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Research Article

Effect of antioxidants and pH on browning and firmness of minimally processed eggplant

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Abstract

Minimal processing of eggplants (*Solanum melongena* L.) generates a rapid onset of enzymatic browning, tissue softening and water loss that limits their shelf life. The aim of this study was to evaluate the effect of 1% ascorbic acid and 2% calcium ascorbate in aqueous solution at natural pH of the antioxidant (pH 2.6 and 7.8, respectively), or adjusted to pH 5 with generally recognized as safe substances, to maintain the quality of minimally processed eggplant stored at 5 °C for 6 d. Water was used as a control. The colour, firmness, polyphenol oxidase activity, and visual quality was evaluated in the freshly cut fruit at 3 and 6 d of storage. No effect of the treatments on firmness or polyphenol oxidase activity was observed. At 3 d of storage, a correlation was observed between polyphenol oxidase activity and the visual evaluation of the cut product. Samples treated with 2% calcium ascorbate and the rest of the treatments at pH 5 had a lower browning index than those treated with 1% ascorbic acid and the control. At the end of the storage period, the visual quality of the eggplant samples treated with 1% ascorbic acid at pH 5 was evaluated above the marketing limit, whereas those treated with 1% ascorbic acid at pH 2.6 had the lowest quality indicators. An adjustment to pH 5 helps to preserve the luminosity and visual quality of the eggplant, however firmness was not affected by calcium ascorbate or the pH of the medium.

Keywords: fresh-cut eggplant; oxidative damage; ready-to-eat; reducing agents; texture

Abbreviations: AA: ascorbic acid; BI: browning index; CA: citric acid; CaAs: calcium ascorbate; GRAS: generally recognized as safe; LSD: Least Significant Difference; N: newton; PPO: polyphenol oxidase; PVP: polyvinylpyrrolidone; SP: sodium phosphate.

Introduction

The fresh-cut produce industry has received a boost in recent years due to the growing demand for healthy foods, which adds additional value for the consumer seeking ready-to-eat foods (Nicola *et al.*, 2009;

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Florkowski, 2014). However, the physical alteration in fresh-cut product can generate changes during storage in various quality attributes such as texture and colour, affecting the consumer's purchasing decision (Barrett *et al*., 2010). Minimal processing modifies the physiology of plant tissue that generates an increase in ethylene production and respiration rate, which accelerates the maturation and senescence process. On the other hand, cell breakdown favours microbial growth and the release of enzymes and substrates responsible for enzymatic browning and tissue softening (Florkowski, 2014).

Eggplant (*Solanum melongena* L.) is important for its richness in phytonutrients with antioxidant capacity, mainly phenolic acids such as chlorogenic acid, caffeic acid and *p*-coumaric acid (Boulekbache-Makhlouf *et al*., 2013; Chumyam *et al*., 2013; Jukanti and Bhatt, 2015). The release of phenolic compounds and polyphenol oxidase (PPO) enzymes during minimal processing induces enzymatic reactions in eggplant tissue that affect the quality. These enzymes catalyse the oxidation reactions of phenolic compounds in the presence of oxygen, first by the hydroxylation of monophenols to *o*-diphenols and their later oxidation to o-quinones, which are polymerized to brown water-soluble melanins that causes what is known as enzymatic browning (Barrett *et al*., 2010; Raghavendra *et al*., 2015).

There are different compounds that allow the control of the enzymatic browning of cut vegetables. These are classified according to their mode of action (i.e. acting on the enzyme, substrate or oxidation products) into the following groups: reducing agents, chelating agents, acidulants, enzyme inhibitors and complexing agents, although some chemical inhibitors can act simultaneously on several of the components of the oxidation process. Among them, calcium ascorbate (CaAs) and ascorbic acid (AA) are reducing agents frequently used to control the browning of minimally processed products. These agents act by reducing *o*-quinones to diphenols (Rodrigues *et al*., 2014). In addition, AA acts as an acidulant by reducing the pH of the medium below the optimal polyphenoloxidase activity. On the other hand, CaAs and other calcium salts are applied as texture enhancer to cut fruits and vegetables with the aim of reducing their loss of firmness (Ngamchuachit *et al*., 2014; Ayón-Reyna *et al*., 2015; Koushesh and Sogvar, 2016).

