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ORIGINAL ARTICLE

Effect of processing on physicochemical composition, bioactive compounds and enzymatic activity of yellow mombin (*Spondias mombin* L.) tropical juice

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Abstract Yellow mombin (*Spondias mombin*, L.) is a tropical fruit that presents exotic taste and aroma, being source of carotenoids and phenolics compounds. It presents a good potential for processing, despite some restriction related with the presence of high amounts of peroxidase (POD) and pectinmethylesterase (PME) which can cause sensory changes in the product. This work addresses the evaluation of changes in POD and PME enzyme activity during the traditional industrial processing used to produce tropical juices in Brazil. The enzyme activity was determined after the main steps of the processing: fruit pulping, homogenization and pasteurization. Although both enzymes presented significant activity loss during processing, the final product showed residual activity for PME (25 %) and POD (2.5 %). PME showed to be more thermal resistant than POD in yellow mombin juice. Considering the compounds with antioxidant activity, yellow mombin presented high amounts of carotenoids and phenolics when compared to other tropical fruits such as passion fruit and pineapple. Although the processing of the fruit resulted in significative phenolic loss, the carotenoids content was not affected significantly by the processing.

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Introduction

Yellow mombin (*Spondias mombin* L.) is a small fruit, elliptical in shape with 3–4 cm in length. The fruit is cultivated in the Northeast of Brazil mainly during the rainy season. Like most regional fruits, yellow mombin is available during a short period of the year. The consumption of commercial products of regional fruits has increased in the last few years in Brazil, due to their year-round availability, and easy preparation. Beyond its flavor characteristics the fruit is a good source of pro-vitamin A (Assis et al. 2006).

Despite the good potential for industrialization, the final product may present changes in the sensory characteristics such as color and flavor, due to changes in composition during the processing.

The action of some enzymes, such as PME, polygalacturonase and POD, might have a pronounced effect on the quality of fresh and processed fruit products (Assis et al. 2001). Cloud loss is a major quality defect occurring in cloudy fruit and vegetable juices. This undesired defect is induced by demethylation of pectin by endogenous pectinmethylesterase (pectinesterase, PME, EC 3.1.1.11) yielding acidic low methoxy pectin, which can cross-link with polyvalent cations such as Ca^{2+} to form insoluble pectate precipitates. To overcome this problem, thermal treatments such as heating (e.g. 1 min at 90 °C for citrus juices) or freezing can respectively be used to inactivate PME or slow down its activity. Unfortunately, these processing steps have a negative influence on the juice flavor (Guiavarc'h et al. 2005).

Consumption of fruit and vegetables is associated with a decreased risk of heart disease and cancer. The antioxidants in

the fruits act by inactivating reactive oxygen species involved in initiation or progression of these diseases (Duthie et al. 2006). In animals and human, carotenoids particularly β-carotene and lycopene, play a role in the protection against photo oxidative processes by acting as singlet molecular oxygen and peroxyl radical's scavengers and can interact synergistically with other antioxidants (Tapiero et al. 2004).

Processing steps may affect the enzyme activity in different ways, and the juice quality may vary due to changes in its physicochemical composition. Usually, thermal treatment and homogenization are the steps that most affect the final quality and composition of processed juices due to heating and to chemicals added to preserve the final product. The purpose of this work was evaluate the effect of processing to produce yellow mombin juice regarding to juice composition (including the compounds with antioxidant activity such as phenolics, anthocyanins, carotenoids and ascorbic acid) and enzyme activity of POD and PME.

Materials and methods

Samples

The processing was carried out in a Brazilian industry of fruit juice. Samples from two different batches of yellow mombin juice were collected immediately after pulping, homogenization and pasteurization (final product) stages as described following.

The fruits were received and weighed and then washed by immersion in chlorinated water (25 ppm) and selected in relation to sanity, physical integrity, uniformity of colour and ripeness. The selected material was then submitted to cutting and to separate the pulp from the peels in an expeller press in an atmosphere of steam, to avoid enzymatic activity in the juice. The pulp need passed through a finisher (0.8 mm) where the juice was separated from the seeds. The resulting material was passed through a finisher (0.8 mm), then through a line filter to reduce the pulp content and the juice was formulated (yellow mombin fruit pulp, preservatives: sodium benzoate and sodium metabisulfite, acidulant: citric acid) continuing to homogenization in a homogenizer of valves under pressure (100 atm) and later deaeration in a scraped surface evaporator (Rossi & Catelli, Parma, Italy) (600 mmHg) at temperature 50 °C. Following the sequence, it was applied the thermal treatment at 90 °C for 60 s in a plate heat exchanger, then the juice was hot filled (85 °C) (Rossi & Catelli, Parma, Italy) in glass bottles 500 mL and immediate closing with plastic caps. After sealing, bottles were cooled on a continuous refrigeration conveyor and stored.

