Influence of refrigeration and cassava starch biofilm use on enzymatic browning in mangaba fruit (*Hancornia speciosa*)

Influência da refrigeração e do uso de biofilme de fécula de mandioca sobre escurecimento enzimático em frutos de mangaba

**Abstract**

The mangaba tree fruit has several nutritional properties and its market interest is growing, although its limited shelf life has made trade over long distances impossible. The present study aimed to quantify peroxidase (POD) and polyphenol oxidase (PPO) enzymes activity in the pulp of mangaba fruits coated by cassava starch biofilm. Fruits were harvested in a home orchard and were evaluated after 3, 6 and 9 days of storage. POD activity was higher than PPO activity in both temperatures and in all storage times tested for the fruits harvested in the home orchard. The temperature of 25 °C was the one that best inhibited the activity of both enzymes. Data showed no statistical difference regarding POD activity in the pulp related to the starch concentration used in the biofilm. As for PPO, the lowest activity was found in the pulp of fruit coated with the highest starch concentration tested (4%).

**Additional keywords:** *Hancornia speciosa* Gomes; native *Cerrado* plant; peroxidase; polyphenol oxidase.

**Introduction**

The mangaba tree (*Hancornia speciosa* Gomes), belonging to the Apocynaceae family, is a fruit species that is part of the diversity of woody communities of the *strictu sensu* cerrado, Southeastern Goiás state (Carvalho, 2011). Its occurrence, however, cover other biomes and regions, and it can be found in natural groupings that facilitate both extraction and conservation (Campos et al., 2011). Therefore, mangaba has great economic exploitation potential, although there are few studies on the crop (Franco et al., 2008).

Mangaba fruits are berries with different sizes, shapes and different colors. They are generally ellipsoidal or rounded, ranging from 2.5 to 6.0 cm in diameter, with yellowish or greenish exocarp, and red or null pigmentation. They also have yellow pulp, with quite smooth, sweet, fleshy-viscous, and acid (Ganga, et al., 2009; Venturini Filho, 2010) taste. In general, it has from 2 to 15 or even 30 discoid seeds, which have flat and light brown staining and 7 to 8 mm diameter (Lederman et al., 2000). They have good digestibility and nutritional value, with higher protein content than that of most fruits used commercially (Veire Neto, 2001). Although having good properties, the mangaba tree fruit is highly perishable, with reduced post-harvest shelf life (Campos et al., 2011). Enzyme biosynthesis, such as peroxidase (POD, EC 1.11.1.7) and polyphenol oxidase (PPO; EC 1.10.3.1), has been considered one of the main factors for quality loss and deterioration of many fruits and vegetables, through...
promotion of oxidative and biodegradation reactions (Lopes & Clemente, 2002). Phenolic compounds oxidation by these enzymes knowingly results in plant tissues browning (Whitehead & Swartd, 1982). Other changes, such as chlorophyll and auxins degradation, indole acetic acid oxidation and lignin biosynthesis are related to the activity of these enzymes. Many of these factors may also be associated with the "flavor", color, texture and nutritional quality of food (Pavinato et al., 2009, Clemente & Pastore, 1998). Peroxidase and polyphenol oxidase activity control is important in preserving and processing food (Galdino & Clemente, 2008).

The most common method to control enzymatic browning is the addition of chemical inhibitors, such as sulfite, in any of its forms (sulfur dioxide, sodium or potassium metabisulfite and sodium or potassium bisulfite) (Sapers, 1993). Cyclodextrins have also been effective to inhibit food enzymatic browning (Carneiro et al., 2006), but chemical preservatives, potentially harmful to humans and the environment, are being gradually replaced. Thus, alternative physical methods have been studied in various fruits and may change post-harvest treatments, besides having good reception from producers and consumers (Edagi et al., 2009).

Physical methods that stand out in perishable plant products preservation are temperature reduction and atmosphere modification by coating fruits and vegetables with protective films (Pineli et al., 2005). Refrigeration is the first step to be adopted, as low temperatures substantially reduce the product metabolism, inhibiting and/or reducing the action of pathogenic microorganisms (Villas Boas, 2002). There is also a growing interest in the development of edible films and coatings that could be applied to the surface of fruits and vegetables. These films, besides extending the product life span, also reduce the use of non-biodegradable disposable packaging (Tanada-Palmu et al., 2002, Chitarras & Chitara, 2005).

In this sense, the aim of the present study was to quantify peroxidase and polyphenol oxidase enzymes activity in mangaba native fruits. In addition, the effect of temperature and cassava protective film coating on the activity of these enzymes, in mangaba fruits harvested in a home orchard, was also assessed.