Although an important relationship has been established between browning speed and the content of phenols and PPO enzymes in several fruits, other factors responsible for browning have been also reported. Thus, for example, Gomes *et al*. (2012) observed greater browning in pears treated at pH 3 than at pH 7 with buffer solutions supplemented with CaAs. Other authors have suggested that an acidic pH generates oxidative stress in the plant tissue accelerating browning reactions, so they have recommended a pH adjustment equal to or above five (Gomes *et al*., 2012; Ghidelli *et al*., 2013; Ghidelli *et al*. 2014a; Gomes *et al*. 2014; Uscanga-Sosa *et al*., 2019). Therefore, the objective of the present study was to evaluate the effect of AA and CaAs in aqueous solution at natural pH or adjusted to pH 5 with generally recognized as safe (GRAS) substances on the quality (colour and firmness) and PPO activity of minimally processed eggplant during storage at 5 °C.

Materials and Methods

Reagents

Sodium phosphate dibasic dihydrate (SP), ascorbic acid (AA), calcium ascorbate (CaAs), 4-methylcatechol, polyvinylpyrrolidone (PVP) anhydrous citric acid (CA) and sodium chloride were used.

Antioxidant treatments

Antioxidant treatments included aqueous solutions of 1% AA and 2% CaAs with a natural pH of 2.6 and 7.8, respectively. Other treatments were obtained from the combination of these antioxidants between them or with other GRAS substances (SP and CA) to reach a pH of 5, resulting in the following combinations: 1% AA + 0.9% SP, 2% CaAs + 0.07% CA, 2% CaAs + 0.2% AA. Water without antioxidants was used as a control.

Biological material and processing

Eggplants were purchased in a local supplier in Valencia, Spain, and processed the same day. The fruits were washed and disinfected with NaClO at 150 ppm for 60 s and dried on absorbent paper at 10 °C. For processing, the ends of the eggplants were removed and cut into cubes with 2-cm sides. In order to ensure randomness and reduce the effect of biological variability among fruits, the eggplant cubes were placed in meshes (one per treatment), distributing material from each individual fruit among all treatments. The eggplant cubes were immersed in the aqueous solutions of the different antioxidant treatments for 2 min, drained and left to dry for 30 min. Finally, 20 cubes (2 cm each side) were packed in polypropylene trays (17.4 x 12.9 x 3.6 cm, 470 mL) with highly-permeable film (64 μm thick, P12-2050PXNP, Ilpra Systems), which was perforated to maintain atmospheric conditions and evaluate only the effect of the treatments. The whole process was carried out in a chamber at a temperature of 10 °C. The treated eggplants were stored at 5 °C for 3 and 6 d to determine of the following parameters: colour, visual quality, firmness and PPO activity. These parameters were also measured in freshly cut fruit. A total of three trays were prepared per treatment and day of analysis, corresponding to three replicates per treatment.

Colour determination

The colour of the eggplant was measure in 20 cubes per treatments with a colorimeter (Konica Minolta, CR-400, Osaka, Japan) equipped with illuminant C and 2° observer, and previously calibrated with a standard white calibration plate. The colour parameters evaluated were L^*, a^*, b^*, *chroma* and *hue* angle in the CIE $L^*a^*b^*$ system. The browning index (BI) was calculated using the following equation (Pilon *et al*., 2013):

$$BI = 100(x - 0.31)/0.172$$

where

$$x = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*)$$

Determination of PPO activity

PPO activity was determined according to the method proposed by Sanchís *et al*. (2015), with slight modifications. Enzyme extraction was performed in three replicates per treatment. For this, 10 g of eggplant were homogenized with 30 mL of Mcllvaine buffer (phosphate-citrate buffer at pH 6.5), to which 1 M NaCl and 5% PVP were added. The homogenate was filtered (41 μm) under vacuum and centrifuged 15 min at 9,961.38 g and 4 °C, and the supernatant was recovered and placed in an ice bath prior to the determination of enzymatic activity. PPO activity was measured using a spectrophotometer (UV1 model, Thermo Electron Corporation, Rugby, UK). For this, 100 μL of the extract and 2.9 mL of 0.05 M 4-methylcatechol, as substrate, were mixed. Finally, the absorbance was measured at 420 nm at 5 s intervals for 2 min at 20 °C, making two measurements per replicate. The substrate at the same concentration was used as a blank. A PPO unit was defined as the change of 0.001 Abs min^{-1} (Zambrano-Zaragoza *et al*., 2014).