The samples were packaged in glass bottles and identified. Except for the pasteurized sample, all other samples were stored frozen (−18 °C).

Physicochemical characterization

pH was directly determined in a Quimis potentiometer calibrated according to the AOAC (2001). Soluble solids content was determined by direct measure in a refractometer (Analitik Jena) and the results were expressed as Brix degrees according to the AOAC (2001). Total acidity was determined by titration with NaOH 0.1 N using phenolphthalein as indicator. Results were expressed as citric acid equivalents. Reducing sugars were determined by DNS method (Miller 1959) using a UV–vis spectrophotometer (Micronal, Model B582, São Paulo, Brazil) for measuring of absorbance. Total sugar was determined by the same method after acid hydrolysis.

Peroxidase (POD) and Pectinmethylesterase (PME) activities

Peroxidase extraction was carried out according to the technique described by Wisseman and Lee (1980). Enzyme activity was determined according to the methodology described by Matsuno and Uritani (1972), using guaiacol 1 g/100 mL added directly to the buffer solution as substrate. Enzyme activity was reported as enzymatic units (U) and is defined as the amount of enzyme that produces a change in absorbance of 1.0/min under the assay conditions at 25 °C.

Pectinmethylesterase activity was measured by titration, estimating free carboxyl groups formed in pectin as a result of enzyme action. One unit of PME was defined as the amount of enzyme which released 1 μmol of carboxyl groups per minute (Assis et al. 2001).

Antioxidant compounds

The total phenolics content was determined by spectrophotometry using the Folin-Denis reagent according to the methods described by Reicher et al. (1981). In dark room, the samples (5 g) were dissolved in a 40 mL of distilled water (6:4 v/v) and for elimination of vitamin C, the samples were placed in a water bath during 2 h, at 85º C, in accordance to Georgé et al. (2005). After cooling the samples were placed in a 100 mL volumetric flask completing the volume with distilled water. The extracts were filtered under reduced pressure through filter paper (Whatman No. 1). An aliquot of 5 mL of extracts was added to 15 mL distilled water, 5 mL of Folin-Denis reagent and 10 mL of a saturated solution of sodium carbonate and completing the volume to 100 mL with distilled water. After standing for 30 min at room temperature, the absorbance was measured at 760 nm using a UV–vis spectrophotometer (Micronal, Model B582, São Paulo, Brazil). All determinations were made in triplicate and values were calculated from calibration curves obtained with a minimum of five tannic acid concentrations. Linearity

