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Chitosan coating with *trans*-cinnamaldehyde improves structural integrity and antioxidant metabolism of fresh-cut melon

Roberta Lopes Carvalho[a], Marilia Freitas Cabral[b], Thais Andrade Germano[a], Winne Moita de Carvalho[b], Isabella Montenegro Brasil[b], Maria Izabel Gallão[c], Carlos Farley Herbster Moura[d], Monica Maria Almeida Lopes[a], Maria Raquel Alcântara de Miranda[a,*]

[a] Federal University of Ceará. Dept. of Biochemistry and Molecular Biology, Av. Mr. Hull 2297, Campus do Pici Bl. 907, CEP 60.440-554, Fortaleza, CE, Brazil
[b] Federal University of Ceará, Departmet of Food Technology, Fortaleza, CE, Brazil
[c] Federal University of Ceará, Department of Biological Sciences, Fortaleza, CE, Brazil
[d] Embrapa Tropical Agroindustry, Rua Dra. Sara Mesquita 2270, CEP 60511-110, Fortaleza, CE, Brazil

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ABSTRACT

This study evaluated a 2% chitosan-based coating with 500 mg L^{-1} *trans*-cinnamaldehyde on quality attributes, pro- and anti-oxidative metabolism, and structural integrity of biological membranes and cell wall of fresh-cut melon (*Cucumis melo* var. Cantalupensis Naud.) stored for 20 days at 4 °C. Coating improved ($P < 0.05$) samples' firmness, colour, soluble solids, total vitamin C and carotenoid content for up to 15 days of storage, thereafter samples showed signs of decay with softening of processed melon resulting from structural changes in both biological membranes and cell walls. Coating also reduced hydrogen peroxide radical levels leading to lower antioxidant enzymatic activity and lipid peroxidation degree and electrolyte leakage levels compared with control. Coating improved produce visual quality due to reduction in the activity of browning-associated enzymes, G-POD and PPO. Results indicate that coating improved quality of fresh cut Cantaloupe melons by acting as a physical barrier to gas exchange decreasing respiration rate and consequently, O_2-dependent processes, while *trans*-cinnamaldehyde acted as free radical scavenger and enzyme inhibitor. The lower respiration rate inhibited the establishment of an oxidative stress induced by fruit processing and senescence with less damage to biological membranes, lower cell wall hydrolase activities and loss of bioactive compounds.

1. Introduction

Melon (*Cucumis melo* L.) is an important crop member of the Cucurbitaceae family. It is well adapted to the soil and agroclimatic conditions of Brazil's Northeastern region where its production reached 575 tons in 2013, with over 30 % being exported (FAOSTAT, 2014). The majority of cultivated melons are consumed as value added, fresh-cut products. In addition, according to IBIS World Industry Report (2015), the global fruit and vegetable processing industry is expected to grow at an annualized rate of 2.8% to US$ 69 billion over the next five years.

Cutting or minimally processing directly affects fruit physiology as cutting increases respiration rates with a greater consumption of carbon substrates and production of reactive oxygen species. Cutting also induces ethylene synthesis, which accelerates senescence. Moreover, minimum processing reduces fruit tissue integrity, triggering deterioration processes such as browning and softening, water loss and production of off-flavours. The removal of the natural protective barrier of epidermis, as well as the increased humidity and solute leakage on fruit surfaces, provides optimum conditions for growth of microorganisms (Oms-Oliu et al., 2008).

Different techniques, such as coating and modified storage atmosphere, have been employed to preserve fresh cut fruit quality and safety. Biofilms or coatings became more and more relevant to the food industry as their edible and biodegradable properties were further investigated, particularly due to the possibility of incorporating bioactive compounds such as antimicrobials (López et al., 2007). Coatings create a modification of the atmosphere

* Corresponding author.
E-mail addresses: isabella@ufc.br (I.M. Brasil), izagalao@gmail.com (M.I. Gallão), farley.moura@embrapa.br (C.F.H. Moura), raquelamiranda@gmail.com, rmiranda@pq.cnpq.br (M.R.A. de Miranda).

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surrounding the fruit acting as a semipermeable barrier that controls gas exchange, reduces water loss and maintains tissue firmness, in addition to inhibiting microbial spoilage (Gonzalez-Aguilar et al., 2009).

Polysaccharide-based coatings modulate internal tissue atmosphere, once they represent an obstacle to moisture, O_2, CO_2, and volatiles movement, slowing down the metabolism, and delaying fruit senescence (Olivas and Barbosa-Cánovas, 2005). Chitosan is a natural polysaccharide considered nontoxic, non-antigenic, and a biofunctional additive and therefore, safe for use in food (No et al., 2007). Additionally, chitosan is an excellent coating matrix for processed fruits due to its film-forming, mechanical and biochemical properties plus biodegradability. Many studies have evaluated the effects of chitosan coatings on the quality and safety of fruits (Chien and Chou, 2006; Chien et al., 2007; Duan et al., 2008; Sangsuwan et al., 2008; Gonzalez-Aguilar et al., 2009; Kong et al., 2010; Brasil et al., 2012). Moreover, the antimicrobial activity of chitosan against a range of foodborne filamentous fungi, yeast and bacteria has attracted attention to it as a preservative of natural origin (Ming et al., 2010). Only a few journal articles explore the effects of chitosan coating on components of oxidative/antioxidant metabolism of fresh fruits (Hong et al., 2012; Wang and Gao, 2013). They reported the maintenance of catalasic antioxidant activity and a delay in increases of browning-associated enzyme activity, guaiacol peroxidase and polyphenoloxidase, as well as delayed vitamin C and phenolic loss. Gonzalez-Aguilar et al. (2009) evaluated chitosan coatings of different molecular weights and their effects on firmness-related enzymes polygalacturonase and pectinmethylesterase in fresh cut "Maradol" papaya, and reported that PG activity and firmness loss decreased as the chitosan molecular weights increased.

In addition, antimicrobial agents such as essential oils may also be incorporated into the coating matrix, and be slowly released onto the surface of foodstuff maintaining effective concentrations for prolonged periods. Essential oils are natural antioxidant and antimicrobial substances extracted from vegetables. Most of them consist of a mixture of terpenes, terpenoids, and other aromatic and aliphatic compounds (Bakkali et al., 2008), but their composition can vary markedly depending on the origin. Cinnamon leaf essential oil has shown to have not only antifungal and antibacterial properties against a broad spectrum of food spoilage microorganisms but also to exhibit in vivo antioxidant activity (Hsu et al., 2012). The main compound of cinnamon leaf essential oil is eugenol (70–95%), followed by cinnamaldehyde that can be present in a proportion of 1–5% (Vangalapati et al., 2012). Although, most essential oils are generally recognized as safe (GRAS) food flavouring compounds, their use as preservatives is limited due to flavour considerations, thus effective antimicrobial doses should not exceed organoleptically acceptable levels.

No previous publications were found that address the effects of chitosan edible coatings with added *trans*-cinnamaldehyde on cell wall enzymes and on components of pro- and anti-oxidative metabolism of fresh-cut fruit, or shelf-life extension of fresh produce. Therefore, the aim of this study was to evaluate the effect of the chitosan-based edible coating on quality attributes, structural integrity, and antioxidant metabolism of fresh-cut melon, as a means to understand its effect on physiology leading to shelf life extension.

2. Materials and methods

2.1. Plant material

Ripe cantaloupe melons (*Cucumis melo* var. *Cantalupensis* Naud.) were purchased at a local market (Fortaleza-CE, Brazil), where they were selected for uniformity of maturation stage, size and absence of defects or injuries. Those with visible mechanical and pathological injuries were discarded. Melons were selected, and ranked as 'commercially' ripe with 10% soluble solid (as described below in Section 2.4).