Firmness determination

Firmness was determined with an Instron Universal Machine texturometer, measuring the force required to penetrate the eggplant a distance of 5 mm with a 7-mm-diameter probe at a speed of 2 mm s^{-1}. Firmness was measured on two sides of each cube in a total of 15 pieces (cubes) per treatment and expressed in newtons (N).

Visual quality

The visual quality of minimally processed eggplants was assessed according to the level of browning and dehydration (visual aspect) by a panel of ten judges, composed of men and women between 25 and 45 years old, who were trained in five, 30-min sessions. A five point scale was used, where 1 was poor, below the limit for consumption: 3 was adequate, at the limit of consumption; 5 was good, limit of commercialization; 7 was very

good, quite fresh; 9 was excellent, freshly cut (Barbagallo *et al*., 2012; Ghidelli *et al*., 2014a; Ghidelli *et al*., 2014b). During training, cut eggplant corresponding to the different levels of the scale, as reference, were presented to the judges. For the evaluation, the judges also had a photograph of different samples of fresh-cut eggplant that exemplifies each level of the scale. Three trays per treatment were used; they were opened and randomly presented for immediate evaluation. Each treatment was numbered with three digits and illuminated with even white light to avoid variability.

Statistical analysis

The quality parameters evaluated in this research were analysed using the Statgraphics Centurion XVI statistical package. Comparison of means was performed using Fisher's Least Significant Difference (LSD) test at a significance level of α=0.05.

$$y_{ij}=\mu+\tau_i+\varepsilon_{ij}$$

where y_{ij} is the observation j in the treatment i, μ is the overall mean, τ_i is the fixed effect due to the treatment, and ε_{ij} is the experimental error with mean 0 and variance σ^2 ($\varepsilon_{ij}\sim N(0,\sigma^2)$).

Results

Effect of antioxidant treatments with different pH on the colour of fresh-cut eggplant

Figure 1 shows changes in the colour parameters L^*, a^*, b^* and *hue* during storage at 5 °C of the fresh-cut eggplants. Tissue browning was accompanied by an increase in a^* and b^* and a decrease in L^* and *hue* during storage. The decrease in L^* was significant in all treatments, with lower values in the control and the samples treated with 1% AA than in the rest of the treatments on the third day of storage. However, no significant differences were observed among treatments in the L^* values after 6 d. At 3 d of storage, no significant differences were observed in a^* and b^* values among treatments; while at 6 d, all the treatments with CaAs had lower values of a^*, without significant differences among them. These treatments with CaAs, together with 1% AA + 0.9% SP, also showed lower values of b^* than 1% AA and the control. The *hue* angle decreased with storage and significant differences were observed among treatments, with 2% CaAs and 2% CaAs + 0.2% AA being the treatments with the highest *hue* and 1% AA the treatment with the lowest *hue* value.

One way to represent the enzymatic browning of some minimally processed fruits and vegetables, such as eggplants and apples, is to calculate the BI, since the equation includes the parameters L^*, a^* and b^*. As shown in Figure 2, the BI of fresh-cut eggplants was not significantly different among treatments at 3 d of storage. However, at the end of the storage period, all the treatments with a pH 5 and 2% CaAs had the lowest BI, with no significant differences between them.

Effect of antioxidant treatments with different pH on the firmness of fresh-cut eggplant

Contrary to expectations, this work showed greater firmness in fresh-cut eggplants at 6 d than at 3 d of storage at 5 °C (Table 1). On the other hand, although at 3 d of storage the control maintained greater firmness than the rest of the treatments, on the sixth day there were no significant differences among treatments.

