International Journal of
Food Science & Technology
www.ifst.org

Original article

Application of edible coatings in minimally processed sweet potatoes (*Ipomoea batatas* L.) to prevent enzymatic browning

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(Received 10 June 2013; Accepted in revised form 8 September 2013)

Summary To control browning phenomenon, an edible coating of cassava starch along with ascorbic acid (AA) (combined treatment) was applied to minimally processed sweet potatoes var cv. 'Blanca Correntina'. The samples were stored at 4 °C for 16 days, and the activities of polyphenol oxidase, peroxidase and phenylalanine ammonia lyase were studied; also, surface colour, antioxidant capacity, ascorbic acid, chlorogenic acid content were evaluated. The combined treatment showed a beneficial impact on the quality of the product, avoiding browning when compared with untreated samples and individual treatments (coating without AA or immersion in AA solution). The improvements were evidenced by the absence of changes in hue, minor changes in a* parameter and a major inhibitory effect of AA on the activity of the three enzymes. Ascorbic acid content and antioxidant capacity increased ($P < 0.05$). The combined treatment not only was effective in preventing enzymatic browning but also retained freshness and improved nutritional value.

Keywords Edible coating, enzymatic browning, minimally processed vegetables, sweet potatoes.

Introduction

Marketing of minimally processed fruits and vegetables has increased rapidly due to an increased demand for fresh and convenient foods with health benefits. Minimal processing basically consists of washing, peeling and cutting of fruits and vegetables with subsequent application of a treatment to prolong shelf-life (Allende *et al*., 2006). However, as a result of these operations, intracellular products such as enzymes might be released, which may have a negative impact on the quality and shelf-life of fruits and vegetables (Chiumarelli *et al*., 2011).

One of the strategies to minimise the undesirable effects of minimal processing is the application of edible coatings, providing an additional protection for fresh-cut vegetables, being complementary to modified atmosphere packaging (MAP). This combined strategy can reduce water losses and gas exchanges rates and act as a carrier for additives that could control undesirable reactions, microbial growth, etc. (Baldwin *et al*., 1995). Polysaccharide-based coatings applied to fruits and vegetables reduce respiratory rate and gas exchange due to their selective permeability to O_2 and CO_2, related to ordered hydrogen-bonded network structure, but they are a poor barrier to water vapour due to their hydrophilic nature (Nísperos-Carriedo *et al*., 1991). Cassava starch used as polysaccharide source has the advantages of low cost and good barrier properties (Famá *et al*., 2007) and has been applied in minimally processed mangoes (Chiumarelli *et al*., 2011) and pineapple (Bierhals *et al*., 2011). To enhance the properties of edible coatings, various products can be added, including antioxidant agents such as ascorbic acid (AA) and L-cysteine, and plasticizers such as glycerol and calcium salts to improve texture (Son *et al*., 2001; Tortoe *et al*., 2007; García *et al*., 1998).

Fresh-cut products derived from sweet potato, which is a highly nutritious vegetable because of its polysaccharide, fibre and antioxidant content, are marketed in a very limited scale till now.

During minimal processing, sweet potatoes suffer browning, a particular problem in vegetables of white or yellow flesh, suberisation and dehydration that negatively affect the quality of the product. The common

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doi:10.1111/ijfs.12381

method for controlling food browning is the surface treatment involving dipping of fresh-cut vegetables in aqueous solution containing antioxidants, such as sulphites, AA and citric acid (Sgroppo *et al*., 2010). McConnell *et al*. (2005) found no changes in surface colour of minimally processed sweet potatoes packed in MAP, and Waimaleongora *et al*. (2008) reported that chitosan-based edible coatings were effective in minimising water losses. Then, the addition of AA to edible coatings could be an attractive strategy because of the role of AA in inhibiting enzymatic browning (De Leonardis *et al*., 2010).