was obtained between 0 and 5 mg/mL corresponding to absorbance values between 0.0 and 0.5. The total phenolics were expressed as mg of tannic acid equivalents (TAE)/100 g of fresh weight. Carotenoids were extracted according to the methodology described by Higby (1962). The quantification was carried out by absorbance at 450 nm (UV–vis spectrophotometer Micronal, Model B582, São Paulo, Brazil). Results were expressed as total carotenoids. Anthocyanins were quantified according to the methods described by Francis (1989). In this assay the anthocyanins were extracted from 1 g fresh pulp of fruits, with 30 mL of 95 % ethanol / 1.5 M HCl (85:15, v:v). The extract was transferred to a 50 mL volumetric flask completing the volume with ethanol-HCL (1,5 M) during 12 h at 4 °C. The material was filtered (filter paper Whatman No. 1) and the absorbance was measured in a UV–vis spectrophotometer at 535 nm (Micronal, Model B582, São Paulo, Brazil), and the total anthocyanin content was calculated as mg/100 g of fresh weight, through the following formula: Absorbance × dilution factor/98.2. Detection limit, sensitivity and between-day reproducibility were 0.11 mg/mL, 98.2 L/mg and 1 %, respectively. Ascorbic acid (AA) was determined using the modified Tillmans methods of the AOAC (2001) by titration with 2,6-dicholorolindophenol, modified by Assunção and Mercadante (2003), where the extractor solvent, metaphosphoric acid, was replaced by 1 g of oxalic acid /100 mL, since the soft yellow colour of the products did not interfere in the colour change turning point. The samples (5 g) were diluted with 40 mL of 1 g/ 100 mL oxalic acid aqueous solution, keeping them in a dark room for 15 min. The solution was titrated by adding the 0.2 g of 2,6-dichlorophenol-indophenol solution/100 mL until a distinct rose-pink color persists. The AA concentration was calculated by comparison with that known L-ascorbic acid standard solution (0.5 mg/mL), prepared and titrated daily. Antioxidant activity was determined by the ABTS method (Re et al. 1999) with few modifications. The ABTS radical cation (ABTS•+) was generated by reaction of 5 mL of aqueous ABTS solution (7 mM) and 88 μL of 140 mM (2.45 mM final concentration) of potassium persulfate solution. The mixture was held in dark at 29 °C for 14 h before use, and then it was diluted with ethanol to obtain an absorbance of 0.7±0.02 units at 734 nm using a UV–vis spectrophotometer (Micronal, Model B582, São Paulo, Brasil). Fruit extracts (30 μL) or Trolox were allowed to react with 3 mL of the resulting blue-green ABTS radical solution in dark condition. The decrease of absorbance at 734 nm was measured at the endpoint at 6 min. The standard curve was linear between 0 and 15 μM Trolox (final concentration). The results were thus expressed as TEAC (Trolox Equivalent Antioxidant Capacity) values. The activity of extracts was estimated at a minimum of three different concentrations. All tests were performed in triplicate.

Statistical analysis

The experimental results were given as mean ± SD of three parallel measurements and the data were statistically examined by ANOVA and Duncan test at 90 % of confidence level. The software *Statistical Analysis System for Windows* version 8.1 (SAS Institute, Inc. SAS User's Guide: version 9.1, Cary, NC: SAS Institute, 2006) was used to process the data.

Results and discussion

Physicochemical characterization

According to the results presented in Table 1 it can be observed that only pH and soluble solids content showed means statistically different. However the difference was only observed between the fruit pulp and the homogenized juice. This difference in soluble solids content is explained by the dilution of the juice during the homogenization step (Silva et al. 1997).

Considering the pulp composition, the values reported in Table 1 are in agreement with the values reported in other published studies (Oliveira et al. 1999; Pinto et al. 2003; Mata et al. 2005). According to Filgueiras et al. (2001), although yellow mombin is an aromatic fruit, its sugar content is low and the fruit presents higher acidity when compared to other tropical fruits, such as passion fruit and pineapple. The results found for yellow mombin juice after homogenization and after pasteurization were lower than the ones reported by Silva et al. (1997) for the yellow mombin juice obtained by enzyme-mechanical extraction. No significant differences in the physicochemical parameters were found between the homogenized and pasteurized juice. Thus, the thermal treatment (pasteurization) did not affect the physicochemical characteristics of the product.

Enzyme activity

The results obtained for POD and PME activities are reported in Table 1. According to these results, the activity of both enzymes decreased during the processing of the juice reaching the lower value after the pasteurization step.

Significant differences were observed for all processing steps. After the homogenization the juice presented an enzyme activity loss of 48.19 % in PME activity and an enzyme activity loss of 88.87 % in POD activity. The pasteurized juice presented losses of 74.10 % in the PME activity and 97.89 % in the POD activity. Filgueiras et al. (2001) reported PME activity of 281.38 U/g for yellow mombin fruits in the ripening stage predominantly green and 305.22 U/g for ripe yellow fruits. Dias et al. (2003) reported mean values of 378.31 U/g for yellow mombin

Table 1 Chemical quality characteristics of yellow mombin samples from the industrial processing

