

Bioactive compounds and enzymatic activity in minimally processed eggplant packed under active modified atmosphere

Compostos bioativos e atividade enzimática em berinjela minimamente processada embalada em atmosfera modificada ativa

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Abstract

The study aimed to assess bioactive compounds and polyphenoloxidase activity of minimally processed eggplants stored in different atmospheres. Eggplants (*Solanum melongena* L.; cv. Ciça) were minimally processed (MP), sanitized and treated with a 2% citric acid solution. They were packed in plastic containers (vacuum) of nylon + transparent polyethylene and submitted to modified atmospheres with the following concentrations of gases: control (atmospheric air), vacuum, 4% O₂ + 5% CO₂, 4% O₂ + 6% CO₂, 4% O₂ + 7% CO₂, and 4% O₂ + 8% CO₂, being stored in a cold chamber (5 ± 1 °C and 90 ± 1% relative humidity) for 10 days. The analyses consisted of the total phenolic compounds, total antioxidant activity, flavonoids, and polyphenoloxidase activity. The experimental design was a completely randomized design in a 6 × 6 factorial scheme (treatment vs. storage period). The data were submitted to analysis of variance and a regression analysis was performed for storage time. The active modified atmosphere with 8% CO₂ is effective in reducing the polyphenoloxidase activity in minimally processed eggplant. The contents of phenolic compounds and flavonoids of minimally processed eggplant decrease with storage.

Key words: Storage. Bioactive compounds. Polyphenoloxidase. *Solanum melongena* L.

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Resumo

Objetivou-se avaliar os conteúdos de compostos bioativos e atividade da polifenoloxidase durante o armazenamento de berinjelas minimamente processadas acondicionadas em diferentes atmosferas. As berinjelas (*Solanum melongena* L.) cv. Ciça foram minimamente processadas (MP), higienizadas e tratadas com solução a 2% ácido cítrico. Foram acondicionadas em embalagens plásticas (vácuo) de nylon + polietileno transparente, e em seguida submetidas à modificação da atmosfera com a aplicação das seguintes concentrações de gases: controle (ar atmosférico), vácuo, 4% O₂+ 5% CO₂, 4% O₂+ 6% CO₂, 4% O₂+ 7% CO₂ e 4% O₂+ 8% CO₂ e armazenadas em câmara fria (5±1°C e 90±1% de umidade relativa) por 10 dias. As análises realizadas foram: compostos fenólicos totais, atividade antioxidante total, flavonoides e atividade da polifenoloxidase. O delineamento experimental utilizado foi inteiramente casualizado (DIC), em esquema fatorial 6 x 6 (tratamento x período de armazenamento). Os dados foram submetidos à análise de variância e fez-se regressão para as análises no tempo de armazenamento. Atmosfera modificada ativa com 8%CO₂ é eficaz em reduzir a atividade da polifenoloxidase em berinjela minimamente processada. Os conteúdos de compostos fenólicos e flavonoides da berinjela minimamente processada diminuem com o armazenamento.

Palavras-chave: Armazenamento. Compostos bioativos. Polifenoloxidase. *Solanum melongena* L.

Introduction

Fruits and vegetables have a variety of bioactive compounds that provide many health benefits, including activation of immune defenses and reduction of inflammatory responses (LANDETE, 2012). Eggplant (*Solanum melongena* L.) is a fruit consumed worldwide and commonly grown in subtropical and tropical regions. It originated in India and was introduced to Brazil by the Portuguese in the 16th century (RIBEIRO et al., 2007). Small farmers have grown this crop in the majority of Brazilian region (FINCO et al., 2009).

