sucrose, a disaccharide of glucose and fructose). These data were previously unknown; hence, this study was designed to address these knowledge gaps.

Material and methods

Sample preparation

Cubiu fruits were randomly, manually harvested from shrubs grown in low-humic gley soil in the municipality of Iranduba, in the Amazonas State of Brazil (03°14'22" south and 60°13'50" west). The determination of the ripening stages for the fruits was based on the four color patterns described by the native population: green (over the entire fruit surface), turning (first appearance of yellow color at the blossom end), ripe (yellow color over the entire fruit surface), and fully ripe (deep red-wine color over the entire fruit surface). In addition to these standard colors, an additional criterion for the inclusion of the fruits in this study was their structural integrity (i.e., the absence of injuries). After harvest, the fruits were transported in plastic containers to the laboratory where the peels were

washed and dried at ambient temperature (22°C). The sample size and mean weight of whole cubiu fruits (peel, pulp, and placenta) at the four ripening stages (the independent variables) included 14 green fruits (167.11 g), 12 turning fruits (204.00 g), 26 ripe fruits (243.23 g), and 18 fully ripe fruits (212.98 g). Each of these four sample groups was then diced, ground, and homogenized in a blender (Arno, Manaus, Amazonas State, Brazil). Part of the homogenized material was immediately used for enzyme extraction as well as the determination of the pectinesterase and polygalacturonase activities, pH, titratable acidity, and soluble solids (the dependent or response variables). The remaining part of the homogenized material was stored in polyethylene bags and kept in a freezer at -20°C until use in physicochemical analyses of other response variables (total fiber content, alcohol-insoluble solids determination, pectin concentration, and reducing and nonreducing sugars). Three replicates were prepared from each sample.

Enzyme extraction

The preparation of the enzyme extracts was similar to the method described by Jen and Robinson (1984) for sweet bell peppers (*Capsicum annuum* L.). The enzyme extracts were prepared from 100 g of each sample obtained as described in the sample preparation section above. Whole cubiu fruits were diced and homogenized in 100 mL of distilled and deionized water at 4°C for 1 minute in a blender. The resultant homogenate was filtered through nylon (pore size 0.45 µm), and the residue was resuspended in 100 mL of a 1 M NaCl solution at 4°C, and then further broken up with a mortar and pestle that were also cooled to 4°C. The resulting suspension was adjusted to pH 6.0 using a 1 M NaOH solution and a pH meter Q400A (Quimis, Manaus, Amazonas State, Brazil). After incubation (with stirring) at 4°C for 1 hour, the solution was clarified by filtration through nylon, and centrifugation (Sorvall Super T21, DuPont Company, Wilmington, DE, USA) at 27,000 x g for 15 minutes at 4°C. The resultant supernatant was used for the enzyme analyses.

Pectinesterase activity

Pectinesterase activity can be assayed based on the release of either free carboxyl groups or methanol from pectin (MCFEETERS et al., 2001). The most common technique is to measure the rate of release of free carboxyl groups from pectin (as in this work) using the pH stat described above (MCFEETERS et al., 2001). Fifty mL aliquots of

1.0% citric pectin (Sigma-Aldrich Inc., St. Louis, MO, USA) diluted in a 0.1 M NaCl solution were used as substrates. The reaction was conducted under a controlled temperature range (29-30°C) with constant stirring. Immediately after adjusting the pH to the narrow range of 6.99-7.00 using NaOH, the enzyme extract (2 mL) was added to the incubation mixture over a period of 30 minutes, which allowed for titration with a 0.025 M NaOH solution. The pectin de-esterification was complete when the pH underwent no further change (LIN et al., 1990). In parallel, a control experiment was performed with enzyme extract that was heat-inactivated by immersion in boiling water (98°C) for 5 minutes followed by immediate immersion in an ice-water bath. The pectinesterase activity was calculated according to the equation proposed by Laratta et al. (1995). The pectinesterase unit was considered to be the amount of enzyme capable of catalyzing the release of 1 µmol of free carboxyl groups per g of fruit tissue per 1 minute (ŞİMŞEK; YEMENİCİOĞLU, 2010).