2.2. Processing and coating conditions

Edible coating solution was prepared using 20 g chitosan (medium-MW 95–98% deacetylated chitin, poly D-glucosamine, with viscosity $<30\ mPa\ s^{-1}$) solubilized in distilled water to 2% (w/v). To achieve complete dispersion of chitosan, was added under agitation for 3 h, at room temperature and then the solution was filtered through cheese cloth to remove any impurities. To improve coating mechanical properties, plasticizer glycerol (1.5%, v/v) and surfactant Tween 20 (0.3% v/v) were added. To achieve the concentration of 0.05% ($500\ mg\ L^{-1}$) antimicrobial *trans*-cinnamaldehyde (99%), an aliquot of 22.5 μL was incorporated to the chitosan coating solution by homogenization for 2 min at $5.000 \times g$ using an Ultra-turrax homogenizer (IKA**,** Germany). The 0.05% concentration was determined in previous work as the minimum inhibitory concentration of the *trans*-cinnamaldehyde against *Listeria innocua* ATCC 33090 and *Escherichia coli* ATCC 25922 BHI, used as representative of Gram-positive and Gram-negative bacteria, respectively (Cabral et al., 2011).

All processing and coating procedures were performed in a sanitized cold chamber (14 °C). Before processing, whole melons were immersed for 5 min in sodium hypochlorite solution ($150\ \mu L\ L^{-1}$, pH 7.0), rinsed in distilled water and then, air-dried for 10 min. Sanitized fruit were manually peeled, de-seeded, and cut into 30 mm cubes (±50 g), dipped in the coating solution for 2 min (treated samples) and then, air-dried for 2 min. Control samples consisted of melon cubes immersed in distilled water under similar conditions. Treated and control samples were placed into rectangular polyethylene terephthalate (PET) trays wrapped with a PVC MWRAP® film and stored at 4 °C for 20 days. Evaluations were performed every 5th d, however, after day 15, samples showed signs of decay, therefore only physiological and structural variables were assayed. The trial consisted of 30 melons that were treated and randomly distributed as twelve pieces per tray; meanwhile a sample consisted of three trays and analyses were done in homogenized pulp tissue in triplicates.

2.3. Chemicals

Chitosan, *trans*-cinnamaldehyde, 2,6-dichloro-indophenol (DFI), trichloroacetic acid (TCA), thiobarbituric acid (TBA), ethylenediaminetetracetic acid (EDTA), nitroblue tetrazolium chloride (NBT), pirocatechol, guaiacol, hydrogen peroxide (H_2O_2) and polygalacturonic acid were purchased from Sigma–Aldrich® (St. Louis, MO, USA). Guaiacol was purchased from Merck® (Darmstadt, Germany). All other chemicals were of analytical grade.

2.4. Quality attributes

Pulp colour was evaluated at two opposite sides of each sample using a handheld digital colorimeter (Konica Minolta® CR-410, Osaka, Japan) with the cylindrical coordinate system and the color space for lightness (L^*), chroma (C^*) and hue angle (h^*).Weight or mass was measured with a Tecnal® balance (São Paulo, Brazil; ±0.05 g) before (A) and after each storage period (B), respectively and calculated as $(A - B)/A$. Soluble solids (SS) content was determined by refractometry as described by AOAC (2005) using a digital refractometer (ATAGO® N1, Washington, USA) with automatic temperature compensation. The results were expressed in % (concentration of sucrose w/w).

2.5. Firmness, histology and associated physiological variables

Processed fruit firmness was measured on a TA.XT2i Stable Micro Systems® (London, UK) automatic texture analyzer with a 6 mm plunger at a shearing speed of 1 mm s^{-1} to a depth of 15 mm. Measurements were carried out at two equidistant points on the pieces and results expressed in Newtons (N).

Integrity of biological membranes as assessed by the degree of cell membrane lipid peroxidation was estimated through malondialdehyde (MDA) formation using the thiobarbituric acid (TBA) method as described by Peixoto et al. (1999). An aliquot of 25 mg of pulp were macerated in 1% trichloroacetic acid (TCA, w/v), and then centrifuged for 15 min at 10,000 × *g*. The supernatant (500 μL) was incubated with 2 mL of 0.5 % (w/v) TBA containing 20% (w/v) TCA at 95 °C for 1 h, and then cooled in an ice bath. The mixture was centrifuged at 9000 × *g* for 10 min, and absorbance of the supernatant was measured at 532 and 600 nm. After subtracting the non-specific absorbance (A_{600}), lipid peroxidation degree was estimated using the molar extinction coefficient of MDA (155 M cm^{-1}) (Heath and Packer, 1968), and the molar concentration of MDA was expressed on a fresh weight basis as μmol kg^{-1}.

Cell membrane integrity was also estimated through electrolyte leakage as described by Serek et al. (1995). Fresh pulp tissue (discs with 1 cm in diameter) was dried with a paper towel and washed thrice with deionized water to remove adsorbed electrolytes. Samples were immersed in 15 mL of deionized water, and kept resting at 25 °C for 24 h, when electric conductivity (C_1) was measured with TEC-4MP® conductivimeter (São Paulo, Brazil). After C_1 was measured, samples were stored in a domestic freezer at -20 °C for 24 h, and then, after defrosting at 25 °C, a second measurement of electric conductivity (C_2) was made. Electrolyte leakage was measured as the difference observed in conductivity using the equation $(C_1/C_2) \times 100$ and expressed as %.

Light microscopic histological analysis started with melon samples fixed with 4% solution of paraformaldehyde in 0.1 mol L^{-1} phosphate buffer, pH 7.2 and 1% glutaraldehyde for 24 h, at ambient temperature (Karnovsky, 1965). The material was then dehydrated in a graded ethanol series, and embedded in Historesin embedding kit (Reichert-Jung®, Germany). The tissue blocks were sectioned at 5 mm on a SLEE CUT® 5062 microtome (Germany). Histochemical reaction was carried out with Toluidine Blue (TB) at pH 4.0 as metachromatic stain to detect anionic radicals (Vidal, 1977). Photomicrographs of the cell structure were taken using an Olympus® BX41 light microscope (Tokyo, Japan) connected to a camera (model UC30) and computer using CELL® software.

Cell wall hydrolases assays were carried out at 4 °C and results were calculated as specific enzyme activity considering the total protein content of each sample, as determined by Bradford (1976), using bovine serum albumin (BSA) as a standard.

Pectinmethylesterase (PME, E.C. 3.1.1.11) was extracted, and its activity determined according to Jen and Robinson (1984). Pulp tissue (5 g) was homogenized with 25 mL of ice-cold 0.2 mol L^{-1} NaCl in a Kinematica Polytron® (Switzerland) The homogenate was filtered through Whatman No. 1 paper, and the filtrate was collected as the enzyme crude extract. For enzyme activity, the reaction mixture contained 5 mL of enzyme crude extract and 30 mL of pectin solution (1% v/w citrus pectin in 0.1 mol L^{-1} NaCl), and the rate of pectin demethylation was monitored through titration with NaOH 0.025 mol L^{-1} for 10 min, at pH 7.0. One unit of PME activity (UA) was defined as the amount of enzyme capable of demethylating pectin corresponding to the consumption in one minute of one nanomole of NaOH per kilogram on a protein basis, and results were expressed as UA kg^{-1}.