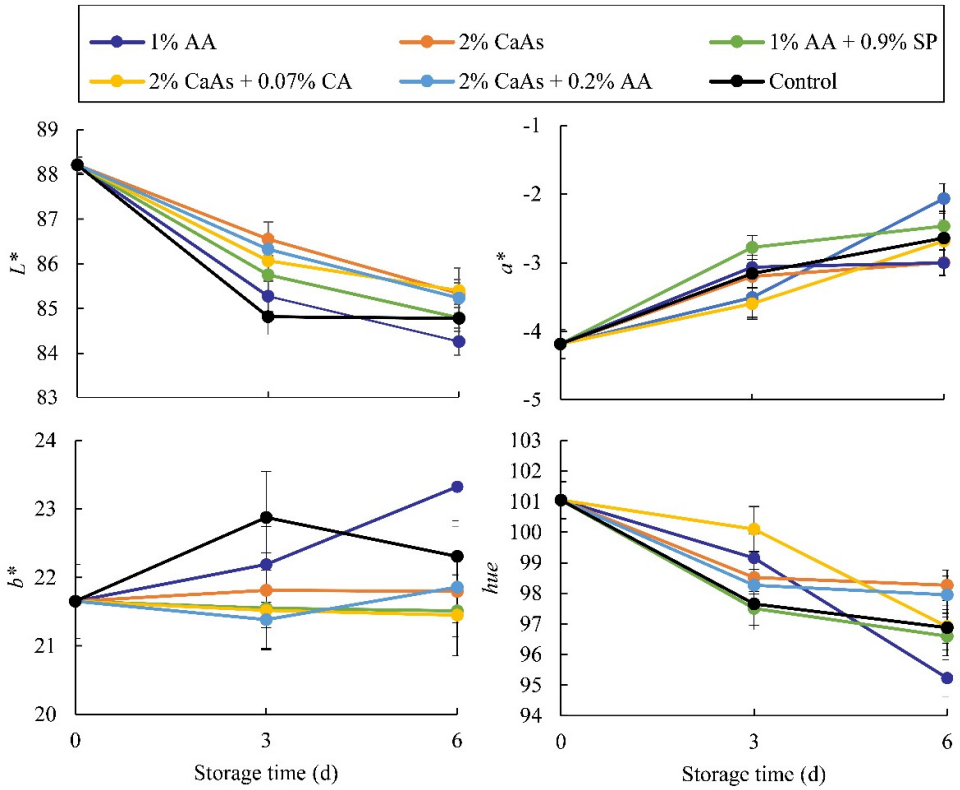

Figure 1. Effect of 1% ascorbic acid (AA) or 2% calcium ascorbate (CaAs) at natural pH or adjusted to pH 5 with sodium phosphate (SP), citric acid (CA) or AA on the colour (*L**, *a**, *b** and *hue)* of fresh-cut eggplant stored for 3 and 6 d at 5 °C. Vertical bars represent standard errors