Parameter	Sample means ± SD		
	Fruit pulp	Homogenized juice	Pasteurized juice
Soluble solids content (°Brix)	9.8±1.06[a]	8.0±0.01[b]	8.0±0.02[b]
Total acidity (g of citric acid /100 g)	1.1±0.13 [a]	1.0±0.04[a]	1.0±0.04 [a]
Reducing sugars (% glucose)	5.2±0.16[a]	5.6±0.23[a]	5.6±0.66[a]
Total sugars (%)	5.7±2.19[a]	5.8±0.19[a]	5.5±0.03[a]
pH	2.7±0.02[a]	2.9±0.04[b]	2.9±0.08[b]
Phenolics (mg/100 g)	112.4±7.30[a]	55.2±2.76[b]	54.6±2.35[b]
Anthocyanins (mg/100 g)	0.3±0.18[a]	0.2±0.04[a]	0.2±0.00[a]
Carotenoids (g/100 g)	1.8±0.74[a]	1.0±0.24[a]	1.4±0.04[a]
Vitamin C (mg/100 g)	13.7±0.16[a]	8.9±0.23[b]	9.8±0.66[b]
Pectinmethylesterase – PME (U/g)	299.0±112.08[a]	154.9 ±5.09[a,b]	77.5±7.64[b]
Peroxidase – POD (U/g)	528.4±0.58[a]	203.8±33.50[b]	11.1±1.90 [b]

Number of replicates for each sample from industrial processing: $n=3$

Values in the same line followed by the same letters are not statistically different according to the Duncan test at 90 % of confidence interval

pulp. Although the values reported in this work are in agreement to the ones reported by Filgueiras et al. (2001), the values were lower than the data reported by Dias et al. (2003). This difference might be attributed to the difference in the ripening stage of the fruits.

The POD is one of the most thermo-resistant enzymes. Thus, when this enzyme is thermally deactivated usually, all the other enzymes are also denatured and pathogen microorganisms are destroyed. According to Sotome et al. (2009), POD is the main responsible for the off-flavor formation during fruit products storage. According to the results presented in Table 1, the thermal treatment applied to the juice can be considered efficient to reduce the POD activity. However, in the present work, despite the high POD enzyme activity reduction, PME was not completely inactivated. The reduction of enzyme activity observed in the homogenization is due to the addition of SO_2 as preserving agent. According to Adams (1997) sulfur dioxide, in its various forms is added to food to decrease and control the growth of microorganisms, to inhibit enzyme-catalysed reactions, to reduce no enzymatic browning, and to act as an antioxidant and reducing agent. It is widely used to help preserve all manner of foods, including fruits and drinks derivates.

Despite the high efficiency of bisulfite its use is limited by food regulation agencies due to its toxicity (Carneiro et al. 2003). Despite the good efficiency of sulfites on POD activity inhibition, the amount allowed by the Brazilian regulation (200 mg SO_2/L) law was not enough to inactivate the enzyme in yellow mombin juice as showed in Table 1.

After pasteurization, a residual activity of 25.90 % and 2.29 % respectively for PME and POD were found in the juice. This result was unexpected since POD is reported as more thermo stable than PME and according to the results found, thermal inactivation of POD was more efficient than thermal inactivation of PME in yellow mombin fruit. This behavior may be attributed to some component of the fruit that might be able to stabilize the enzyme PME. Chagas et al. (2007) found higher enzyme stability for dextransucrase in cashew apple juice than in the synthetic medium used to produce the enzyme. According to the authors, cashew apple juice might contain some component that stabilizes the enzyme. A similar system might occur with PME in yellow mombin juice. According to Versteeg et al. (1980), PME is more thermal resistant in orange juice than POD, presenting the same behavior found in the present study. Assis et al. (2001) also reported different thermal resistance for PME in different fruits. According to the authors, PME found in acerola juice requires higher temperatures and processing times to be inactivated when compared to the enzyme found in citric fruits, such as orange. Rudra et al. (2008) indicated the presence of label and resistant forms of POD. Assis et al. (2001) also suggested the presence of different PME isoenzymes, with different thermal stability, in acerola fruits due to the non-linear thermal inactivation. Despite the knowledge of the existence of different PME iosoenzymes in orange juice, information about their structure and thermal resistance are scarce (Tribess and Tadini 2006).

Antioxidant compounds

According to the results presented in Table 1, significant differences were found between the fruit pulping and the homogenization step for total phenolics and ascorbic acid in yellow mombin juice. According to Lima et al. (2002), anthocyanins are pigments responsible for the red and purple color of fruits and other vegetables. Lima et al. (2002) reported anthocyanins values ranging from 14.11 to 16.23 mg/ 100 g of fruit in acerola fruits and Bobbio et al. (2000), reported anthocyanins values of 50 mg/ 100 g for açai. Acerola and açai are fruits with intense colors in the red

and blue spectra, which are characteristics of anthocyanins pigments. On the other hand, yellow mombin is a yellow fruit and low content of anthocyanins was expected for this fruit. Kuskoski et al. (2005) reported low anthocyanins content for fruits ranging from white to yellow such as graviola, pineapple, cupuaçu and passion fruit.