Eggplant is considered a good source of minerals and vitamins, in addition to being rich in fiber and low in lipids. The interest in this vegetable has been growing rapidly because it is a good source of antioxidants, such as anthocyanins and phenolic acids, which are beneficial to human health (GAJEWSKI et al., 2009). Eggplant phenolic compounds have the potential to reduce intestinal glucose uptake and provide cell antioxidant protection, preventing the oxidation and complications of diabetes, and the flavonoids present in this vegetable have been attributed to a reduction of plasma lipids (SUDHEESH et al., 1999; CHEREM et al., 2007).

Despite the antioxidant benefits of phenolic compounds to human health, it is important to recognize that a high phenolic acid content has some disadvantages for the industry, such as an accelerated browning of fruits in the minimum processing (TAN et al., 2016). Mechanical stresses caused by processing increase the rate of biochemical reactions responsible for changes in color, taste, texture, and nutritional quality of minimally processed products (ROCHA et al., 2003). In addition, the exposure of minimally processed products to low O₂ levels and very high CO₂ levels can lead to anaerobic respiration and fermentation, with the production of undesirable metabolites and the occurrence of physiological disorders, such as enzymatic browning, decreasing product quality (GHIDELLI; PEREZ-GAGO, 2018). The enzymatic browning of eggplant fruit pulp is caused by the action of polyphenoloxidases (PPOs), which catalyze the conversion of phenolic acids (stored in vacuoles) into quinones, which react with oxygen to give rise to brown color compounds, causing a decrease in quality of the final product (KAUSHIK et al., 2017). Therefore, the O₂ and CO₂ range in the package, as well as the handling and/or processing characteristics, should be well defined to maintain quality and nutritional value in minimally processed eggplants.

Thus, the aim of this study was to assess the contents of bioactive compounds and enzymatic activity during storage of minimally processed eggplants stored in different atmospheres.

Material and Methods

Eggplants (cv. Ciça) were harvested at the commercial maturation stage from a farm located in Campinas, SP (Brazil). These fruits were transported to the Laboratory of Post-Harvest of Fruits and Vegetables, School of Agronomic Sciences, UNESP, in Botucatu (SP), where they were selected for physical and biological integrity. Afterwards, the fruits were washed in running water to remove dirt from harvest and transportation and immersed in a sodium hypochlorite solution (200 mgL^{-1}) for 20 minutes.

The fruits were cut into 1.5 cm thick slices and the inedible parts (piece of fruit next to the peduncle) were removed. The slices received 50 mg L^{-1} sodium hypochlorite solution for 20 minutes. The solution was discarded and the slices were washed in running water. After this process, the slices were immersed in 2% citric acid solution for 10 minutes to avoid their browning. The drainage was carried out in a domestic drainer and finished in a domestic centrifuge (Consul®) at a rotation of 2800 rpm for 40 seconds.

Samples of 150 g were conditioned in plastic containers (vacuum) of nylon + transparent polyethylene of high oxygen barrier ($10 \text{ cm}^3 \text{ day}^{-1}$) and water vapor ($5 \text{ m}^2 \text{ day}^{-1}$), and then submitted to modified atmosphere treatments with the following concentrations of gases: Treatment 1, atmospheric air; Treatment 2, vacuum; Treatment 3, 4% O_2 + 5% CO_2 ; Treatment 4, 4% O_2 + 6% CO_2 ; Treatment 5, 4% O_2 + 7% CO_2 ; and Treatment 6, 4% O_2 + 8% CO_2 . The packages were kept for 10 days in a cold chamber with a temperature of $5 \pm 1 \text{ }^\circ\text{C}$ and a relative humidity of $90 \pm 1\%$. Every 2 days, three replications from each treatment were analyzed for the total antioxidant activity, total

extractable phenolic compounds, flavonoids, and polyphenoloxidase activity.

A pre-test was performed in order to find the best extractor (ethyl alcohol and acetone) for the analysis of phenolic compounds of the minimally processed eggplant. The best extraction was observed in 80% ethyl alcohol, with the extract obtained from 1 g of eggplant pulp and 10 mL of the extractor (ethyl alcohol 80%). The mixture was homogenized in Turrax and then in an ultrasonic bath for 15 minutes, being centrifuged at 6000 rpm at $4 \text{ }^\circ\text{C}$ and for 20 minutes. The supernatant was removed with an automatic pipette and stored in an amber flask at $8 \text{ }^\circ\text{C}$ until the beginning of the biochemical analyses.