Polygalacturonase (PG, E.C. 3.2.1.15) was extracted and its activity determined according to Pressey and Avants (1982) and Buescher and Furmanski (1978), respectively. Pulp tissue (12.5 g) was homogenized with 25 mL of ice-cold water, and centrifuged for 20 min at 6000 × *g* at 4 °C. The precipitate was re-suspended in 10 mL ice-cold distilled water, and centrifuged under the same conditions. This step was repeated three times before the precipitate was re-suspended in 10 mL of 1 mol L^{-1} NaCl, stirred for 1 min, then the solution was adjusted to pH 6.0 and left for 1 h. The solution was centrifuged for 20 min at 6000 × *g* at 4 °C, and the supernatant was collected as the extract for activity assay. For enzyme activity, the reaction mixture consisted of 200 μL enzyme crude extract plus 50 μL 0.25 % polygalacturonic acid in 37.5 mmol L^{-1} sodium acetate buffer, pH 5.0. The reaction mixture was incubated for 3 h at 30 °C followed by immersion in a boiling water bath to stop the reaction. The reducing groups liberated were determined according to Somogyi technique (Nelson, 1944). One unit of PG activity corresponds to one micromole of reducing sugars produced on protein basis, and results were expressed as UA kg^{-1} s^{-1}.

2.6. Oxidative stress marker and antioxidant metabolites

The content of hydrogen peroxide (H_2O_2), an oxidative stress marker, was determined as described by Alexieva et al. (2001). Pulp tissue (500 mg) were homogenized in ice bath with 5 mL 5 % TCA (w/v), and then centrifuged at 12,000 × *g* for 15 min at 4 °C. The 0.5 mL supernatant was added to 0.5 mL of 10 mmol L^{-1} potassium phosphate buffer (pH 7.0) and 1 mL of 1 mol L^{-1} KI, absorbance was monitored at 390 nm, and H_2O_2 content was given on a standard curve expressed as mol kg^{-1} on a fresh weight basis.

Bioactive antioxidant compounds were determined. Total carotenoids were measured as described by Lichtenthaler and Welburn (1983) based on the chlorophyll content after absorbance was read at 470, 646 and 663 ηm, and calculated as follows: chlorophyll a (g L^{-1}) = 12.25 × (A663) – 2.81 × (A646); chlorophyll b (g L^{-1}) = 20.31 × (A646) – 2.81 × (A663) and carotenoids (g L^{-1}) = (1000 × (A470) – 3.27 × [chlorophyll a] – (104 × [chlorophyll b])/227, and results were expressed as mg kg^{-1} on a fresh weight basis. While, total vitamin C content was determined by titration with 0.02% 2,6-dichloro-indophenol (DFI) (Strohecker and Henning, 1967), with one gram of pulp homogenized in 100 mL of 0.5 % oxalic acid. Then, 5 mL of this solution was diluted to 50 mL with distilled water, titrated, and results were expressed as mg kg^{-1} on a fresh weight basis.

Antioxidant enzymes activity assays, except PPO, used as enzymatic extract two grams of fruit pulp homogenized in 10 mL of 0.1 mol L^{-1} potassium phosphate buffer (pH 7.0) containing 0.1 mmol L^{-1} ethylenediaminetetracetic acid (EDTA) for 1 min, followed by centrifugation at 3000 × *g* for 40 min at 4 °C (Yang et al., 2009). The supernatant fraction was used as a crude extract, and all the procedures were performed at 4 °C. Results were also calculated as specific enzyme activity considering the total protein content of each sample (Bradford, 1976).

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined spectrophotometrically based on inhibition of the photochemical reduction of nitroblue tetrazolium chloride (NBT) (Giannopolitis and Ries, 1977). The reaction mixture absorbance was measured by the Biotek Sinergy® Mx Microplate Reader (Vermont, USA) spectrophotometer at 560 nm, and one unit of SOD activity was defined as the amount of enzyme required to cause a 50% reduction in the NBT photo-reduction rate on protein basis. Results were expressed as UA kg^{-1}.

Catalase (CAT, EC 1.11.1.6) activity was measured according to Beers and Sizer (1952). The reaction started by adding the enzyme extract, and then the decrease in hydrogen peroxide (H_2O_2) monitored through absorbance at 240 nm, and quantified by its molar extinction coefficient (36 mol L^{-1} cm^{-1}). One unit of CAT

activity was defined as the amount of enzyme required to decompose H_2O_2 on protein basis, and the results were expressed as $\mu mol\ kg^{-1}\ s^{-1}$.

Ascorbate peroxidase (APX, EC 1.11.1.1) activity assayed according to Nakano and Asada (1981). Enzyme activity was measured using the molar extinction coefficient for ascorbate ($2.8\ mol\ L^{-1}\ cm^{-1}$), considering that 1 mol of ascorbate is required for a reduction of 1 mol H_2O_2 on protein basis, and results were expressed as $\mu mol\ kg^{-1}\ s^{-1}$. Guaiacol peroxidase (G-POD, EC 1.11.1.7) activity assayed according to Amanko et al. (1994). Initially, $100\ mmol\ L^{-1}$ phosphate buffer (pH 7.0), $0.1\ mmol\ L^{-1}$ EDTA were incubated at 30 °C for 10 min, and then $0.2\ mol\ L^{-1}$ guaiacol, $0.03\ mmol\ L^{-1}$ H_2O_2 and 10 μL enzyme extract were added to complete the reaction mixture (2 mL). Enzyme activity was measured using the molar extinction coefficient for tetraguaiacol ($26.6\ mmol\ L^{-1}\ cm^{-1}$) on protein basis, through absorbance at 470 nm, and the results expressed in $\mu mol\ kg^{-1}\ s^{-1}$.

Polyphenoloxidase (PPO, EC 1.14.18.1) activity was determined, and extract prepared according to the methodology described by Wissemann and Lee (1980). One gram of pulp homogenized for 5 min with 10 mL of $0.1\ mol\ L^{-1}$ phosphate buffer, pH 7 plus 1 % (w/v) of PVPP. The enzyme solution was centrifuged for 40 min at $5000 \times g$ in a SIGMA® 6 K15 centrifuge at 4 °C. The supernatant was in hot water bath (37 °C) for 10 min, centrifuged for 20 min at $2000 \times g$ at 4 °C, and this supernatant was used as the crude extract for further activity assay. PPO activity was determined spectrophotometrically at 395 nm, after the reaction mixture consisting of 0.1 mL of enzyme extract, 0.08 mL of $0.1\ mol\ L^{-1}$ phosphate buffer (pH 6.0) containing pirocatechol $0.1\ mol\ L^{-1}$ was incubated at 30 °C for 30 min. The reaction was stopped by the addition of 0.8 mL of perchloric acid $2\ mol\ L^{-1}$, and absorbance at 395 nm was monitored. One unit of enzyme activity was defined as the amount of enzyme required to increase absorbance in 0.001 units on protein basis, and was expressed as $UA\ kg^{-1}\ s^{-1}$.

2.7. Statistical analysis

The experimental design was completely randomized in factorial 2×5 (treatment × storage time) with three replications consisting of three trays with twelve melon cubes, each. The data was subjected to analysis of variance (ANOVA) using the computer program SISVAR 3.01, and the averages were compared by the Tukey's test at 5 % probability.