The inhibitory effect of AA on one of the key enzymes of enzymatic browning, polyphenol oxidase (PPO, EC 1.14.18.1), has been studied in partially purified enzymes from mangoes and peaches (Guerrero-Beltrán *et al*., 2005). Another enzyme related to this phenomenon is peroxidase (POD, EC 1.11.1.7), which can also oxidise phenols to quinones and promote lignin synthesis; however, its role in browning is not yet clear. Santos de Araujo *et al*. (2004) found that sweet potatoes present high activity of POD compared with other roots, and Aquino-Bolaños & Mercado-Silva (2004) reported the important role that POD could play in enzymatic browning. However, some studies on potatoes showed that browning was not rate-limited by either the enzymes associated with browning or polyphenol substrate concentration (Cantos *et al*., 2002), although Walter & Purcell (1980) suggested that there was a critical relationship between them in sweet potatoes.

The major polyphenols reported in sweet potatoes are chlorogenic acid and its isomers 4, 5- dicaffeoylquinic acid, 3, 5- dicaffeoylquinic acid and 3, 4 dicaffeoylquinic acid, and caffeic acid (Padda & Picha, 2008a; Zheng & Clifford, 2008). The synthesis of these polyphenols can be increased due to the higher activity of the enzyme phenylalanine ammonia lyase (PAL, EC 4.3.1.5) (Gerasimova *et al*., 2005) whose synthesis may be increased in response to tissue injury generating *trans* cinnamic acid, which is a precursor for chlorogenic acid.

The main objective of this work was to analyse the effect of a cassava starch coating along with ascorbic acid (AA) on the surface colour of fresh-cut sweet potatoes, during refrigerated storage, in comparison with a coating without the addition of AA. The specific objectives include the analysis of the relationships between colour measurements and activities of the enzymes related to enzymatic browning (polyphenol oxidase and peroxidase) and several chemical and biochemical determinations in the sweet potato tissue (ascorbic acid content, antioxidant capacity, concentration of chlorogenic acid and activity of phenylalanine ammonia lyase as precursor of chlorogenic acid). This analysis is considered important to determine the influence of the different factors on the performance of the coating method.

Materials and methods

Plant material

The tests were performed using sweet potatoes (*Ipomoea batatas* Lam) from the variety 'Blanca Correntina', with white flesh, brown skin and elongated shape, cultivated in Corrientes (Argentina) 27° 8′ 16″ S, 58° 50′ 25″ W. Twenty kilograms for each sample was purchased from the local market during April and May 2011. The roots were selected by weight (average weight = 151.88 ± 45.93 g), washed, sanitised (150 ppm sodium hypochlorite/5 min) and immersed in an ice bath at 4 °C per 30 min, hand-peeled and diced with sharp knives into pieces of 2 cm. These samples were considered as control (without treatments). Immediately after dicing, the samples were submitted to the treatments, details of which are given in the following sections.

Cassava starch coating

The cassava starch coating (EC) was prepared with 2.5 g of commercial cassava starch (Molinos Argentinos S.R.L), 0.2 g of potassium sorbate and 2.5 g of glycerol solubilised in 100 mL of distilled water. The mixture was stirred until complete dissolution, diluted to pH = 5 with 25% citric acid (Famá *et al*., 2007) and heated to 80 °C with constant stirring until gelatinisation and allowed to cool before applying to sweet potatoes.

Application treatments

The previously peeled and diced pieces underwent the following treatments: (i) application of the cassava coating (EC), (ii) immersion in 0.5 M ascorbic acid solution (AAS) and (iii) application of a cassava coating added with 0.5 M ascorbic acid, at pH = 3 (EC + AA). The sweet potato pieces (2 × 2 × 2 cm) were immersed for 10 min in the selected treatment mixture in a ratio 3:1 (treatment solution: sweet potatoes); after that, they were drained using a manually operated commercial salad spinner. All these procedures were performed at room temperature. Then, 200 g of sweet potatoes was packed in polystyrene trays (15 × 10 × 3 cm), coated with self-adhesive film of polyvinyl chloride (thickness, 10 μm; O_2 permeability, 10.78 $cm^3\ m^{-2}\ atm^{-1}\ day^{-1}$; CO_2 permeability, 51.32 $cm^3\ m^{-2}\ atm^{-1}\ day^{-1}$; water vapour permeability, 38 $g\ m^{-2}\ day^{-1}$) and stored at 4 ± 1 °C for 16 and 12 days for treated and control products, respectively. Samples were analysed at time zero and every 4 days during storage. The concentration of AA 0.5 M was selected on preliminary tests. The antibrowning effect of AA added to the coating in a range of 0.1–0.8 M was tested in minimally processed sweet potatoes and evaluated using surface colour. The samples were stored at 4 °C and visually evaluated. The

lowest concentration that achieved browning inhibition for 2 weeks was 0.5 M.