To determine the total extractable phenolic compounds, the spectrophotometric method described by Singleton et al. (1999) was used, in which 0.5 mL of extract was mixed in 2.5 mL of Folin-Ciocalteu solution (20%), standing for 5 minutes and then 2.0 mL of sodium carbonate (4%) was added. The reaction was conditioned at ambient temperature and in the dark for 120 minutes. The absorbance was measured at 740 nm by using gallic acid as a standard and the results were expressed as mg of gallic acid 100 g^{-1} of pulp.

The total antioxidant activity was assessed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as the methodology described by Mensor et al. (2001), in which 3 mL of ethyl alcohol (80%), 0.5 mL of ethylic extract, and 0.3 mL of DPPH solution (prepared in ethyl alcohol PA) was added to a test tube and the reaction was incubated in the dark for 45 minutes. The reading was performed at 517 nm and the results were expressed as the ability to scavenge the radical in%.

The polyphenoloxidase enzyme activity (EC: 1.10.3.1) was determined following the Kar and Mishra (1976) methodology with adaptations. The extract was made with 0.2 g of the sample and 5 mL of the sodium phosphate buffer pH 6.0 (after testing with different buffers and pH), being homogenized in a Turrax and centrifuged at 6000 rpm at $4 \text{ }^\circ\text{C}$ for

25 minutes. The supernatant was removed with an automatic pipette and placed in an amber glass. The reaction was performed with 0.3 mL of extract and 1.85 mL of 0.1M catechol solution (prepared with a buffer solution pH 6.0), being water bathed at 30 °C for 30 minutes and added 0.8 mL of perchloric acid at the end to stop the reaction. The reading was performed at 395 nm after absorption spectrum scanning and the results expressed in μmol catechol transformed into $\text{min}^{-1} \text{g}^{-1}$ fresh mass.

The quantification of flavonoid contents followed the recommendations of Santos and Blatt (1998) and Awad et al. (2000). For this, 4 mL of acidified methanol was added in a 0.2 g of eggplant sample, followed by an ultrasonic bath for 30 minutes. The sample was removed from the bath and 1mL of 5% aluminum chloride solution (w/v) in methanol was added. Subsequently, the sample was incubated for 30 minutes and centrifuged for 20 minutes at 6000 rpm. The supernatant was removed and read in a spectrophotometer at an absorbance of 425nm. The values were expressed as mg in quercetin equivalent 100g^{-1} of pulp.

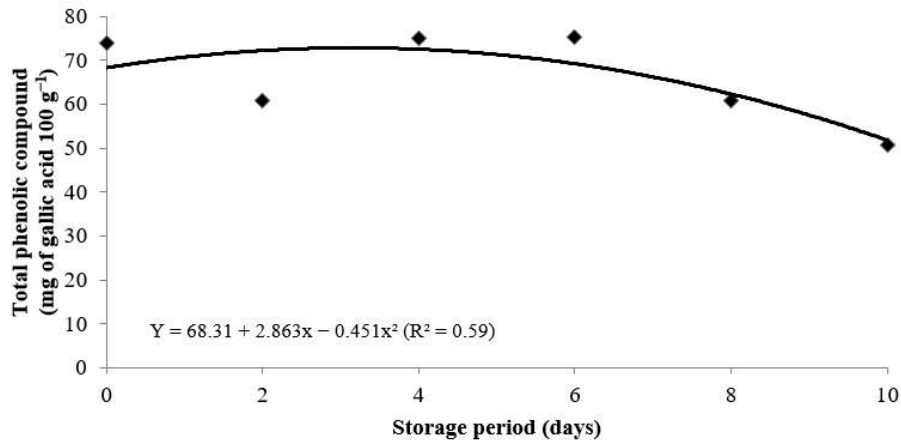
The experimental design was a completely randomized design in a 6×6 factorial scheme

(treatment \times storage period). The data were submitted to analysis of variance and the means compared by the Tukey's test at 5% significance level. The regression analysis was performed for analyzing the storage time.