Polygalacturonase activity

To measure the polygalacturonase activity, test tubes containing 100 µL of 37.5 mM sodium acetate buffer, pH 5.0, and 800 µL of galacturonic acid (0.1%) (Sigma-Aldrich Inc., St. Louis, MO, USA) were placed in a thermo-stabilized bath for 5 minutes at 30°C. After the addition of 100 µL of the enzyme extract and incubation for 1 hour, the reaction was stopped by immersion of the tubes in a boiling water bath for 5 minutes and then in an ice-water bath. Throughout the process, the tubes were sealed using polyvinyl chloride (PVC) film (Vitafilm, Goodyear, Manaus, Amazonas State, Brazil). To quantify the reducing groups that were released, the Somogyi-Nelson method, as described by Southgate (1991), was used. Anhydrous glucose (Merck, São Paulo, São Paulo State, Brazil) was used as a standard for the calculation of the linear correlation. At the same time and under the same conditions, a control experiment was performed using an enzyme extract that was heat-inactivated by immersion in boiling water (95°C) for 5 minutes followed by immediate immersion in an ice-water bath. Distilled water was also used as a control to account for the interference by any non-enzymatic release as well as the reagent colors. One unit of polygalacturonase was considered to be the amount of enzyme capable of catalyzing the release of 1 µmol of reducing groups per 1 mL of enzyme extract per 1 minute of incubation under the conditions defined above.

Determination of alcohol-insoluble solids

The procedure for the determination of alcohol-insoluble solids (AIS) followed that of Cantor et al. (1992) with certain modifications. The AIS content was determined for 10 g aliquots prepared as described above. Previously ground whole cubiu fruits were added to 100 mL of alcohol (92.8%), and this mixture was kept immersed and thermally stabilized at 85°C for 20 minutes. The mixture was then immediately filtered through paper (with a known dry weight) while still hot. The residual material on the filter paper was consecutively washed with 300 mL of alcohol and 100 mL of hexane (both chemicals were purchased from Vetec, Rio de Janeiro, Rio de Janeiro State, Brazil). The residue on the filter paper was dried at 65°C in a furnace equipped with an air circulation system until a constant weight was reached. The resultant dry material (AIS) was crushed with a mortar and pestle until it turned into a light-brown powder that was easily granulated at ambient temperature (22°C). To minimize the uptake of moisture, the AIS samples were stored in desiccators (Laborglas, São Paulo, São Paulo State, Brazil). The AIS content was expressed in g 100 g⁻¹ fresh weight.

Pectin concentration

Using the method of Ahmed and Labavitch (1977) as described by Chang et al. (1996), the pectin concentration was determined from the AIS (10 mg) obtained as outlined above. The samples were digested with 2 mL of concentrated H₂SO₄ (*d* = 1.84) in an ice bath and then 50 mL of deionized water was slowly added over approximately 1 hour. The pectin concentration of the clear solution containing the digested sample was analyzed using the m-hydroxydiphenyl method and was expressed as anhydrogalacturonic acid. The absorbance was evaluated at 520 nm on a spectrophotometer (SP-2000 UV, Spectrum, Shanghai, China). The pectin concentration was expressed in g 100 g⁻¹ fresh weight.

Total fiber content

In this work, the method applied to measure the total fiber content (cellulose, hemicellulose, lignin, and resistant starch) focused on the indigestibility of the fiber fraction and used procedures that simulate intestinal digestion (SOUTHGATE, 1999; MARÍN et al., 2011). The total fiber content was determined from the samples (2 g) prepared as initially described in the sample preparation section above. Petroleum ether (Vetec, Rio de Janeiro, Rio de Janeiro State,

Brazil) was used to remove lipids from the pre-homogenized and pre-dehydrated whole cubiu fruits in a Soxhlet extractor (Marconi, São Leopoldo, Rio Grande do Sul State, Brazil). The samples were successively treated with a 0.1275 M H₂SO₄ solution and a 0.313 M NaOH solution, and then intercalated by filtration through paper. The residual material on the filter paper was thoroughly washed with boiling distilled water. After washing with petroleum ether and evaporating the solvent, the residue on the filter paper was dried at 65°C in a furnace supplied with an air circulation system until the sample reached a constant weight. The total fiber content was expressed in g 100 g⁻¹ fresh weight.

pH and titratable acidity

The pH was measured using a calibrated pH meter. The determination of the titratable acidity was performed by titration with 0.1 M NaOH using phenolphthalein as the indicator (INSTITUTO ADOLFO LUTZ, 2008). The results were expressed as the percentage of citric acid (WOODS; AURAND, 1977).