Weight loss

After removing samples from the tray, the samples were weighed and weight losses were calculated.

Polyphenol oxidase and peroxidase activities

Five grams of fresh tissue was homogenised with 20 mL of cold acetone and vacuum-filtered, and the residue was left overnight in a vacuum desiccator. A sample of 0.2 g of acetone powder was resuspended in a solution of 10 mL of 0.1 M phosphate buffer (pH = 6.0) and 10 mL of 1 M NaCl 2 mM phenylmethylsulfonyl fluoride (PMSF) under magnetic stirring in ice bath for 45 min. The product was centrifuged (4000 g/10 min) and filtered. This product was named as enzyme extract.

To determine the activity of the enzyme PPO, 1 mL of enzyme extract was mixed with 1 mL of 40 mM catechol (Biopack, Argentina) in 10 mM phosphate buffer (pH = 7.0). The reaction mixture was incubated at 25 °C, and absorbance was read every 15 s for 2 min at 420 nm. Enzyme activity was expressed as variation in units of absorbance, ΔUA min^{-1} mg $protein^{-1}$ (Chikezie, 2006).

The peroxidase (POD) activity was quantified by mixing 1 mL of enzyme extract with 1 mL of 40 mM guaiacol (Fluka AG, Switzerland) in 50% ethanol solution and 1 mL of 25 mM hydrogen peroxide. The reaction mixture was incubated at 25 °C (Fehrmann & Diamond, 1967). Readings were taken every 15 s for 5 min at 470 nm. Enzyme activity was expressed as ΔUA min^{-1} mg $protein^{-1}$.

Phenylalanine ammonia lyase (PAL) activity (EC 4.3.1.5)

Acetone powder (0.4 g) was dissolved in 20 mL of 0.1 M borate buffer (pH = 8.8) containing polyvinylpyrrolidone 1% (w/v) and 5 mM β-mercaptoethanol, with constant stirring in ice bath for 20 min (Walton & Sondheimer, 1968). The product was centrifuged (4000 g per 5 min) and the supernatant was filtered. For the determination of PAL activity, 1 mL of extract was mixed with 2 mL of 0.1 M borate buffer (pH = 8.8) and 1 mL of 100 mM L-phenylalanine (Biopack, Zárate, Buenos Aires, Argentina). The reaction mixture was incubated at 37 °C, and readings were taken at 0, 60, 120, 180 and 240 min. Results were expressed as ΔUA h^{-1} mg $protein^{-1}$.

Protein content

Total protein content in the enzymatic extracts was determined by the method proposed by Lowry *et al.* (1951) using bovine albumin as a standard.

Chlorogenic acid

Five grams of fresh tissue was homogenised with 80% methanol (v /v); extraction was carried out at 80 °C (Padda & Picha, 2008a) for 20 min and filtered. The extract was filtrated through 0.45-μm nylon membrane before injection into HPLC (Shimadzu LC-10 A, Tokyo, Japan). The mobile phase was 1% (v/v) formic acid/methanol (70:30) (pH = 2.5), with Hypersil ODS column 250 × 4.6 mm, 5 μm particle size (Thermo Scientific, Walthman, MA, USA), flow rate 0.8 mL min^{-1} and detection at 320 nm with UV-visible detector (Shimadzu, SP 10A, Tokyo, Japan). Two extracts were made from each sample, and injections were performed in triplicate. Results were expressed as mg chlorogenic acid per 100 g fresh tissue (FT).