Material and methods

Plant Material Obtainment

Mangaba fruits were collected between December 2011 and April 2012 at Fazenda Gameleira, located in the municipality of Montes Claros, Goiás state (16°06′20″S and 51°17′11″W), at 592 m altitude, in a strictu sensu cerrado area, in order to quantify peroxidase (POD) and polyphenol oxidase (PPO) enzymatic activity in the pulp of these native fruits. Other fruits were obtained at Fazenda Jatobá, located in the region of the municipality of Caçu, GO (18°33′S e 51°08′W), which is a mangaba domestic cultivation area, in order to assess the effects of temperature and different cassava starch concentrations on the activity of POD and PPO enzymes activity.

Fruits were collected at an early stage of physiological maturity. Fruits were ellipsoidal or rounded, ranging from 3.0 to 5.0 cm in diameter, with greenish to yellowish color. Fruits were directly collected from the tree, with collectors, during the morning, and were immediately packed in an insulated Styrofoam box, in order to prevent fruit respiration rate increase during transport to the laboratory. In the laboratory, fruits were selected according to size and color. Subsequently, fruits were treated with commercial sodium hypochlorite at 2% for 15 minutes and were placed in aluminum racks, in order to drain sanitizing solution excess. Then, fruits were subjected to the following tests: (1) POD and PPO activity analysis in the pulp of native mangaba fruits; (2) POD and PPO activity analysis in mangaba fruit pulp stored at 10 °C and 25 °C; (3) POD and PPO activity analysis in mangaba fruit pulp coated with 1, 2, 3 and 4% of cassava starch. For this test, mangaba fruits were immersed in cassava starch solution at different concentrations, in order to reduce ripening impact on these fruits, which were stored at 25 °C. Evaluations were conducted after 3, 6, and 9 days. For the temperature test, fruits were packed in polystyrene trays covered with PVC film (15 µm thickness), which were subsequently stored in a polystyrene box. For this test, mangaba fruits were subjected to the following treatments: (1) POD and PPO activity analysis in mangaba fruit pulp stored at 10 °C and 25 °C; (2) POD and PPO activity analysis in mangaba fruit pulp coated with 1, 2, 3 and 4% of cassava starch.

Sample Collection and Preparation

2 grams of fruit pulp samples subjected to the different treatments were immediately frozen in liquid nitrogen and kept at -80 °C in an ultra-freezer (Terroni UFV 120 model, Brazil), until the enzymatic activity assessment was conducted. For mangaba fruits stored at 10 °C and 25 °C, samples were taken at each storage time, that is, 0, 3, 6, 9 days. For fruits coated with different cassava starch concentrations, samples occurred at the end of the experiment, after 9 days of storage.

The crude extract was obtained by frozen pulp homogenization in 10 mL of 0.05 M (pH 7.0) phosphate buffer containing 1 mg of polyvinylpyrrolidone. The homogenate was filtered and centrifuged at a temperature of 4 °C at 4,000g for 20 minutes. After this period, the supernatant was transferred to tubes kept at 4 °C, according to Campos & Silva (2003), and the precipitated material was discarded. Afterwards, the crude extract was used to determine POD and PPO enzymatic activity. In order to avoid changes in the activity of the enzymes analyzed, all glassware was left in a freezer at -18 °C for at least 4 hours, in addition to being kept in ice bath for the entire process.
Peroxidase Enzyme Assay

The enzyme assay was carried out according to Campos & Silveira (2003), in triplicate, and tubes were vortexed after each step. First, 2.5 mL of reaction buffer (phosphate-citrate buffer, containing dibasic sodium phosphate solution at 0.2 M and citric acid at 0.1 M, pH 5.0), 1.5 mL of crude extract and 0.25 mL of guaiacol 0.5% were put in each tube. Then, 0.25 mL of H2O2 at 3% was added to the mixture and tubes were incubated at 30 °C for 15 minutes. After incubation, 0.25 mL of sodium metabisulfite solution at 2% was added to the tubes, which were left for 10 minutes at 4 °C. For enzymatic activity quantification, absorbance readings were performed at 450 nm, using a spectrophotometer (Thermo Scientific UV 60S, Germany). As enzymatic reaction, water was used. Enzyme activity was expressed as enzyme unit (EU). An enzyme unit corresponds to the enzyme extract amount that registered an absorbance increase of 0.001 unit per minute.