Soluble solids

Soluble solids were measured in drops of undiluted juice from freshly harvested cubiu fruits, using a refractometer coupled with a thermometer (A. Krüss Optronic, AR 2008, Nuremberg, Germany). The results were corrected to 20°C and expressed in °Brix units (INSTITUTO ADOLFO LUTZ, 2008). The Brix/acid ratio was calculated according to Ranganna (1986).

Reducing and nonreducing sugars

The reducing sugars were extracted with distilled water and quantified at 510 nm in a spectrophotometer (Spectrum, SP-2000, Shanghai, China) according to the Somogyi-Nelson method (SOUTHGATE, 1991). Glucose was used as the standard. Fructose was extracted with distilled water, separated with amylic alcohol, and measured at 640 nm in the aforementioned spectrophotometer (RIBÉREAU-GAYON; PEYNAUD, 1966). Fructose was then used as the standard. The glucose content was calculated as the difference between the measured concentration of the reducing sugars and the concentration of fructose. The determination of nonreducing sugars (sucrose) was performed by acid hydrolysis with HCl and heating in a water bath, as described by Southgate (1991). All sugar contents were expressed in g 100 g⁻¹ fresh weight.

Statistical analyses

The data were analyzed using descriptive statistics, correlation coefficients, and multiple linear regressions obtained with the SPSS computer program (SPSS Statistical Software Inc., Chicago, IL, USA). The ripening stages were translated from the fruit peel colors to numerical ranges as follows: green (1-2), turning (2-3), ripe (3-4), and fully ripe (4). A $p \leq 0.05$ was considered statistically significant.

Results and discussion

Pectinase activities

The multiple linear regression models confirmed the strong correlation between the pectinase activities and pectin concentrations at all ripening stages investigated in this study. The minimum activities of these enzymes correlated with the highest pectin concentrations at the green stage. The maximum activity of pectinesterase was measured at the turning stage, whereas the maximum activity of polygalacturonase was measured at the fully ripe stage (Figure 1). These pectinase profiles closely resembled those of the aforementioned sweet bell pepper, which is also a species of the Solanaceae family (JEN; ROBINSON, 1984). The apparent coordination of these enzyme activities has led to the assumption that they exert combined effects on pectin degradation (JEN; ROBINSON, 1984; KETSA, 2003; KUMAR; CHAUHAN, 2010; NEGI; HANDA, 2008). Recent work has strengthened this hypothesis (CHENG et al., 2011). Nevertheless, it is plausible that cell wall modification is a multifactorial coordinated process, including nonenzymatic solubilization (NEGI; HANDA, 2008; SASIDHARAN; PIERIK, 2010). Furthermore, pectin depolymerization is a more specialized process than solubilization, and evidence suggests that these two processes are not correlated (MERCADO et al., 2011). Many aspects of pectinase activities require further investigation; however, the characterization of their changes in ripening cubiu fruits presented herein is a fundamental step towards preserving the postharvest quality of these fruits and thus meeting consumer satisfaction.

Dietary fibers

In general, the structural components decreased as ripening progressed, although some specific differences in this decrease between the diverse types of fibers were also present (Figure 2). The statistical analysis of the dietary fiber content

showed only one modal value in the AIS at the green and turning stages. AIS are mainly composed of pectins and other cell wall materials (BURNS; ALBRIGO, 1998). Therefore, AIS and pectin displayed similar profiles, with the highest levels occurring at the green and turning stages, and the lowest levels observed at the fully ripe stage. Additionally, pectin levels ($\text{g } 100 \text{ g}^{-1}$ fresh weight) were higher in ripe cubiu fruits than in certain other popular fruits consumed worldwide, such as plums at 0.70, lemons at 0.51, oranges at 0.46, and apples at 0.38 (SOUTHGATE, 1991). Pectin is a high-value functional food ingredient and the substantial pectin content in whole cubiu fruits deserves further investigation, such as the analysis of the different tissues of these fruits (WILLATS et al., 2006). The total fiber contents ($\text{g } 100 \text{ g}^{-1}$ fresh weight) were generally lower than those of pectin in this study, although they were not negligible compared with the fiber contents of other edible solanaceous crops of Brazil, such as tomatoes at 1.20 (NEPA-UNICAMP, 2011).