Ascorbic acid (AA)

The determination of AA was performed by high-performance liquid chromatography (HPLC; Shimadzu LC-10 A) in an extract prepared with phosphoric acid (Sgroppo *et al.*, 2010). Before the injection, the extracts were filtered through a nylon membrane of 0.45 μm. Mobile phase used was acidified (pH = 2.5) water with H_2SO_4, with flow rate 0.8 mL min^{-1}, Hypersil ODS column 250 × 4.6 mm, 5 μm particle size (Thermo Scientific) and detection at 260 nm with UV-visible detector (Shimadzu, 10A SP). Two extracts were prepared from each sample, and the measurements were taken in triplicate. Results were expressed as mg AA per 100 g FT.

Antioxidant Capacity (AOC)

Ten grams of fresh tissue was homogenised with 30 mL of methanol 80% (Padda & Picha, 2008a), magnetically stirred at 4 °C/10 min and filtered and centrifuged (4000 g per 5 min). AOC was determined by mixing 6 mL of 0.076 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, St. Louis, MO, USA) in pure methanol with 0.4 mL of the extract. The mixture was allowed to react for 120 min in the dark, and absorbance was read at 517 nm. Percentage of inactivation (% I) was calculated as $\%I = (A_0 - A_{120})\, A_0^{-1}$, where A_0 is the absorbance of a blank performed with 0.4 mL of methanol and A_{120} is the absorbance of the sample. Results were expressed as mg chlorogenic acid eq. g FT^{-1}.

Surface colour

Surface colour was evaluated in three sites on each side of 20 sweet potato pieces, for each treatment and storage time. The parameters L^*, a^* and b^* were measured with a chromameter (Minolta CR400, Osaka, Japan); hue and ΔE were calculated. The reference

values L_0*, a_0* and b_0* were measured in fresh-cut sweet potatoes (immediately after peeling and cutting).

Statistical analysis

The experiments were performed in triplicate for each condition. All the determinations were done in triplicate, and the results were statistically analysed using analysis of variance (ANOVA) ($\alpha = 0.05$). The differences found were tested by multiple-range least significant difference (LSD) using Info Stat-Statistical Software 2009 (Córdoba, Argentina).

Results and discussion

PPO and POD activities

Browning reactions are generally assumed as a consequence of polyphenol oxidase (PPO) and peroxidase (POD) action on polyphenols to form quinones, which polymerise and produce colour changes in fresh-cut fruit and vegetable products. After peeling and cutting vegetables, the compartmentalisation of the cells disappears and PPO and/or POD can act on phenol substrates, leading to browning reactions (Olivas *et al.*, 2007). In potatoes, PPO activity is strictly connected with the changes in colour due to the formation of coloured polymers (Severini *et al.*, 2003); however, Castillo León *et al.* (2002) reported a high activity of POD in sweet potatoes, and this high activity could be involved in browning.

In fresh sweet potatoes, the obtained values of activity were 2.412 ± 0.311 and $5.79\ 10^{-1} \pm 6.29\ 10^{-2}$ ΔUA min^{-1} mg protein^{-1} for POD and PPO, respectively (Figs 1 and 2).

In control samples, PPO activity doubled at day four and decreased after 8 days of storage; this result could be related to the inhibition by reaction products, because they can interact with the active site of the enzyme (Golan-Goldhirsh & Whitaker, 1984) (Fig. 2).

In addition, the activity of POD enzyme increased at day four, remaining constant until the end of the storage period. Further increase in this activity was not observed, and this result can be attributed to the fact that the raw material is a storage tissue and it is not specialised in wound response.

The activity of PPO and POD in EC samples (with cassava coating) was similar to that of the control samples, showing that the edible coating itself, without the addition of AA, would not be efficient for inhibiting enzymatic browning (Fig. 1) despite the activity of POD decreased significantly ($P < 0.05$) at the end of the experiment.