3. Results and discussion

3.1. Postharvest quality and bioactive compounds content

Fresh cut melons coated with chitosan with added *trans*-cinnamaldehyde, as well as uncoated control fruit showed no signs of decay, and therefore were acceptable for consumption for up to fifteen of the twenty days of cold storage, during which they were evaluated for quality attributes. Chitosan coating did not affect weight loss, as both control and treated fruit lost similar weight during the first 15 d of storage, averaging 13% (Fig. 1A). However, throughout the fifteen days of storage, the soluble solids content remained constant and higher ($p < 0.05$) in coated fruit, 7.8 %, when compared to control which declined significantly to 5.3 % (Fig. 1B). Bioactive antioxidant compounds relevant to human health, such as vitamin C and carotenoids, were also evaluated as representations of produce quality. Total carotenoids (Fig. 1C) and vitamin C (Fig. 1D) contents showed similar patterns regarding the influence

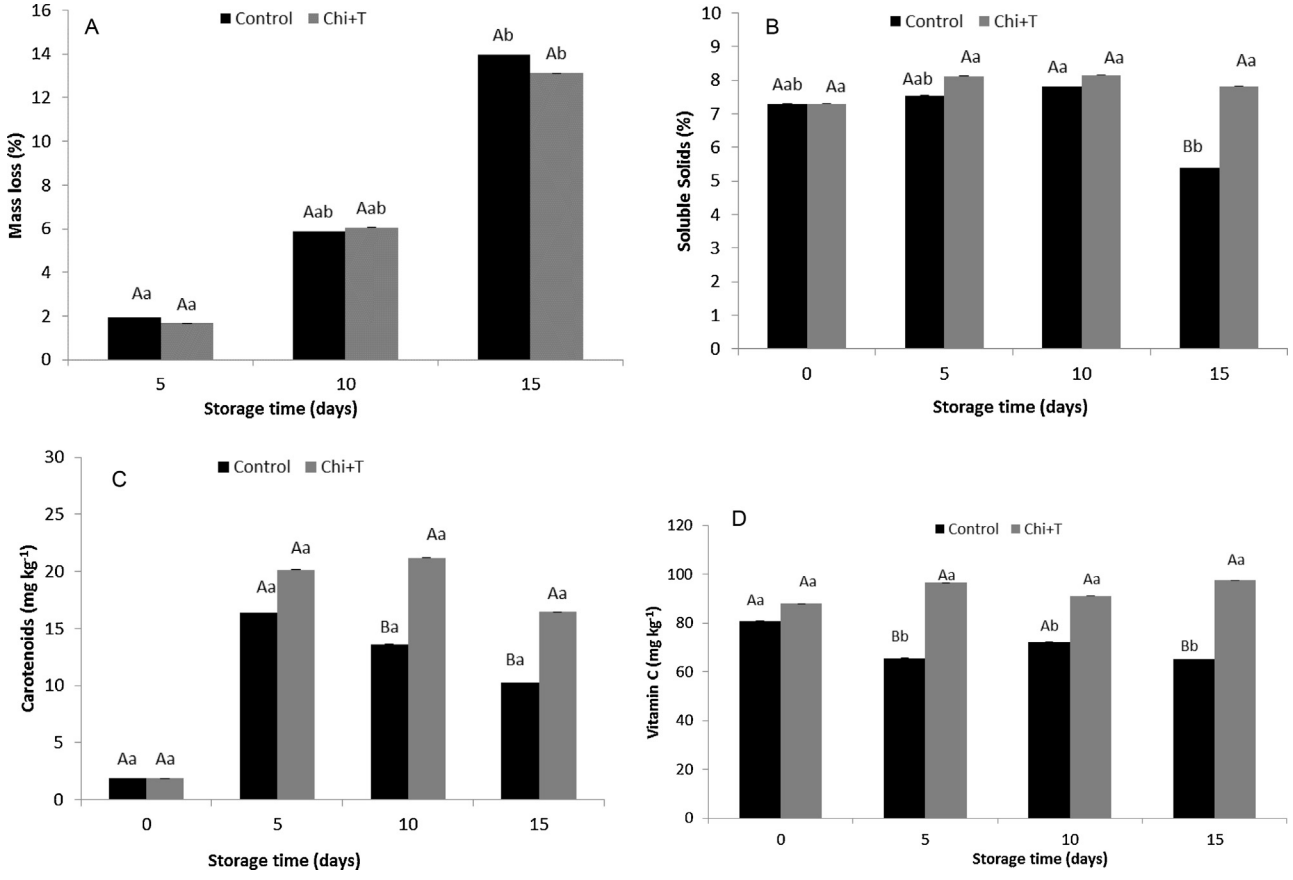

Fig. 1. Mass loss (A), soluble solids (B), total carotenoid (C) and total vitamin C (D) contents of fresh-cut melon coated with chitosan plus *trans*-cinnamaldehyde and stored at 4 °C for 15 days. Different capital and lowercase letters indicate significant differences at $P < 0.05$ between coating treatments and storage periods, respectively.

of coating, with significantly ($P < 0.05$) higher levels of such variables being maintained, throughout storage. Thus, at the end of storage period, total carotenoids and vitamin C of coated melon were 27 and 33 % higher than for uncoated control fruit, respectively.

These results show that, although the chitosan plus *trans*-cinnamaldehyde coating did not prevent any mass loss during storage of processed melons, it positively influenced melon quality through maintenance of important components necessary for sensorial quality such as SS and with nutritional value such as carotenoids and vitamin C. These results may be explained by a restriction in gas exchange due to coating that inhibited the oxidation of these antioxidants; however, it did not affect water evaporation from fruit tissue, justifying the mass loss observed. The efficacy of chitosan coating on reducing water loss depends on its molecular weight and additives (Gonzalez-Aguilar et al., 2009)

Thus, by reducing respiration, the coating may have preserved the bioactive compounds concentrations longer due to reduced exposure to oxygen inhibiting their oxidization, as shown by Rivera-Lopez et al. (2005). Indeed, chitosan coating has been associated with reducing vitamin C loss to only 11% compared to 29% found for control, in Cavendish banana (Suseno et al., 2014) and to 30% compared to 42% for control, in "Earliglow" strawberry (Wang and Gao, 2013). However, results in this study indicate that incorporation of *trans*-cinnamaldehyde exerted an even greater influence on preservation of such compounds, probably due to its antioxidant potential. As Hsu et al. (2012) showed, this essential oil component is a major contributor to oxidative stress resistance in vivo.

3.2. Structural organization of fresh cut melon

The coating showed effectiveness in maintaining original firmness of fresh cut melons for up to 20 days (Fig. 2A), in spite of the weight loss (Fig. 1A). Firmness of control fruit decreased almost 40% by day 20, while coated samples were significantly firmer ($P < 0.05$) throughout storage. Thus, chitosan coating prevented firmness loss and delayed the softening process that is dependent on oxygen availability. The observation in this study is probably due to the effect of the surface coating acting as a gas barrier, since surface coatings have been reported to increase resistance of fruit surface to gas permeability, creating a modified