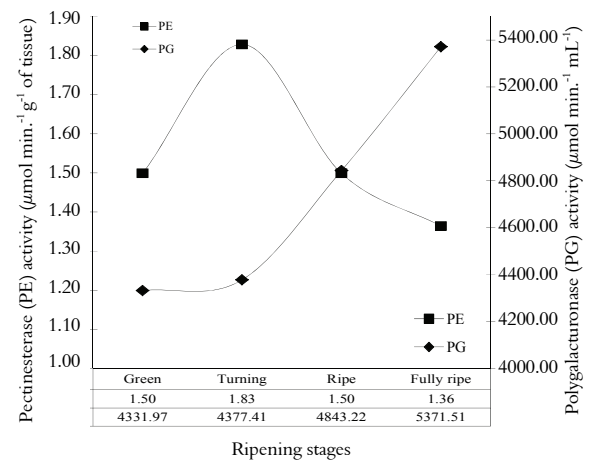


Figure 1. Pectinase activities during cubiu fruit ripening.

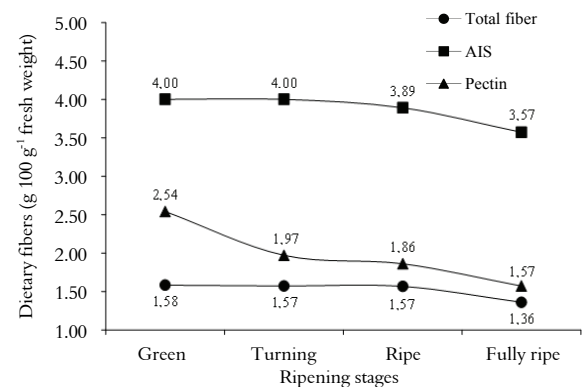


Figure 2. The dietary fiber contents of cubiu fruits at four ripening stages.

Physicochemical indices related to the fruit flavor

The pH, titratable acidity, concentration of soluble solids, Brix/acid ratio, and reducing and nonreducing sugar contents are not only important ripening indices, but are also related to the flavor of the fruits (PEDRO; FERREIRA, 2005; PRETEL et al., 2008; PURKAYASTHA; MAHANTA, 2011; SADLER; MURPHY, 2010). The statistical analysis of these indices showed no modal value. The titratable acidity increased from the green stage to the fully ripe stage, whereas the pH decreased from the green stage to the turning stage, varying slightly until the fully ripe stage (Figure 3). This profile contradicted the general axiom in which there is a considerable decrease in organic acid content during the ripening process (WORKNEH; OSTHOFF, 2010). For example, studies have shown that in the solanaceous species tomato, the concentration of citric acid may exhibit a ten-fold decline during ripening (WORKNEH; OSTHOFF, 2010). The soluble solid content increased throughout the four ripening stages, partially due to the simultaneous increase in the levels of organic acids (Figure 4). Organic acids affect flavor by acting on the perception of sweetness (HOUNSOME et al., 2008). As a result, the Brix/acid ratio is a better predictor of the flavor impact of an acid than are the Brix or acid values alone (SADLER; MURPHY, 2010). In addition, the reducing sugars reached the highest level at the turning stage, with glucose being the major sugar (Figure 5). Fructose reached a maximum at the green stage. Sucrose remained low at all investigated ripening stages, with a profile similar to that found in other solanaceous fruits and in agreement with its role

as a source of glucose and fructose in the intermediary metabolism of fruits (HALL et al., 2011; KAWABATA et al., 2002; NAVARRO et al., 2006; SAUER, 2007). Given the present data, cubiu may be considered to be a very acidic fruit with a small degree of sweetness.

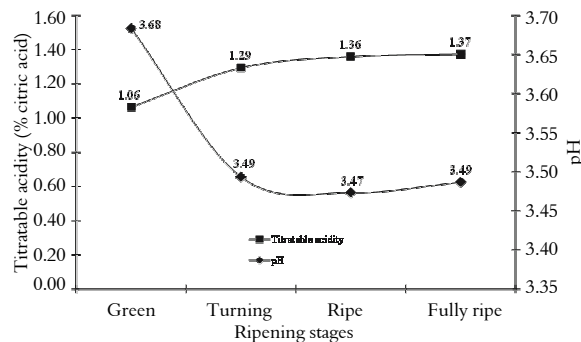


Figure 3. Changes in the titratable acidity and pH during cubiu fruit ripening.