Immediately after applying the treatments including AA (in the coating EC + AA or in solution AAS), an inhibitory effect on the activities of PPO and POD was

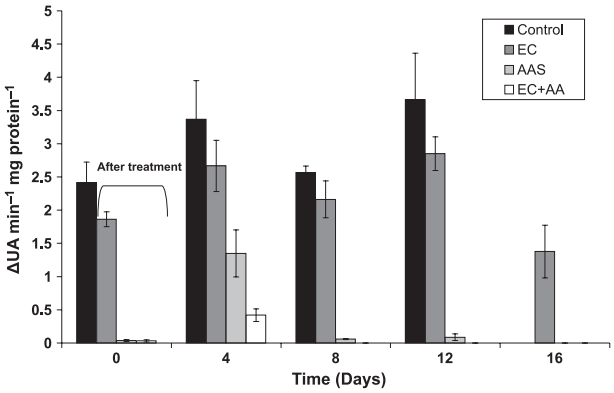

Figure 1 Changes in POD activity during storage of minimally processed sweet potatoes 'Blanca Correntina' at 4 °C. Each value is the mean of three replicates, and error bars indicate the standard deviation. EC, edible coating; AAS, ascorbic acid solution; EC + AA, edible coating with ascorbic acid.

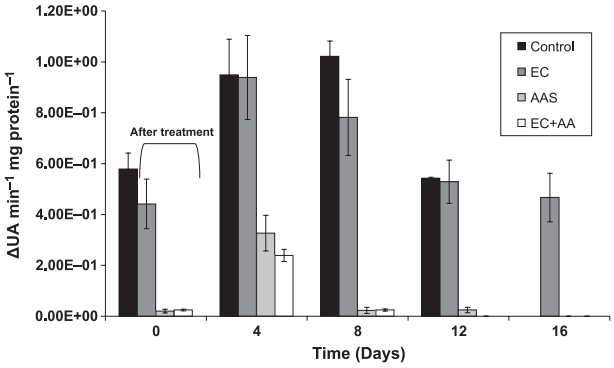

Figure 2 Changes in PPO activity during storage of minimally processed sweet potatoes 'Blanca Correntina' at 4 °C. Each value is the mean of three replicates, and error bars indicate the standard deviation. EC, edible coating; AAS, ascorbic acid solution; EC + AA, edible coating with ascorbic acid.

observed. The measured values were $2.26\ 10^{-2} \pm 5.38\ 10^{-3}$ and 0.034 ± 0.016 ΔUA min^{-1} mg protein^{-1} for PPO and POD, respectively (Figs 1 and 2). Both enzymes showed a slight increase at day four, more important in AAS than in EC + AA samples, and then, the activities decreased with inhibition values greater than 95% until the end of experiments. Samples submitted to the combined treatment (EC + AA) showed no activity of POD since day eight and remained inhibited until the end, while AAS samples presented total inhibition just at the end of the experiment. For PPO, no activity was detected since day twelve in samples submitted to EC + AA treatment, while for AAS samples, its activity was inhibited only at day sixteen.

PPO and POD showed similar behaviour throughout the experiment; this result is in agreement with

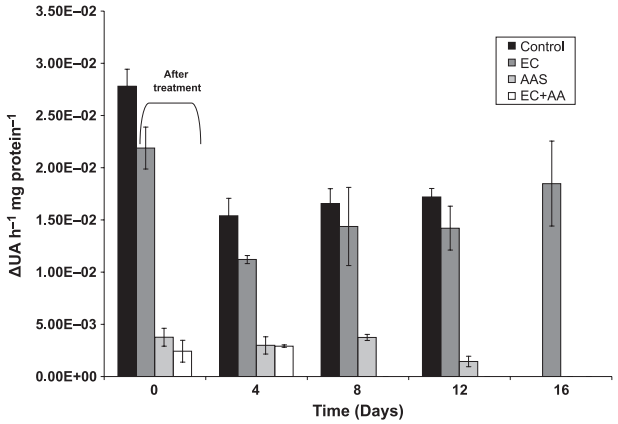

Figure 3 Activity of PAL in minimally processed sweet potatoes 'Blanca Correntina' during storage at 4 °C. Each value is the mean of three replicates, and error bars indicate the standard deviation. EC, edible coating; AAS, ascorbic acid solution; EC + AA, edible coating with ascorbic acid.

Richard-Forget & Gauillard (1997) who reported that the role of POD in enzymatic browning in apples is highly related to PPO activity. The activities of both enzymes were affected ($P < 0.05$) by the treatments with AA, probably because AA decreases pH or sequestrates metal ions (Jang & Moon, 2011).