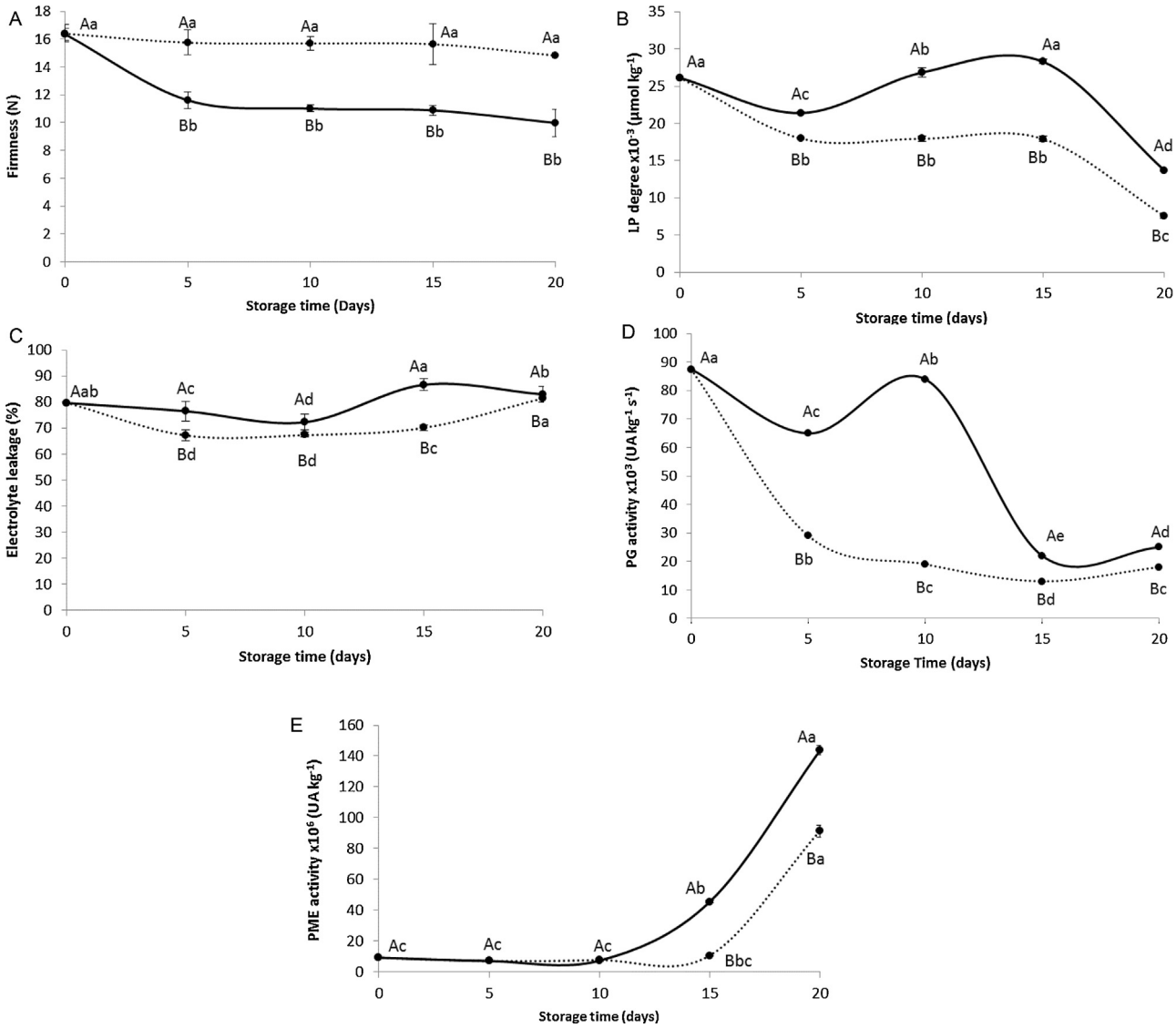

Fig. 2. Firmness (A), biological membrane lipid peroxidation—LP degree (B), electrolyte leakage (C) and activity of cell wall hydrolases polyglacturonase—PG (D) and pectinmethylesterase—PME (E) of fresh cut melon coated with chitosan plus *trans*-cinnamaldehyde (·····) and uncoated (—), stored at 4 °C. Different capital and lowercase letters indicate significant differences at $P < 0.05$ between coating treatments and storage periods, respectively.

internal atmosphere, and reducing the respiration rate (Marpudi et al., 2011).

Two main factors influence fruit tissue integrity and firmness: cell wall and biological membrane structures. The degree of biological membrane damage through lipid peroxidation (LP) was significantly ($P < 0.05$) reduced in coated samples (18×10^{-3} μmol kg^{-1}) while in control groups after 15 days of storage showed a maximum LP of 28×10^{-3} μmol kg^{-1} (Fig. 2B). The increase in MDA values found in control fruit could be explained by an imbalance between oxidative/antioxidative systems, which occurs during normal ripening and senescence ontological processes (Lacan and Baccou, 1998), and which affect the structural organization of fruit tissue. Thus, the results here could be associated to firmness data (Fig. 2A) that showed the control fruit became softer in the end of storage due to higher lipid peroxidation.

The lipid peroxidation (LP) process initiates with a reaction between a free radical and unsaturated fatty acid, propagated by peroxyl radicals forming lipid hydroperoxides and aldehydes, such

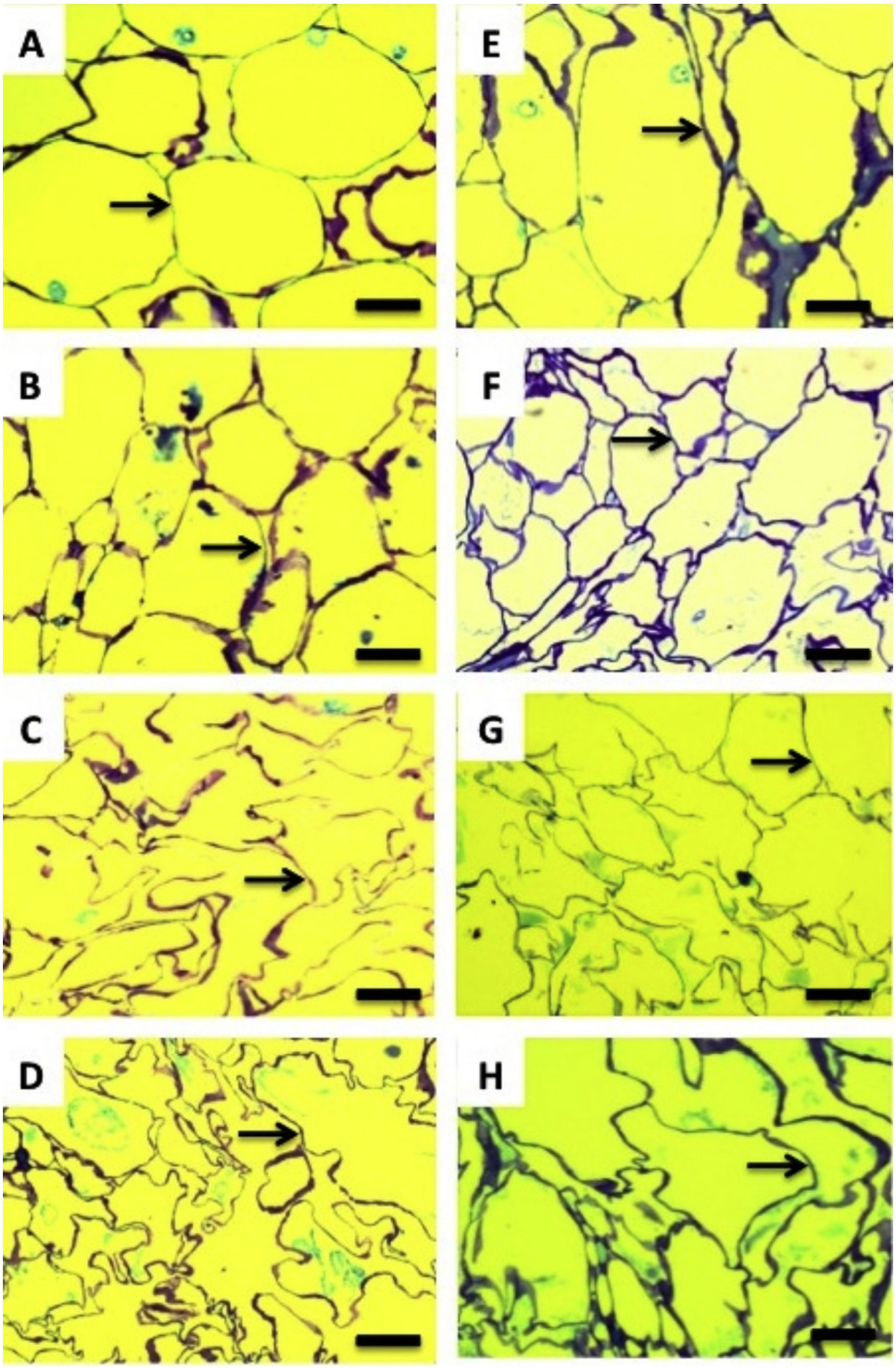

Fig. 3. Histology of fresh cut control uncoated melon stored for (A) 0; (B) 5, (C) 10 and (D) 15 days and of chitosan plus *trans*-cinnamaldehyde-coated melon stored for (E) 0; (F) 5, (G) 10 and (H) 15 days at 4 °C stained with toluidine blue. Cell wall (→). Bar: 100 μm.

