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ORIGINAL ARTICLE

Storage quality of fresh-cut apples treated with yerba mate (*Ilex paraguariensis*)

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Abstract Dipping fresh-cut fruits in antioxidant solutions is a useful method to avoid enzymatic browning. Yerba mate extracts have a high content of antioxidant compounds and could be a natural alternative to control browning and improve the bioactive properties of fresh-cut apples. Therefore, this study aimed to evaluate the performance of an antioxidant solution of yerba mate (1.2%), citric acid (0.9%) and ascorbic acid (1.0%) with water as control, on fresh-cut 'Granny Smith' apples during storage at 2 °C (18 days) and 10 °C (15 days) under MAP. Physicochemical characteristics, bioactive properties, sensory attributes, microbial quality as well as the gas composition within the packages were analyzed throughout storage. Samples from both treatments showed a slower quality loss at 2 °C than at 10 °C. The antioxidant solution increased the lag-phase of molds, mesophilic and psychrotrophic microorganisms stored at 2 °C. The phenolic compounds of yerba mate together with ascorbic acid, not only increased the antioxidant capacity of the fresh-cut apples but also reduced the enzymatic browning at both temperatures, increasing the storage time in 2–5 days with an acceptable appearance, when compared to control samples. The antioxidant solution containing yerba mate provided the fresh-cut apples with a higher content of healthy compounds throughout storage at both temperatures.

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Keywords Minimally processed · Antioxidant · Sensory · Microbial growth · Browning

Introduction

Fresh-cut fruits are convenient products with high nutritional quality and increased consumption and production in response to consumer demand for ready-to-eat healthy foods (Alves et al. 2017; Luo et al. 2011). Nevertheless, browning is a serious technological problem affecting many minimally processed fruits and vegetables, including apples. Several alternatives have been proposed to control enzymatic browning, but dipping the cut produce in solutions of ascorbic acid (AA) alone or combined with other chemical agents, is still the most used method (Rojas-Graü et al. 2007).

Ilex paraguariensis St. Hil. Aquifoliaceae dried and milled leaves, commonly known as 'yerba mate' (YM) are widely consumed as an infusion in South America. YM extracts are rich in phenolic compounds (at concentrations similar to red wines and higher than green tea), especially in chlorogenic acids, with high antioxidant activity (Bracesco et al. 2011). The use of YM extracts could be an alternative to prevent quality loss in fresh-cut fruits and vegetables, given the rising interest of consumers for food additives of natural origin and their bioactive properties. In a previous study, Rodríguez-Arzuaga and Piagentini (2018) optimized the concentrations of YM, AA and citric acid (CA) in a dipping solution, to reduce browning development (at 25 °C) and increase the antioxidant capacity of 'Granny Smith' fresh-cut apples, without negatively affecting their flavor. The acceptability study showed that more than 78% of the surveyed consumers liked the fresh-cut apples dipped in the optimal solution (1.2% YM +

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0.9% CA + 1% AA). However, minimally processed fruits and vegetables must be stored under refrigeration and the quality changes throughout these storage conditions should be evaluated.

Several changes occur during cold storage of fresh-cut produce, which may affect their quality and, therefore, their shelf life. Softening is one of the most undesirable defects occurring throughout processing and storage. Softening can occur during ripening; be promoted by wound-induced ethylene, by water loss that leads to a decrease in turgor and crispiness, or by the enzymatic hydrolysis of cell wall pectic substances, due to mechanical stress of plant tissues (Cortellino et al. 2015). Browning alters the appearance of the product, and hence strongly affects the purchase decision (Toivonen and Brummell 2008). Enzymatic browning occurs when polyphenol oxidase and peroxidase catalyze the oxidation of phenolics to *o*-quinones, which then polymerize to dark compounds (Capotorto et al. 2018). This reaction takes place when the subcellular compartmentalization is disrupted at the cut surfaces, putting the oxidative enzymes in contact with their substrate (Toivonen and Brummell 2008). Besides texture and appearance, the flavor also affects the sensory quality of fresh-cut apples. The characteristic flavor of fresh-cut produce depends on the nutritional composition, genetic factors, maturity degree, and postharvest treatments (Ma et al. 2017). During storage, the characteristic flavor can be altered due to biochemical reactions (including enzymatic browning), senescence processes, microbial growth, and physiological reactions (Rux et al. 2017; Putnik et al. 2017a; Machado Azevedo et al. 2018). In addition to biochemical and physiological reactions, fresh-cut fruits are particularly susceptible to microbial growth (Ma et al. 2017). Furthermore, the natural microflora of fruits, which includes bacteria, yeasts, and molds, is largely responsible for the spoilage of the produce. The storage temperature of the fresh-cut apples plays an important role in determining the outcome of the final microflora found after refrigeration and leading to a selection for psychrotrophic over mesophilic microorganisms (Ayala-Zavala et al. 2008).