PAL activity

The phenylalanine ammonia lyase (PAL) is the first key enzyme involved in the biosynthesis of phenols in vegetables and is induced by wounding in minimal processing.

The initial activity of PAL in control samples was $2.78\ 10^{-2} \pm 1.64\ 10^{-3}$, decreasing at day four ($P < 0.05$), which could be related to the phenomenon inhibition by products (Fig. 3). Meanwhile, in samples coated with cassava starch (EC), the activity of PAL was slightly reduced to $2.19\ 10^{-2} \pm 2.00\ 10^{-3}$ after the coating was applied following a similar behaviour to control samples during storage. As observed for PPO and POD, the coating alone had no significant effect on the activity of PAL ($P > 0.05$).

The PAL activity in sweet potatoes after both treatments with AA (EC + AA and AAS) was $3.76\ 10^{-3} \pm 8.60\ 10^{-4}$ and $2.4310^{-3} \pm 1.04\ 10^{-3}$ ΔUA h^{-1} mg $protein^{-1}$, respectively; these values were five times lower than that of control. Until day 8, PAL activity remained constant for AAS samples, then decreased and disappeared at the day 16 of storage (Fig. 3). However, in samples coated with AA (EC + AA), there was no activity detected since day 8. The loss of the enzymatic activity of PAL may also be attributed to the pH decrease produced by the presence of AA, as this enzyme requires a pH close to 8 for its optimal activity.

The behaviour of PPO and POD enzymes was opposite to that of PAL activity; while PPO and POD presented the highest activities at days 4 and 8 (Figs 1 and 2), PAL had no significant changes in control samples and EC (Fig. 3). In samples treated with AA, the inhibitory effect was observed since day 8 for PAL and POD (Figs 1 and 3) and since day 12 for PPO (Fig. 2) in EC + AA samples. Samples treated with AA solution had inhibition of POD and PPO since day 12 and at day 16 for PAL.

Chlorogenic acid content

In sweet potatoes, chlorogenic acid and its isomers are the most representative phenols (Padda & Picha, 2008a; Zheng & Clifford, 2008). The synthesis of these phenylpropanoids could be induced by mechanical stress (Ishiguro *et al.*, 2007) and act directly as a defence response.

The chlorogenic acid content of fresh-cut sweet potatoes was 7.707 ± 0.736 mg 100 g FT^{-1}, in the order of those reported by Padda & Picha (2008a,b). There were no significant differences during storage ($P > 0.05$), even though we found that the activity of PAL decreased and the activity of PPO and POD showed a maximum. This could suggest that there might be other factors involved in chlorogenic acid synthesis and degradation. Padda & Picha (2008a) reported that PPO enzyme oxidises phenolic compounds during the first days of refrigerated storage of sweet potatoes.

In coated samples (EC), the chlorogenic acid content increased during the first 4 days of storage ($P < 0.05$). This behaviour could be related to the O_2 availability necessary to oxidation reactions due to barrier effect of the coating; it must be taken into account that cassava starch coating has low permeability to oxygen.

Sweet potatoes treated with AA (EC + AA and AAS) showed initial values of chlorogenic acid almost 2.5 times higher than that of the control, reaching minimum content after 8 days of storage (Table S2). This result correlates with the activities of the oxidative enzymes PPO and POD, especially with PPO that showed its maximum value at day 8.

Ascorbic acid (AA) concentration and antioxidant capacity (AOC)

Generally, white-fleshed sweet potatoes have lower AA content than fruits; it is around 20 mg 100 g FT^{-1} (FAO, 2012), and in 'Blanca Correntina' variety, it was 1.415 ± 0.282 mg AA 100 g FT^{-1}.

In controls and samples coated only with cassava starch (EC), the AA content showed a decrease during storage, and at the end of the experiment, it was almost ten times lower than in samples initially treated with AA (Table S1).

When ascorbic acid treatments were applied (EC + AA and AAS), a significant increase in its concentration ($P < 0.05$) was observed, mainly in samples coated (EC + AA) (Table S1). Although AA content was significantly reduced on day 8, it remained significantly higher ($P < 0.05$) during storage, suggesting a protective effect of the coating.