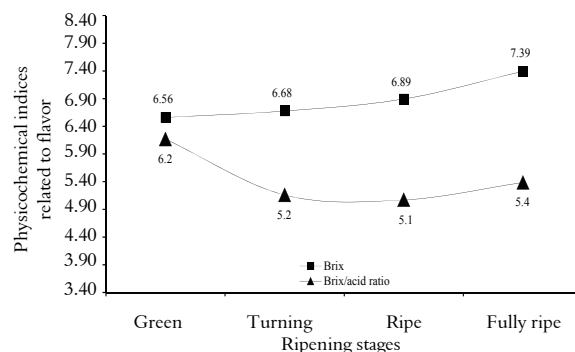


Figure 4. Changes in the soluble solids concentration (Brix) and the Brix/acid ratio during cubiu fruit ripening.

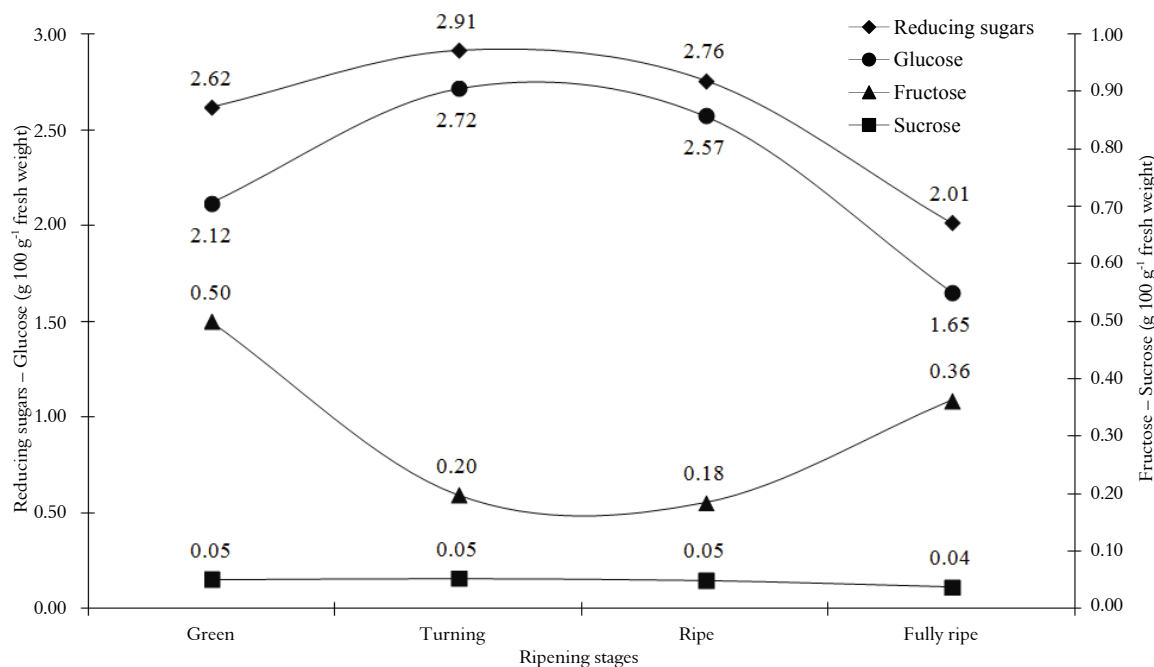


Figure 5. Changes in the sugar contents during cubiu fruit ripening.

Conclusion

Changes in the pectinase activities were strongly correlated with variations in the dietary fiber contents during cubiu fruit ripening. These fruits were an important source of dietary fibers and pectin, in particular, at all investigated ripening stages, partially explaining the recognition of cubiu as a medicinal fruit. The flavor of cubiu fruit may be characterized as very acidic with a small degree of sweetness according to the physicochemical indices investigated herein.

Acknowledgements

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) for their financial support of this study.

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Received on January 30, 2013.

Accepted on April 15, 2013.

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