Phenolic compounds in fruits are important because they may be involved in the browning reactions during and after fruit processing, as well as, due to their influence on the fruit flavor and astringency (Filgueiras et al. 2000). Besides, phenolic and carotenoids are powerful antioxidants. The phenolic contents found in the present work are in agreement with data for yellow mombin pulp (110–120 mg/100 g of fruit) reported by Filgueiras et al. (2000), but the values were lower than found by Tiburski et al. (2011) and Vasco et al. (2008) for yellow mombin pulp (249 mg/100 g). However, the phenolic levels in yellow mombin are very low compared to the values reported for other tropical fruits such as acerola (580.1 mg/ 100 g of fruit) and mango (544.0 mg/ 100 g of fruit) as reported by Kuskoski et al. (2005). The ascorbic acid content of yellow mombin is also low when compared to other fruits and thus, the juice is not considered a good source of ascorbic acid (Pinto et al. 2003).

Because color is a decisive factor in consumers' preference for a given food, for a long time, the major concern, in relation to carotenoids in industrial processing, was the maintenance of color and measurement of total carotenoid content is usually sufficient. The actual emphasis on nutritive value of food, determining the levels of specific provitamin A carotenoids has became very important. Carotenoids are responsible for the pleasing yellow, orange or red color of many foods (Rodriguez-Amaya et al. 2008).

The importance of carotenoids in food goes beyond as natural pigments and biological functions and actions have increasingly been attributed to these pigments. These biological effects are independent of the pro-vitamin A activity and have been attributed to the antioxidant property of carotenoids, through deactivation of free radicals and singlet oxygen quenching (Sharma et al. 2012).

No significant change during fruit processing was found for carotenoids in yellow mombin fruit, meaning that this compound was not affected by processing. According to Hamano and Mercadante (2001) the composition of yellow mombin presents β-criptoxhantine as the major carotenoid, corresponding to approximately 64 % of the total carotenoids found in yellow mombin. According to the same authors, yellow mombin fruit also presents α-carotene, β-carotene e a criptoflavine as provitamin compounds.

Considering the provitamin activity of the carotenoids in yellow mombin fruit, the values found herein represents in terms of retinol equivalents (RE) 154.98 RE/100 mg in yellow mombin pulp and 112.98 RE/100 mg in the pasteurized juice. Hamano and Mercadante (2001) found a content of 88.7 RE/100 mg, conferring to the product a provitamin A characteristic.

According to Sagar and Suresh (2010) a more number of vitamins such as A, C and thiamine are heat sensitive and sensitive to oxidative degradation. However according to Rodriguez-Amaya et al. (2008) despite their susceptibility to decomposition, carotenoids may be retained during industrial processing if good technological practices are followed, mainly with regard to temperature and time of processing.

The antioxidant activity was determined only in the fruit pulp since there is an addition of sulfite in yellow mombin juice in the homogenization step, which may cause interference in the analysis results. Yellow mombin pulp presented an antioxidant activity of 2.45±0.07 μmol Trolox equivalents (TEAC). Kuskoski et al. (2005) reported values of 2.0±0,1 TEAC for cupuaçu pulp, 3.4±0.3 TEAC for pineapple pulp and 2.7±0.1 TEAC for passion fruit pulp. Thus the antioxidant activity of yellow mombin pulp is comparable to the values reported for other tropical fruits. However, the values found in this study were lower than those described by Tiburski et al. (2011) and Vasco et al. (2008) for yellow mombin pulp (17 μmol TEAC/g).

Conclusion

The thermal treatment (pasteurization) did not affect the physicochemical characteristics of the product and the final product was in accordance to the quality standard required by the Brazilian norms. The thermal treatment did not assure total inactivation of POD and PME in the final product. The processing of the fruit did not cause significant loss in antioxidant content of yellow mombin, except for phenolic and ascorbic acid content.

The antioxidant capacity of yellow mombin is probably due to its high content of carotenoids and phenolics.

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