as malondialdehyde (MDA). Therefore, LP represents a cascade of reactions initiated by free radicals as reactive oxygen species (ROS), which are byproducts of normal respiratory process, on cell membrane lipids leading to changes in its structure/fluidity, failure of metabolite transport mechanisms, and ultimately to cell death (Mittler, 2002). Such changes in membrane fluidity will eventually lead to rupture and leakage of solutes from the cell interior to the apoplast. Oms-Oliu et al. (2008) observed that LP increased when "Piel de Sapo" melons were stored under refrigeration and the authors credited this to activation of the enzyme superoxide dismutase and inhibition of catalase and ascorbate peroxidase, which are associated with protective mechanisms against chilling injury. Lacan and Baccou (1998) studied ripening of "Jerac" melons and reported that membrane lipid peroxidation by free radicals resulted in increased leakage of intracellular material due to increased activity of lipolytic enzymes.

The chitosan and *trans*-cinnamaldehyde coating was efficient in reducing the undesirable but unavoidable postharvest process of electrolyte leakage due to cell membrane rupture in fresh cut melons. Thus, Fig. 2C shows that electrolyte leakage corroborated with previous LP result (Fig. 2B), as control samples showed significantly higher ($P < 0.05$) electrolyte leakage values (86.63%) than coated samples (70.11 %), after 15 days of cold storage. Loss of membrane integrity, and subsequent increase in electrolyte leakage are determined by each plant species' ability to withstand physiological disorders. Plant tissues normally respond to stresses such as mechanical injury or extreme temperatures with an increase in electrolyte leakage (Mittler, 2002).

The activity of cell wall hydrolytic enzyme pectinmethylesterase (PME) remained low and constant until the 10th day of storage, thereafter increased significantly ($P < 0.05$) in control fruit (Fig. 2D). After 20 days of storage, control fresh cut melon showed a much higher PME activity per mass of protein, 144×10^6 UA kg^{-1} than the coated samples, which had 91×10^6 UA kg^{-1}. Unlike PME, polygalacturonasic (PG) activity was initially high, especially in uncoated fruit, and decreased during storage and was significantly ($P < 0.05$) inhibited by chitosan coating (Fig. 2E). Thus, after five days, PG activity per mass of protein of control and coated fruits was 65 and 29×10^3 UA kg^{-1} s^{-1}, respectively. Immediately after processing of fresh cut melon, PG activity seemed more strongly associated to firmness loss (Fig. 2A), whereas PME was more important as storage time progressed.

PME is known to catalyze the demethylation of polyuronide chain from the pectin matrix enabling PG hydrolysis of 1,4-β linkages between galacturonic acid residues. A strong correlation between PG activity and firmness was also reported for "Piel de sapo", "Galia" and "Charentais" melons. (Chisari et al., 2009). Gonzalez-Aguilar et al. (2009) coated fresh cut "Maradol" papaya with chitosan, and observed a 30% reduction in PG activity, which the authors regarded as the main enzyme associated with fruit softening. Moreover, studies on cell wall hydrolysis demonstrate that, due to the complexity of wall structure, a collective and synergistic action of several enzymes are needed to promote any relevant textural changes, not of one single specific enzyme (Ali et al., 2004).

Results presented here indicate that softening of processed melon (Fig. 2A) is due to structural changes that occur in both biological membranes (Fig. 2B and C) and cell wall (Fig. 2D and E). Moreover, the chitosan-based coating with *trans*-cinnamaldehyde reduced the damage to membrane structure by free radicals and the activity of hidrolases involved with cell wall dissolution. Such effects positively influenced fresh cut melon firmness probably through inhibition of respiration-dependent event imposed by the coating itself and/or the antioxidant potential of *trans*-

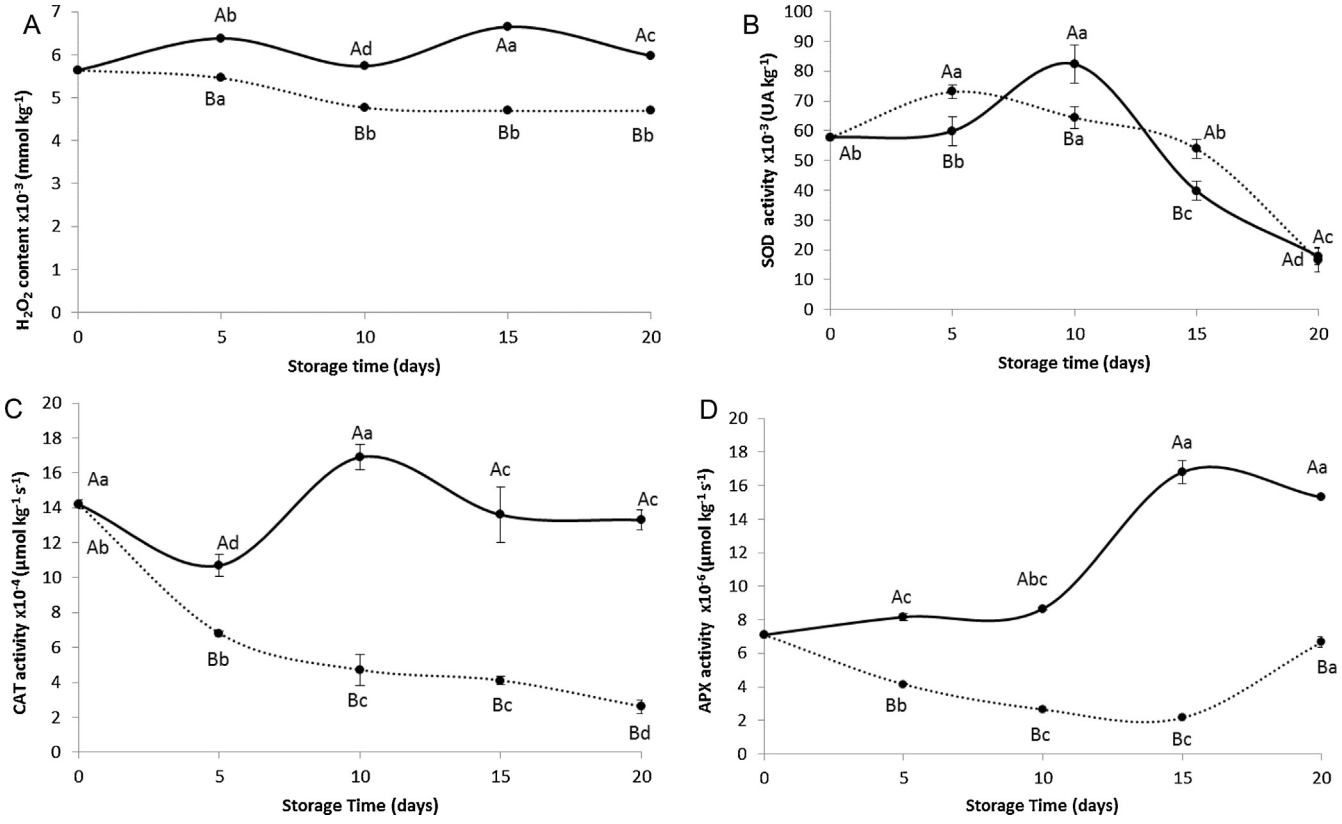

Fig. 4. Hydrogen peroxide (H_2O_2) content (A) and activity of antioxidant enzymes superoxide dismutase-SOD (B), catalase-CAT (C) and ascorbate peroxidase-APX (D) of fresh cut melon coated with chitosan plus *trans*-cinnamaldehyde (·····) and uncoated (—), stored at 4 °C. Different capital and lowercase letters indicate significant differences at $P < 0.05$ between coating treatments and storage periods, respectively.

cinnamaldehyde which is known to inhibit the activity of cell wall hydrolases (Bang et al., 2000).