The measurement of the antioxidant activity of fruits and vegetables is nutritionally important; fresh-cut vegetables are exposed to oxidative stress, probably affecting composition and content of antioxidant compounds. It has been demonstrated that the increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. In vegetables, AA and phenolic compounds are the most representative compounds that contribute to AOC.

In control samples, the initial AOC was 29.132 ± 9.567 mg chlorogenic acid eq. g FT^{-1} and increased more than twice at the end of storage. For coated samples (EC), the AOC was 42.520 ± 0.374 mg chlorogenic acid eq. g FT^{-1} and showed a slight increase. Padda & Picha (2008a,b) attributed the increase in AOC to an activation of the synthesis of phenol compounds. Even though we cannot compare absolute values, we compared the behaviour of this parameter, finding similar results to those reported by Padda & Picha (2008b).

After applying the treatments with AA, the AOC values were 949.731 ± 95.091 for EC + AA and 650.652 ± 92.341 mg chlorogenic acid eq. g FT^{-1} for AAS; however, these values decreased significantly during storage ($P < 0.05$) in coincidence with the decrease in AA observed in Table S1.

Weight losses

After peeling and cutting the sweet potatoes, the underlying tissue was exposed to the environment and it could lose weight during storage by dehydration or respiration rate. Control samples lost more than 3.5% of their weight ($P < 0.05$). Waimaleongora *et al*. (2008) reported similar weight losses for *Beauregarde* sweet potatoes after storage at 4 °C. Lower weight losses were observed for treated samples. In fresh-cut sweet potatoes coated or treated with AA, weight losses were close to 2%.

Surface colour

There is no general consensus about the best colour parameter that describes browning, particularly in the case of products with heterogeneous colour. Some authors suggest the use of ΔE, L^* or hue, as seen in apples, pears and potatoes. However, it is important to determine the parameter that best fits each product to achieve the best description of the phenomenon (Piagentini *et al*., 2012).

The L^* value measured immediately after cutting was 89.126 ± 1.913; in our experiments, the pieces exposed to air showed immediate browning, but coating or immersion in AA solution was effective in controlling it (Table S2). Similarly, EC samples showed a slight increase in L^*; however, using AA in the coatings led to higher L^* values; this result would suggest that the coating alone is not effective in preventing browning. The highest L^* values corresponded to samples coated with EC + AA (Table S2) with a better freshness appearance than the other samples.

During refrigerated storage, L^* decreased significantly for all the treatments, showing significant differences between them ($P < 0.05$). However, in *Beauregard* fresh-cut sweet potatoes, Waimaleongora *et al*. (2008) reported no significant differences in L^* during 17-day storage at 4 °C.

The other two parameters evaluated, a^* and b^*, showed an opposite behaviour; a^* tends to increase and b^* to decrease during storage. In recently cut sweet potatoes, a^* and b^* coordinates were −0.970 ± 0.423 and 26.328 ± 2.433, respectively, and both increased when treatments were applied. A significant increase was observed for control and EC ($P < 0.05$) (Table S2). Samples treated with AA (EC + AA and AAS) presented slight changes in a^* parameter throughout the experiment. In the same way, b^* parameter showed important differences for control and EC samples; however, it slightly changed in samples coated with AA (EC + AA). Coincidently, in fresh-cut apples treated with AA and citric acid, L^* values were higher and a^* values lower than in nontreated samples (Piagentini *et al*., 2012).

In fresh-cut sweet potato cvs. *Beauregard* and *Hernandez* stored in MAP (5–21% O_2 and 0.4–4% CO_2), McConnell *et al*. (2005) reported some significant changes in L^*, a^* and b^* values, without extreme browning and Walter & Purcell (1980) found that minimal processes such as cutting and slicing did not induce discoloration in sweet potatoes.

When hue angle was evaluated, a marked decrease ($P < 0.05$) was observed for control and EC after days 4 and 8, respectively, because a* parameter changed from negative to positive values. Both AA treatments did not significantly change hue angle values during storage (Table S2). There were no marked differences for ΔE (data not shown), indicating that this parameter would not be the most useful for assessing colour changes in this product.