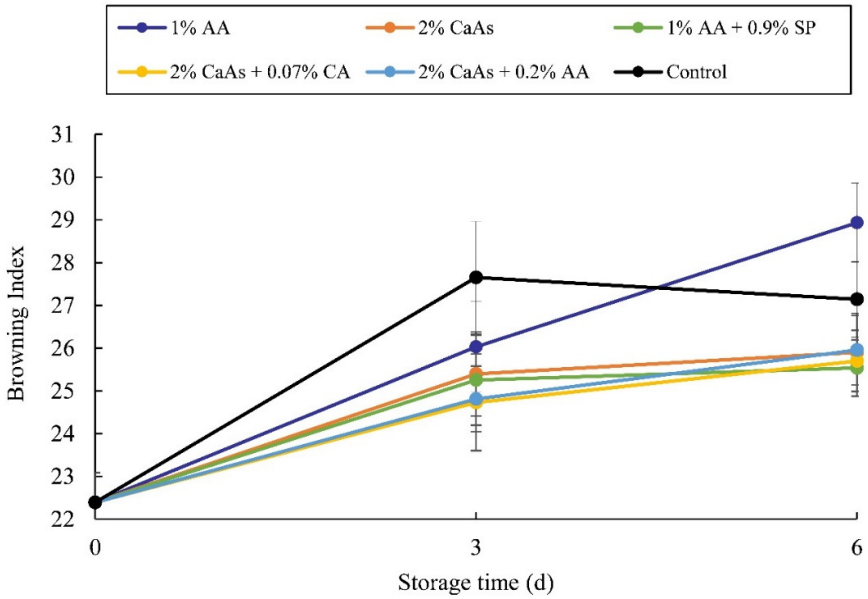

Figure 2. Effect of 1% ascorbic acid (AA) or 2% calcium ascorbate (CaAs) at natural pH or adjusted to pH 5 with sodium phosphate (SP), citric acid (CA) or AA on the browning index (BI) of fresh-cut eggplant stored for 3 and 6 d at 5 °C. Vertical bars represent standard errors

Table 1. Firmness (N) and PPO activity (ΔA_{420} min^{-1} mL^{-1}) of fresh-cut eggplant, treated with 1% ascorbic acid (AA) or 2% calcium ascorbate (CaAs) at natural pH or adjusted to pH 5 with sodium phosphate (SP), citric acid (CA) or AA, during storage at 5 °C

Antioxidant	pH	Firmness (N)		PPO activity (ΔA_{420} min^{-1} mL^{-1})	
		Storage time		Storage time	
		3 d	6 d	3 d	6 d
1% AA	2.6	13.26 ± 0.61 a †	16.23 ± 0.83 a †	0.090 ± 0.011 d ††	0.070 ± 0.013 a ††
2% CaAs	7.8	14.30 ± 0.60 ab†	17.00 ± 0.90 a †	0.082 ± 0.006 bc††	0.071 ± 0.012 a ††
1% AA + 0.9% SP	5	14.63 ± 0.76 ab†	15.98 ± 0.68 a †	0.076 ± 0.004 ab††	0.072 ± 0.006 a ††
2% CaAs + 0.07% CA	5	12.79 ± 0.61 a †	14.79 ± 0.79 a †	0.085 ± 0.003 cd††	0.077 ± 0.003 a ††
2% CaAs + 0.2% AA	5	13.15 ± 0.61 a †	16.74 ± 0.93 a †	0.070 ± 0.007 a ††	0.074 ± 0.007 a ††
Control	7	15.52 ± 0.73 b †	15.52 ± 0.67 a †	0.086 ± 0.003 cd††	0.082 ± 0.005 a ††

†Mean ± standard error. ††Mean ± standard deviation. Control: water.

For each storage time, different letters indicate significant differences between treatments ($p \leq 0.05$).

Firmness at the time of cutting: 15.89 ± 0.86 N.

PPO activity at the time of cutting: 0.098 ± 0.003 min^{-1} mL^{-1}.

Effect of antioxidant treatments with different pH on the visual quality of fresh-cut eggplant

As shown in Figure 3, the visual quality of the eggplant in the control samples was evaluated below the limit of commercialization at 3 and 6 d of storage, whereas the best evaluated treatments at 3 d were those with pH 5, being 2% CaAs + 0.07% CA the best evaluated. On day six, the only treatment that maintained the visual quality of fresh-cut eggplants above the limit of commercialization was 1% AA + 0.9% SP (pH 5), whereas the treatment with the lowest score was 1% AA, which corresponds to the treatment with an acidic pH (pH 2.6).

Effect of antioxidant treatments with different pH on the PPO activity in fresh-cut eggplant

The PPO activity of fresh-cut eggplants stored at 5 °C was significantly higher at 3 d of storage in most of the treatments, with greater enzymatic activity in samples treated with 1% AA and the control untreated (Table 1). At the end of the evaluated storage period, no significant differences in PPO activity among treatments were observed.