Histological analysis of fresh cut melon showed that the mesocarp consisted of parenchyma with large cells and intercellular spaces (Fig. 3A–H). Fig. 3A and E show that, at time zero, turgor of intact, tightly packed cells from both control and coated tissues, respectively, is probably the main factor of mechanical resistance, as at this stage, fruit were firm (Fig. 2A). After the first 10 d of storage (Fig. 4C and G), tissue disorganization was observed as cells suffered plasmolysis, cell walls loosened and intercellular spaces enlarged due to a reduction in cell-cell adhesion, in both samples. These changes in turgor and cell wall structure imply a wrinkled appearance resulting from dehydration and respiration, which would have been lower in chitosan-coated fruit. Therefore, fresh cut melon tissue softening (Fig. 2A) may be explained by enlargement of intercellular spaces, decline in cell turgor and cell wall, and middle lamella loosening, which was slowed down through inhibition of cell hydrolases by chitosan plus *trans*-cinnamaldehyde coating.

Cell walls of fleshy fruit pulp are a framework of cellulose microfibers joined by hemicellulose molecules, embedded in a matrix of pectin, which also constitute the middle lamella. Cell wall loosening was evidenced by the dye, which reacts with the negative charges of pectin matrix, and results from dissolution of the pectic substances as well as breakage of cross-links between other wall components. In Fig. 4A–H, arrows indicate cell wall undergoing a dissolution process with enlarged intercellular spaces. This can be associated with the activity of PG (Fig. 2E), which remained high in control fruit up to day 10, corroborating to the idea that this enzyme is primary responsible for dissolution of ripe melon cell wall, and demonstrating why the chitosan-coated melon were firmer than control (Fig. 2A).

3.3. Oxidative stress marker and antioxidant enzymes

Hydrogen peroxide (H_2O_2) is a ROS commonly used as an oxidative stress marker, and its contents were directly influenced by coating of fresh cut melons (Fig. 4A). After twenty days of storage, control showed statistically ($P < 0.05$) higher H_2O_2 levels than coated samples, 5.9 and 4.7×10^{-3} mmol kg^{-1}, respectively, explaining the higher LP levels (Fig. 2B) and consequently, faster softening (Fig. 2A). Thus, chitosan plus *trans*-cinnamaldehyde coating reduced the oxidative stress as indicated by the lower H_2O_2 levels justified by atmospheric changes surrounding fruit tissue which slowed down the cellular respiratory process with lower ROS production. Another ROS, anion superoxide ($O_2^{\bullet -}$) was evaluated in "Pearl" guava stored at 11 °C with different chitosan coatings, and results showed the increase in $O_2^{\bullet -}$ levels was slowed down by chitosan on a dose-dependent manner (Hong et al., 2012). However, it has also been reported that *trans*-cinnamaldehyde is able to scavenge H_2O_2 (Dalcanale and Montanari, 1985). Therefore, chitosan coating with added *trans*-cinnamaldehyde would inhibit the establishment of an oxidative stress, which is defined as an imbalance between production and neutralization of reactive species as ROS, and to our knowledge, there is no previous publication associating H_2O_2 content to coating of fruit with chitosan or *trans*-cinnamaldehyde.

Under normal physiological conditions, molecular oxygen (O_2) receives two electrons from cytochrome C oxidase in the respiratory chain and is reduced to water (H_2O), but occasionally and in different sites, O_2 may receive only one electron forming reactive species as superoxide ($O_2^{\bullet -}$). Thus, radicals are byproducts generated from normal respiratory metabolism in plant mitochondria; however, stressful conditions as those induced by processing or extreme temperatures may result in enhanced production of

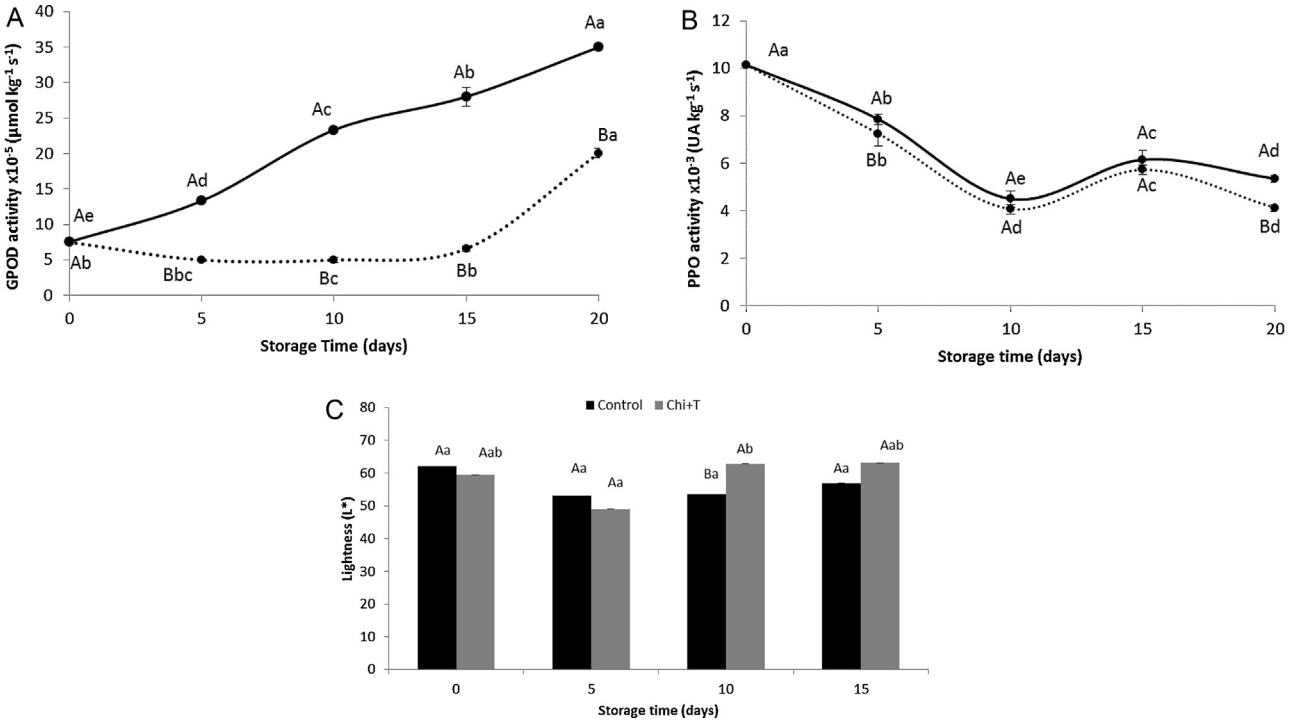

Fig. 5. Activity of browning enzymes guaiacol peroxidase (GPOD) (A) and polyphenoloxidase (PPO) (B) and lightness L^* (C) of fresh cut melon coated with chitosan plus *trans*-cinnamaldehyde (·····) and uncoated (—), stored at 4 °C. Different capital and lowercase letters indicate significant differences at $P < 0.05$ between coating treatments and storage periods, respectively.

these ROS. Nevertheless, plant cells have developed an efficient enzymatic antioxidant defense mechanism that fight oxidative stress by dismutating $O_2^{\bullet-}$ into less reactive species as H_2O_2, which eventually will be scavenged or neutralized (Mittler, 2002).