Results and Discussion

The total phenolic compounds in eggplant extracts were assessed by the Folin method and the results are shown in Figure 1. The values of total phenolic compounds initially found in eggplants cv. *Çiça* were high (73.8 mg of gallic acid 100g^{-1}) when compared to several other eggplant cultivars (KAUR et al., 2014). These compounds are found in many fruits and vegetables and their identification reveals important information regarding food quality and potential health benefits (antioxidant and antitumor activity) (DU et al., 2009; WANG et al., 2011). In eggplants, this action mechanism of phenolic compounds plays an important role in reducing lipid oxidation in plant and animal tissues. When incorporated into human food, it not only preserves food quality (color, taste, and odor) but also reduces risks of developing pathologies such as atherosclerosis and cancer (NAYANATHARA et al., 2016).

Figure 1. Contents of total extractable phenolic compounds (mg of gallic acid 100g^{-1}) in minimally processed eggplant over a 10-day storage at $5 \pm 1 \text{ }^\circ\text{C}$ and $90 \pm 1\% \text{ RH}$. Botucatu, 2015.



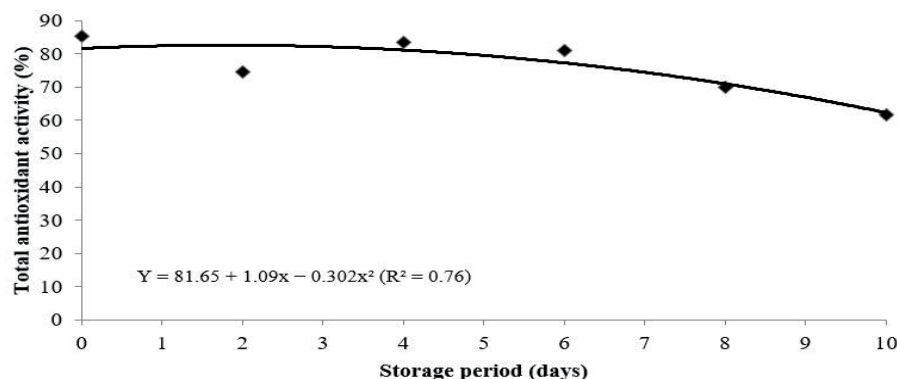
The content of phenolic compounds increased until the fourth day of storage (Figure 1), decreasing after this period and reaching the final value of 50.7 mg of gallic acid 100 g⁻¹. This behavior was similar in all gas concentrations, with no interaction effect between treatments and storage period. In minimally processed plants, injuries in the plant tissue caused by cutting the fruits and/or vegetables lead to an increase of respiratory rate and ethylene production, causing a stimulus to the formation of secondary metabolites, which are produced as a plant defense mechanism (ALARCÓN-FLORES et al., 2015; KLUGE et al., 2006), and may increase the functional food value. In addition, the biosynthesis of phenolic compounds may continue after harvest and increase during storage at low temperatures (CONCELLÓN et al., 2007; HOLCROFT et al., 1998) and modified atmosphere (SELCUK; ERKAN, 2015).

Concellón et al. (2007) described changes in the contents of phenolic compounds of eggplants stored at a low temperature (0 and 10 °C) and reported a decrease in their levels at the end of storage. According to Lee et al. (2003), a prolonged storage may promote chemical and enzymatic oxidation of phenolic compounds, contributing to their reduction. The increase of enzymatic activity catalyzes oxidation and hydroxylation reactions of some phenolic compounds (phenols and catechols)

into quinones and then in melanin, which is no longer characterized as phenolic compounds (ZIYAN; PEKYARDIMCI, 2003; SERRADELL et al., 2000). Such a loss of phenolic compounds may also be related to their function in acting against reactive oxygen species, which could be produced during storage.