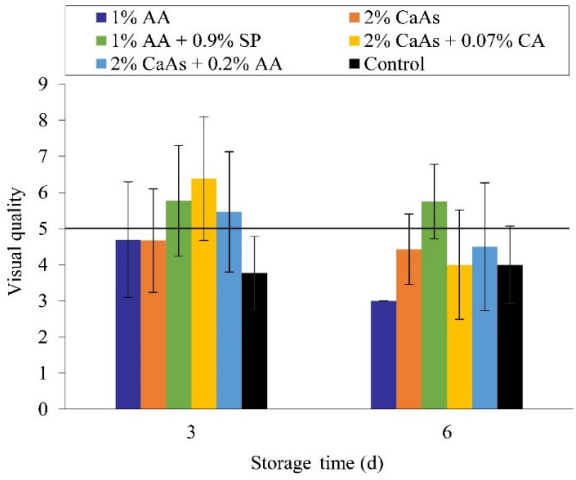

Figure 3. Visual quality of fresh-cut eggplant treated with 1% ascorbic acid (AA) or 2% calcium ascorbate (CaAs) at natural pH or adjusted to pH 5 with sodium phosphate (SP), citric acid (CA) or AA, during storage at 5 °C. The dividing line indicates the limit of marketability (five points on the scale, from 1 = very bad to 9 = excellent, freshly cut). Vertical bars represent standard deviations

Discussion

Effect of antioxidant treatments with different pH on the colour of fresh-cut eggplant

The application of 1% AA resulted in a higher browning of the eggplant cubes than the rest of the antioxidant treatments, suggesting a possible oxidative damage caused by the low pH. In fact, the treatment with 1% AA + 0.9% SP did not induce browning of the cut tissue as the pH of the solution was brought to pH 5. This result confirms what was previously observed by our group, suggesting that the application of AA as an antioxidant shows better antioxidant activity when the pH is adjusted to values close to 7 (Uscanga-Sosa *et al*., 2019). In the present work, a pH value around 5 also seems appropriated to reduce browning of AA treated eggplant tissue. This can be achieved by combining the AA with other GRAS ingredients that would contribute to adjust the pH to higher values or by reducing the concentration of AA. In this sense, Ghidelli *et al*. (2014a) reported that AA concentrations below 0.8% reduced browning in fresh eggplant as compared with the control, while higher concentrations induced tissue browning, likely due to oxidative damage caused by a pH of the antioxidant solution below 3. The oxidative stress at low pH has also been reported in other tissues such as pear (Gomes *et al*., 2012) and artichokes (Ghidelli *et al*., 2013).

Effect of antioxidant treatments with different pH on the firmness of fresh-cut eggplant

Calcium plays an important role in the firmness of plant tissue as it forms bonds with the carboxyl groups of pectin homogalacturonans, thereby increasing cell wall stiffness (Hussain *et al*., 2012; Salazar Iribe and Gamboa de Buen, 2013). Generally, retention of the firmness of fresh-cut vegetables through the application of calcium agents has been more effective when this has been combined with other technologies. In this sense, calcium agents have been proven with heat treatments in papaya, melon and eggplant (Silveira *et al*., 2011; Barbagallo *et al*., 2012; Ayón-Reyna *et al*., 2015); chitosan coatings in melon and papaya (Eryani-Raqeeb *et al*., 2009; Chong *et al*., 2015); and other edible coatings in apple (Freitas *et al*., 2013; Koushesh and Sogvar, 2016); among others.

On the other hand, the loss of firmness of minimally processed horticultural products such as tomatoes, persimmon or pears can be enhanced by acidic pH (Gomes *et al*., 2010; Schouten *et al*., 2010; Sanchís *et al*., 2015). However, Gomes *et al*. (2012) observed that the pH of a calcium ascorbate solution did not affect the softening kinetics of cut pear, unlike previous observations showing that acid solutions enhanced the softening of freshly cut pears (Gomes *et al*., 2010).

The present study showed contradictory results in tissue firmness, with a slight increase with storage time and no effect of AsCa or low pH values. This result may be due to the high heterogeneity of eggplant tissue. Eggplants are characterized by having the central part of the fruit, where the seeds are, more porous and rather soft than the area near the epicarp (Santacatalina, 2011; Uscanga-Sosa *et al*., 2019). Considering that each treatment contained cubes of different parts of the fruit to maintain randomness, this might have contributed to reduce the possibility of detecting significant differences among treatments.