The antioxidant activity of superoxide dismutase (SOD, Fig. 4B) per mass of protein increased with significant ($P < 0.05$) differences between treatments until day 10 to 82.3×10^{-3} UA kg^{-1} in control and until day 5, to 73.1×10^{-3} UA kg^{-1} in coated fruit. SOD dismutates $O_2^{\bullet-}$ to O_2 and H_2O_2, which can be scavenged by catalase and different peroxidases, thereby SOD is considered the first line of enzymatic antioxidative defense. In control samples, the increase in SOD activity explains the higher H_2O_2 levels (Fig. 4A), a reactive species that oxidizes relevant homeostatic molecules corroborating to the structural disintegration characteristic of senescence.

Chitosan plus *trans*-cinnamaldehyde-coated fresh cut melons showed significant ($P < 0.05$) inhibition of catalase activity when compared to control (Fig. 4C). The ubiquitous enzyme catalase (CAT) catalyzes H_2O_2 decomposition to O_2 and H_2O, and its activity levels declined in control after day 10, from 16.9 to 13.3×10^{-4} μmol kg^{-1} s^{-1} while in coated fruit, it declined after day 5 from 6.8 to 2.6×10^{-4} μmol kg^{-1} s^{-1}. In coated fruit, the lower catalasic activity is justified by the lower H_2O_2 levels (Fig. 4A) due to the atmospheric changes induced by chitosan coating, and scavenging potential of *trans*-cinnamaldehyde. Corroborating the idea that low O_2 atmosphere affects antioxidant enzymatic activity, Meng et al. (2012) observed that cell membranes remained intact with inhibition of LP, of membrane permeability and of catalase and peroxidase activities when minimally processed green peppers were stored under an argon (low O_2) atmosphere. Wang and Gao (2013) also reported that chitosan-coated strawberry showed lower CAT activity under storage at 5 °C, due to its protective barrier property.

Peroxidases reduce H_2O_2 to water (H_2O) using different electron donors such as ascorbate or phenolic compounds (guaiacol). The activity per mass of protein of ascorbate peroxidase (APX, Fig. 4D) was much lower than that of catalase (Fig. 4C), and was also significantly ($P < 0.05$) inhibited by coating to 6.66×10^{-6} μmol kg^{-1} s^{-1} at the end of storage, while control was 15.30×10^{-6} μmol kg^{-1} s^{-1}. The lower APX activity indicates CAT is the primary responsible for H_2O_2 neutralization in melons. The activity found for guaiacol peroxidase (G-POD, Fig. 5A) confirms this statement, as coating significantly inhibited G-POD activity to 20×10^{-5} μmol kg^{-1} s^{-1}after 20 d of storage, while control was 35×10^{-5} μmol kg^{-1} s^{-1}. This enzyme reduces H_2O_2 by oxidizing phenolics to dark-coloured compounds, leading to loss of visual quality and furthermore, contributing to undesirable changes in flavour, aroma, texture and nutritional composition.

Another browning-responsible enzyme, polyphenol oxidase (PPO) had a statistically ($p < 0.05$) lower activity per protein mass in coated fruit, 4.11×10^{-3} UA kg^{-1} s^{-1}, when compared to control, 5.35×10^{-3} UA kg^{-1} s^{-1}, at the end of storage (Fig. 5B). PPO catalyzes the *o*-hydroxylation of monophenols to *o*-diphenols and, further, oxidation of *o*-diphenols to dark-coloured quinones. Among the colour variables evaluated (data not shown), only lightness (L^*) presented significant differences ($p < 0.05$), and decreased throughout storage with the coated samples showing higher values than control, darker fruits (Fig. 5C). Therefore, visual quality of fresh cut melon was improved by the chitosan-*trans*-cinnamaldehyde coating, and agrees with results found for browning-associated enzymes especially for G-POD (Fig. 5A). Chisari et al. (2009) studied the enzymatic browning of minimally processed melon from different varieties, and concluded that G-POD activity was more relevant to this process than PPO.

These results also indicate that chitosan-coating reduced O_2 availability, and the initial increase observed in PPO activity may be explained by processing of pulp tissue, which allows enzymes to join the substrates localized in distinct subcellular compartments. Moreover, *trans*-cinnamaldehyde would also play a role inhibiting enzymes activities as shown by its effects on tyrosinase, an enzyme

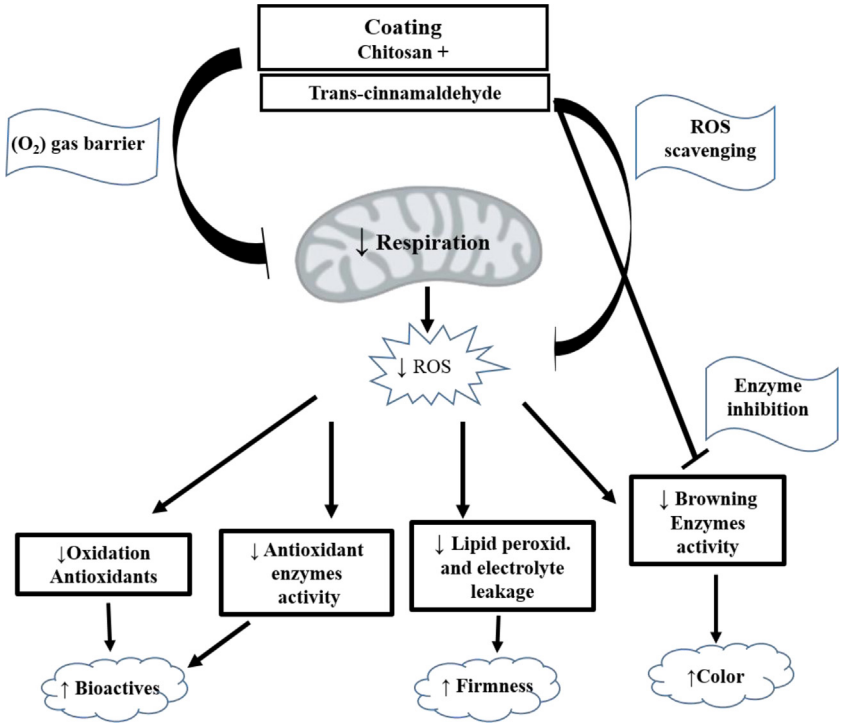

Fig. 6. Proposed mode of action of chitosan coating with incorporated *trans*-cinnamaldehyde on fresh cut melon physiology.

involved with pigment synthesis, especially on its monophenolase and diphenolase activity due to its action as a reversible inhibitor (Zhu et al., 2009)

4. Conclusion

The 2% chitosan-based coating with 500 mg L^{-1} antimicrobial *trans*-cinnamaldehyde maintained quality of fresh cut Cantaloupe melons during storage at 4 °C by preserving total vitamin C, carotenoid, lightness and firmness. These results were a consequence of lower free radical production, which led to stability of biological membranes with prolonged integrity of fruit tissues as well as lower activity of antioxidant and browning-associated enzymes with therefore, lighter samples.

It is proposed that, besides chitosan coating acting as a physical barrier to gas exchange decreasing respiration rate and consequently, O_2-dependent processes, *trans*-cinnamaldehyde acts as free radical scavenger and enzyme inhibitor (Fig. 6). The lower respiration rate inhibits the establishment of an oxidative stress induced by fruit processing and senescence leading to less damage to biological membranes, and lower cell wall hydrolytic enzyme activity. Another indication that coating prevented oxidative imbalance was the lower browning enzymatic activity resulting in better visual quality of coated fruit.

The results presented in this manuscript present new information concerning the influence of edible coatings on fruit at a physiological level, since many of the previous studies focused on quality or, specific metabolic processes. Further technological challenges, such as scaling and aseptic manipulation, should be addressed before application in industrial operations of fresh-cut fruits.

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