The antioxidant activity of eggplant extracts at the end of storage decreased by about 30% (Figure 2), regardless of the used gas concentrations (atmospheric air, vacuum, 4% O₂ + 5% CO₂, 4% O₂ + 6% CO₂, 4% O₂ + 7% CO₂, and 4% O₂ + 8% CO₂). Ayhan and Esturk (2009) also reported an increase in the antioxidant activity of pomegranate under modified atmosphere (up to the 9th day of storage), followed by a reduction over time. This decrease in the antioxidant activity from the fourth day of storage probably occurred due to a decrease in the phenolic compounds, which are mainly responsible for the antioxidant properties of eggplants (YAMASAKI et al., 1997; SELCUK; ERKAN, 2015). The antioxidant capacity and the content of phenolic compounds have a high positive correlation in eggplant fruits. These compounds have a high reducing power and possibly play an important role as antioxidants and regulators of the redox state of cells. In vitro experiments show that phenolic compounds can eliminate free radicals present in cells (GÜRBÜZ et al., 2018).

Figure 2. Total antioxidant activity (%) measured by the DPPH method in minimally processed eggplant over a 10-day storage at 5 ± 1 °C and 90 ± 1% RH. Botucatu, 2015.



In this study, flavonoid contents of 1.4 to 3.04 mg in quercetin equivalent 100 g^{-1} were observed in minimally processed eggplants packed in different modified atmospheres. Xiang-Min et al. (2014) observed higher values (about 50 mg in quercetin equivalent 100 g^{-1}) in intact eggplant fruits. The authors identified four different flavonoids (quercetin, apigenin, kaempferol, and isorhamnetin) in leaves and fruits of eggplant. Fategbe et al. (2013) reported contents of flavonoids in eggplant pulp from 70 to 150 mg in quercetin equivalent 100 g^{-1} .

A decrease in flavonoid contents from 2.89 to 2.13 mg in quercetin equivalent 100 g^{-1} was observed during the storage period (Figure 3), probably due to an increase in the enzymatic activity during processing (cutting). According to Matheis (1983), the polyphenoloxidase (PPO) oxidizes phenolic compounds into quinones in the presence of oxygen, forming brown or black pigments. An increase in the enzymatic activity of PPO of minimally processed eggplants was observed up to the sixth day of storage at all CO_2 concentrations (Figure 4), except for 8%, reinforcing the hypothesis of oxidation of phenolic compounds by the enzyme.

Figure 3. Flavonoid contents (mg in quercetin equivalent 100g^{-1}) in minimally processed eggplant over a 10-day storage at $5 \pm 1\text{ }^\circ\text{C}$ and $90 \pm 1\%$ RH. Botucatu, 2015.