PPO Enzymatic Assay

The enzymatic assay was performed in triplicate, and tubes were vortexed after each step, according to Khan & Robinson (1994). First, 3.6 mL of reaction buffer (0.05 M phosphate buffer, pH 6.0), 1 mL of the enzyme extract and 0.1 mL of 0.1 M catechol were added to the tubes. Then, tubes were incubated in a water bath at 30 °C for 30 minutes. Afterwards, 0.2 mL of 1.4% perchloric acid was added to the tubes, which were left for 10 minutes at 4 °C. In order to quantify enzymatic activity, absorbance readings were conducted at 395 nm, using a spectrophotometer (Thermo Scientific UV 60S, Germany). As enzymatic reaction control, the enzyme extract was replaced by water. Enzyme activity was expressed in enzymatic unit (EU), as used for peroxidase.

Experimental Design

Experiments were conducted in a completely randomized design, with five repetitions, in which each repetition corresponds to a tray with three fruits. In the test 1, evaluation between POD and PPO concentrations in native fruits was carried; for tests 2 and 3, a completely randomized design (CRD) consisting of two temperatures (10 °C and 25 °C) and four storage times (0, 1, 2, 3 and 4%) was used; for test 3, the mangaba fruit coating with cassava starch was tested in five concentrations (0, 1, 2, 3 and 4%).

POD and PPO enzymatic activity data in fruit pulp were subjected to analysis of variance by F test, and regression models were fitted when necessary. Means were compared by Tukey test at 5% probability, with the help of SISVAR software (Ferreira, 2003).

Results and discussions

POD and PPO enzymatic activity analysis in mangaba native fruit pulp:

The mean peroxidase and polyphenol oxidase activity found in the pulp of native fruits were of 2.57 EU min⁻¹ mg⁻¹ (± 0.26 mean standard error) and 2.37 EU min⁻¹ mg⁻¹ (± 0.089), respectively, which was not statistically different between each other. This result may be related to the nature of the fruits used in the test, i.e., fruits obtained in cerrado environment. Native plants in general are characterized by high diversity (Clement, 2001), which contributed to increase the repetition deviations compared to the mean. Thus, actual differences in the activity of these two enzymes in the fruit pulp of the species in question could not be accessed.

POD activity increase is commonly associated with cell wall compounds biosynthesis, in response to mechanical tissue damage, disease resistance and healing or injury repair mechanisms (Chitarra & Chitarra, 2005). Polyphenol oxidase activity is generally high in foods that had their skin peeled or were injured by any other procedure (Nguyen et al., 2003).

Mangaba fruits analyzed showed no wounds or cuts that could stimulate oxidative enzymes activity. Even so, its pulp showed high oxidative enzyme levels compared with those found in pequi fruits. In this species, PPO levels ranged from 0.042 to 0.078 EU min⁻¹ mg⁻¹ in fruits kept intact (Rodrigues et al., 2011). When pequi fruits were sliced, this PPO enzyme activity increased from 0.049 to 0.091 EU min⁻¹ mg⁻¹. POD activity was lower in whole pequi fruits (Rodrigues et al., 2011).

POD and PPO activity analysis in mangaba fruit pulp stored at 10 °C and 25 °C for 0, 3, 6 and 9 days:

POD and PPO enzymatic activities showed interaction between temperature and storage time in mangaba fruits. However, it was not observed in isolate for PPO enzyme storage time (Table 1).

POD activity means were always higher than PPO means, in both temperatures and at all storage times tested. Analyzing each enzyme in particular, data show higher POD activity after 9 days of storage at 10 °C, while the minimum point calculation shows that the lowest activity of this enzyme, at this temperature was achieved on the seventh day of storage. At 25 °C, the highest activity for that enzyme was observed in the 3-day storage period (Figure 1A). At 25 °C, POD activity in the fruit pulp was reduced after 9 days of storage. The same behavior was also evidenced by (Detoni et al., 2005), while assessing “Niagara Rosada” grapes. For this species, POD activity decreased at the end of the storage period for the three temperatures used (1, 14 and 24 °C).

For PPO, the third day of storage at 10 °C showed the highest rates of this enzyme in the fruit pulp (Figure 1B). For this PPO enzyme, minimum point calculation showed lower activity in the seventh day, as for POD. At 25 °C, there was no significant difference in relation to the storage time. That is there was not enzymatic biosynthesis in higher concentrations than those occurring at the initial time, in any of the periods. However, the minimum curve point was obtained in the fifth day.
**Table 1** - Analysis of variance for peroxidase (POD) and polyphenoloxidase (PPO) enzymes activity related to temperature (10 °C to 25 °C) and storage time (0, 3, 6 and 9 days) on *Hancornia speciosa* Gomes fruit pulp.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Degrees of freedom</th>
<th>POD</th>
<th>PPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperatures</td>
<td>1</td>
<td>8.46**</td>
<td>1.95**</td>
</tr>
<tr>
<td>Storage time</td>
<td>3</td>
<td>3.85**</td>
<td>0.11ns</td>
</tr>
<tr>
<td>Temperatures x Storage time</td>
<td>3</td>
<td>5.66**</td>
<td>0.31**</td>
</tr>
<tr>
<td>Residue</td>
<td>24</td>
<td>0.51</td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>19.45</td>
<td>24.07</td>
</tr>
</tbody>
</table>