Relationships between the measured variables in coated and uncoated sweet potato samples

The initial event in the enzymatic browning of sweet potatoes is the breakdown of membranes within the cells of plant tissues, and it is evidenced as changes in the surface colour. In the process of browning, the oxidative enzymes PPO and POD are involved in the oxidation of phenolic compounds such as chlorogenic

acid, whose synthesis depends on the activity of PAL. The condition needed for these enzymatic reactions to occur is that not only enzymes and substrates should be in contact, but also other factors, such as pH and oxygen provision, should be appropriate.

In control sweet potatoes, brown colours developed intensely, as was observed from a^* and hue angle changes, and coincidentally, the highest activities of the oxidative enzymes were detected. As no changes in chlorogenic acid levels were found, this would suggest that other phenolic compounds could be implicated in browning.

PPO enzyme can act on different substrates to produce browning. For example, in the case of potatoes, it was reported that the best substrate is 4-methylcatechol instead of chlorogenic acid (Mercado Silva & Aquino Bolaños, 2005). Oxidation of chlorogenic and caffeic acids produces yellow pigments, while tyrosine produces red colours. This would suggest that there are other substrates involved in the browning phenomenon in addition to chlorogenic acid.

The use of cassava starch coating (EC) without AA in minimally processed sweet potatoes produced a decrease in the activity of oxidative enzymes and PAL, and higher content of chlorogenic acid. However, the coating without the addition of AA had only a partial effect in controlling browning by delaying the appearance of these phenomena. Changes in the values of a* (from -1.208 ± 0.791 to 0.576 ± 0.331) and hue angle (from 178.563 ± 0.520 to 1.546 ± 0.015) during storage confirmed these findings.

The application of an edible coating along with AA (EC + AA) resulted in a good combination to prevent browning during refrigerated storage, as indicated by the analysed parameters. Browning-related enzymes (PPO and POD) were inhibited, as well as the PAL activity and slight variations were found in chlorogenic acid content.

In addition, AA levels and antioxidant capacity were the highest for EC + AA treatment, indicating the protective effect of the cassava starch coating on AA. In this case, the surface of sweet potatoes showed no signs of browning, as demonstrated by the values of a* (from -1.775 ± 0.557 to -1.218 ± 0.813) and the constant values of hue angle (178.511 ± 0.039).

A similar effect in colour was observed when the samples were immersed in AA solution (AAS treatment); instrumentally, no colour variations were detected ($a^* = -1.142 \pm 0.327$, hue = 178.496 ± 0.048) although the activities of PPO, POD and PAL were higher. However, these samples looked less fresh than those coated with cassava starch and AA (EC + AA); additionally, there were no significant differences in weight losses, but AA levels and AOC were lower at the end of the experiment.

Conclusions

The results obtained in the present work showed that the use of cassava starch coating along with ascorbic acid during refrigerated storage at 4 °C increased the effectiveness of ascorbic acid as an antibrowning agent of minimally processed sweet potatoes, also improving its nutritional value.

Browning-related enzymes polyphenol oxidase and peroxidase were inhibited by the application of this coating in minimally processed sweet potatoes, and colour parameters (a^* and hue) did not change. Meanwhile, phenylalanine ammonia lyase was inhibited, and slight variations in chlorogenic acid content were found.

The ascorbic acid levels and antioxidant capacity were the highest for the combined treatment (EC + AA), indicating the protective effect of the cassava starch coating containing AA. The individual treatments, ascorbic acid solution or cassava starch coating, were not as efficient as the combined treatment in retaining the quality of sweet potatoes.

Acknowledgments

This work was supported by Universidad Nacional del Nordeste (Argentina) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Ascorbic acid content, antioxidant activity and chlorogenic acid content evaluation in minimally processed sweet potatoes submitted to different treatments stored at 4 °C.

Table S2. Evaluation of L^*, a^*, b^* and hue in minimally processed sweet potatoes submitted to different treatments during storage at 4 °C.