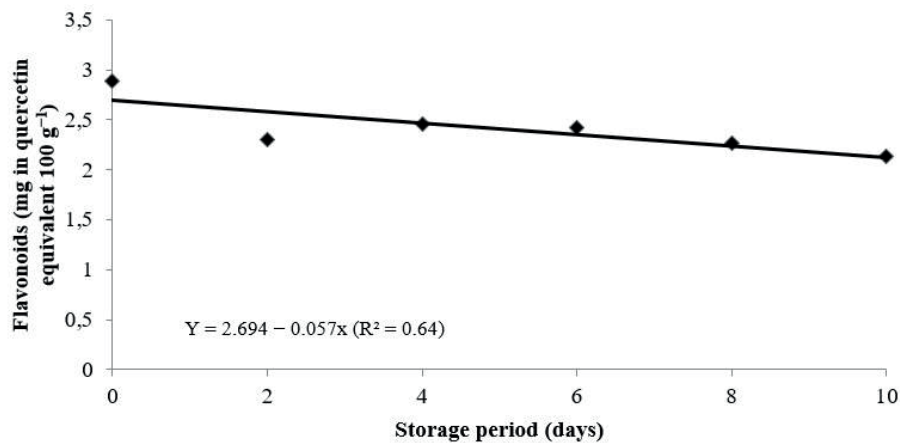
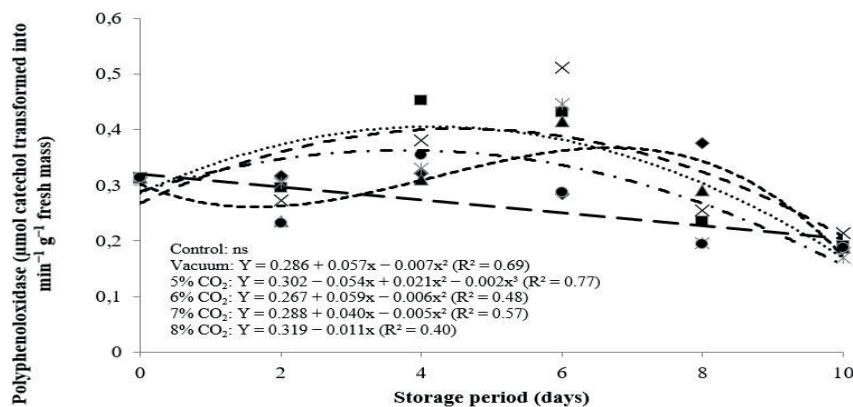


Figure 4. Polyphenoloxidase activity (μmol catechol transformed into $\text{min}^{-1}\text{ g}^{-1}$ fresh mass) in minimally processed eggplant in different modified atmospheres over a 10-day storage at $5 \pm 1\text{ }^\circ\text{C}$ and $90 \pm 1\%$ RH. Botucatu, 2015.



The activity of the enzyme polyphenoloxidase increased during the storage period between the fourth and sixth day (Figure 4), except in the control treatment and 8% CO₂, and after the sixth day, the activity presented a decrease. After eggplant cutting, there is an increase in the contact surface with oxygen and a tendency to increase the polyphenoloxidase (GASULL; BECERRA, 2006), resulting in the browning of the eggplants.

The change in the atmosphere with an increase in CO₂ concentration and a decrease in O₂ of minimally processed products can cause a decrease in the respiratory rate and ethylene production, with a consequent inhibition or reduction of the enzymatic activity (e.g. polyphenoloxidase), avoiding post-harvest deterioration by physiological disorders (MORAES et al., 2008; CHITARRA; CHITARRA, 2005). However, this behavior was not observed in our study with minimally processed eggplant because the control did not change over the storage period, but presented a descending linear behavior with 8% CO₂, reducing the polyphenoloxidase activity over the storage period (10 days) and showing to be effective in its control when compared to the other treatments.

The minimally processed eggplant packed in vacuum presented by the fourth day of storage the highest enzyme activity when compared to other active modified atmospheres, probably in response to the initial processing of the product. Snoeck et al. (2011) observed an increase in the enzymatic activity by the third day in vacuum-packed processed potatoes, suggesting that the cutting of the plant tissue may have induced the polyphenoloxidase synthesis occurring at the beginning of storage. The reduction of activity from the third day (vacuum) may have occurred due to a low oxygen concentration in the atmosphere. In minimally processed 'Ágata' potatoes, the vacuum was the most effective method to control browning by the enzymatic action when compared to other gas concentrations (PINELI et al., 2005).

Conclusions

The active modified atmosphere with 8% CO₂ is effective in reducing the polyphenoloxidase activity in minimally processed eggplant.

The contents of phenolic compounds and flavonoids of the minimally processed eggplant decrease with storage.

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