**Significant effect (p <0.01) by F test; "ns" – not significant; CV (%): coefficient of variation.**

**Figure 1** - Peroxidase (A) and polyphenoloxidase (B) enzymes activity related to temperature (10 °C to 25 °C) and storage time (0, 3, 6 and 9 days) of *Hancornia speciosa* Gomes fruit pulp harvested in a domestic cultivation area.
By comparing the two temperatures, the 25 °C temperature best inhibited the activity of the two enzymes in question in mangaba fruit pulp (Figure 1). Data of this study indicates that mangaba fruits, when subjected to low temperatures, may have suffered injuries from cold. Physiological disorders are common in tropical and subtropical species stored at temperatures between 5 and 15 °C (Fernández-Trujillo et al., 1998). In tests conducted with batata-baroa (Arracacia xanthorrhiza) stored at 5 °C and 10 °C (Menolli et al., 2008), POD activity increase related to storage time was also found. Thé et al. (2001), studied the influence of storage at 7 °C and 10 °C in oxidative enzymes activity in pineapple, concluding that refrigeration intensified enzyme activity.

Another tissue response to injury by cold is browning (Menolli et al., 2008), which may explain the higher PPO levels found in mangaba fruit pulp samples stored at 10 °C, compared to those stored at 25 °C. Polyphenol oxidases promote phenolic compounds enzymatic oxidation, initially producing quinone, which is quickly condensed, forming insoluble and dark pigments called melanin. In addition, they may non-enzymatically react with amino acids, proteins or other compounds (Araujo, 1990).

**POD and PPO activity analysis in mangaba fruit pulp coated with different cassava starch concentrations:**

The cassava starch concentration significantly affected PPO enzymatic activity (Table 2).

POD enzymatic activity was kept above PPO activity, regardless of the cassava starch concentration used as mangaba fruit edible coating, as shown in Figure 2.

The lowest PPO activity was found in the pulp of fruit coated with the highest starch concentration tested, that is, 4%. These results agree with those found by Castricini et al., 2010, in "Golden" papaya fruit, in which cassava starch coating at 3 and 5% influenced maturation, reducing fresh matter and firmness loss, aiding in the maintenance of the green color during storage. In the study by Lima et al., 2010, with lychee (Litchi chinensis), cassava starch film reduced POD and PPO activity in the fruit skin. However, this technique was less efficient than the simple coating of fruits with PVC film, perforated or not.

**Table 2 - Analysis of variance for peroxidase (POD) and polyphenoloxidase (PPO) enzyme activity in relation to mangaba fruit coating with different cassava starch concentrations (0, 1, 2, 3 and 4%).**

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>Degrees of freedom</th>
<th>Mean Square</th>
<th>POD</th>
<th>PPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Starch</td>
<td>4</td>
<td>2.63&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.27*</td>
<td></td>
</tr>
<tr>
<td>Residue</td>
<td>10</td>
<td>1.47</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>24.40</td>
<td>32.90</td>
<td></td>
</tr>
</tbody>
</table>

* Significant effect (p <0.05) by F test; **ns** - not significant; CV (%): coefficient of variation.

**Figure 2** – Peroxidase (POD) and polyphenol oxidase (PPO) enzymes activity in mangaba fruits (Hancornia speciosa Gomes) coated with cassava starch (0, 1, 2, 3 and 4%).
Data shown in this study open perspectives for new studies related to mangaba tree fruits post-harvest conservation to be developed, since the market interest on these fruits is increasing. Therefore, their low lifetime limits the mangaba production chain, reducing it to regional markets (Mota et al., 2008).

Conclusions

POD enzymatic activity was always higher than PPO activity, affecting mangaba fruit quality on its shelf life reduction. The temperature of 10 °C is not recommended as inhibitor mechanism for POD and PPO in mangaba fruit pulp, and increased activity of these oxidative enzymes may indicate stress and injuries caused by low temperature. Mangaba fruit coating with cassava starch at 4% may be an alternative to reduce PPO activity.

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