Effect of antioxidant treatments with different pH on the visual quality of fresh-cut eggplant

Visual quality was determined by a panel of judges who assessed the degree of browning and the overall appearance, including dehydration, of the tissue and other visual changes. After 3 d of storage, only the samples treated with the antioxidants at pH 5 were evaluated above the marketability threshold. At day 6, the results confirmed the negative effect of 1% AA at low pH in colour, while 1% AA + 0.9% SP at pH 5 was the only treatment above the limit of marketability.

Effect of antioxidant treatments with different pH on the PPO activity in fresh-cut eggplant

Among the enzymes that can have deleterious effects in fresh-cut products, PPO can be considered the most important since it negatively affects the colour of cut tissues. The PPO enzymes are found in chloroplasts, mitochondria and peroxisomes, whereas phenolic compounds are found in plant cell vacuoles. The cutting process of fruits and vegetables results in the release of the PPO enzymes and phenolic substrates, allowing contact between them, which results in the oxidation of phenolic compounds in the presence of oxygen with the formation of dark pigments (Barbagallo *et al*., 2009; Scuderi *et at.*, 2011; Shetty *et al*., 2011). In addition, some works have reported an increase in the biosynthesis of phenolic compounds by the action of the enzyme phenylalanine ammonia lyase in response to the lesion produced by peeling and cutting of the tissues, which increases browning even with moderate PPO activity (Lichanporn *et al*., 2009). On the other hand, in the case of eggplants, Concellón *et al.* (2004) reported a continuous increase in PPO activity and the release of phenols during 12 d of storage of whole fresh fruits at 10 °C. This was attributed to the loss of cell integrity at that temperature, indicating a possible increase in the susceptibility of this tissue to browning when processed.

It is well known that CaAs and AA are effective in controlling browning by reducing *o*-quinones to diphenols (Remorini *et al*., 2015; Koushesh and Sogvar, 2016; Derardja *et al*., 2017). Furthermore, AA may also contribute to inhibiting PPO activity by reducing the pH below the optimal level, that in the case of eggplants is between 4.8 and 7 (Todaro *et al.*, 2011; Rodrigues *et al*., 2014).

In the present study, the highest PPO activity was reported at 3 days of storage in samples treated with 1% AA at pH 2.6, whereas the lowest was found in samples treated with 2% CaAs + 0.2% AA at 3 days of storage and 1% AA + 0.9% SP at pH 5 at 6 days of storage. These results suggest that in eggplants, low pH values, even below the optimum activity of the PPO activity, induce oxidative stress in the tissue and accelerate browning reactions. Similarly, Gomes *et al*. (2014) reported discrepancies among enzymatic browning, pH and PPO activity in pear slices. These authors concluded that the pH of the antioxidant solution was the main factor affecting browning of fresh-cut pear, recommending pH values equal to or greater than 6 to control the oxidative stress observed at acid pH values. On the other hand, at the end of the evaluated storage period no significant differences in PPO activity were observed among treatments, which could indicate that eggplant browning is not only influenced by PPO activity.

In the present study, differences in PPO activity among treatments were only observed after 3 days of storage, probably because reducing agents were consumed in the oxidation reaction providing only a temporary effect (Rodrigues *et al*., 2014). The control samples also had high PPO activity, which correlated with the visual evaluation that resulted in lower values for these treatments.

Conclusions

The results of this study confirm the effect of pH on the browning of fresh-cut eggplant treated with AA or CaAs as antioxidants during storage at 5 °C. Despite the antioxidant effect of AA, the acidic pH of the 1% aqueous solution generated oxidative stress in the plant tissue accelerating browning reactions. An adjustment to pH 5 helps to eliminate the negative effect of acidic pH by maintaining the visual quality of the fresh-cut eggplant above the limit of marketability for up to 6 d of storage at 5 °C. Similarly, combination of CaAs with another antioxidant to achieve pH 5 helps preserve the luminosity and visual quality of the fresh-cut eggplants. Although a relationship is observed between browning and PPO activity in minimally processed eggplant at 3 d, this relationship is lost with storage time. Firmness was not affected by the application of CaAs or the pH of the medium. Further studies will be carried out to improve the quality and extend the shelf life of minimally processed eggplants, including the combination of antioxidant agents adjusted at an effective pH value with other technologies such as edible coatings.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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