All the above-mentioned quality aspects and their changes throughout refrigerated storage must be evaluated when assaying new dipping treatments for fresh-cut produce. The objective of the present study was to evaluate the quality changes of 'Granny Smith' fresh-cut apples treated with a dipping solution of 1.2% YM, 0.9% CA and 1.0% AA, during storage at two different temperatures.

Materials and methods

Plant material and dipping solution preparation

'Granny Smith' apples (pH = 3.4, acidity = 5.2 g malic acid kg^{-1} FW, SSC = 11.8 °Brix, firmness = 68.6 N) were purchased at a local market of Santa Fe (Argentina) and stored at 2 °C until processing. Apples of uniform size and color were used in the experiments.

Argentine commercial YM, with not less than 65% of dried and milled leaves and not more than 35% of milled stems, was used (total phenolic content: 55.20 mg GAE g^{-1}YM). The antioxidant solution (1.2% YM, 0.9% CA and 1.0% AA) was selected after an optimization procedure carried out by Rodríguez-Arzuaga and Piagentini (2018) and prepared under the same conditions (pH = 2.8, SSC = 2.3 °Brix).

Sample preparation

Whole fruits were washed with a 100 mg L^{-1} solution of NaClO (pH = 7.0, T = 4 °C) in a 1:3 (m/v) ratio for 2 min, peeled with a sharp stainless steel knife, cored and cut into eight wedges. The wedges were disinfected with a 30 mg L^{-1} solution of NaClO (pH = 7.0, T = 4 °C) in a 1:3 (m/v) ratio for 2 min. After disinfection, the wedges were dipped for 3 min in a 1:3 (m/v) ratio in water (C: control samples) or in the antioxidant solution (T: treated apples). Then, T and C samples were drained, let stand on absorbing paper for 2 min and packed in polyethylene terephthalate (PET) clamshell containers (80–100 g). The containers were made of PET of 0.42 mm thick, with a surface area of 0.045 m^2, transmission rates of 4.73 10^{-15} – 9.617 10^{-15} kg m^{-2} s^{-1} Pa^{-1} for O_2 (at 23 °C and 0% RH) and 1.3 10^{-7} – 2.08 10^{-7} kg m^{-2} s^{-1} for water vapor (at 38 °C and 90% RH). C and T samples were stored at 2 °C for 18 days or at 10 °C for 15 days. The temperature of the processing room was about 12–15 °C. The storage temperatures were selected to simulate industrial storage conditions (2 °C) and domestic or commercial storage conditions (10 °C).

Quality assessment

Firmness

Firmness was determined on the apple wedges, through penetration tests. A TA-XT Plus texture analyzer (Stable Micro System, Surrey, UK) using a cylindrical probe of 11-mm diameter, a 1 mm s^{-1} penetration speed, and up to 10-mm penetration distance. The maximum force

was recorded and the results were expressed in Newton (N).

Color

Color (CIELAB values) was measured with a Minolta spectrophotometer (Model CM-508d/8, Minolta, Tokyo, Japan) calibrated using the standard white tile. Measurements were made at the middle area of the two cut faces of each apple wedge as described by Piagentini et al. (2012), with D65/10° as the illuminant/viewing geometry and specular component excluded. Chroma value [$C^*_{ab} = (a^{*2} + b^{*2})^{0.5}$] was also determined.

Total phenolic content and antioxidant capacity determination

Extraction procedure

Two fruit extracts per sample were obtained as described by Piagentini and Pirovani (2017). Briefly, 5 g of crushed apples were homogenized with 50 mL of acetone:water (80:20) in an ultrasound bath for 15 min and then centrifuged at 4 °C and 12,000 × g for 15 min. The supernatant was used as the extract for the total phenolic content and antioxidant capacity determinations.

Total phenolic content (TPC)

TPC was determined by the Folin-Ciocalteu method according to Piagentini and Pirovani (2017). The absorbance was measured at 760 nm in a spectrophotometer (Genesis 5, Milton Roy, Ivyland, USA), after 30 min of reaction at room temperature (ca. 25 °C). The obtained results were corrected according to Cortez et al. (2018) to compensate for the overestimation produced by the AA interference. The results were expressed as milligrams of gallic acid equivalents by kilogram of fresh weight (mg kg^{-1} FW).

Antioxidant capacity

The antioxidant capacity was determined by the DPPH* and FRAP methods, as described by Rodríguez-Arzuaga and Piagentini (2018). The DPPH* results were expressed as AA equivalent antioxidant capacity (AEAC, mg kg^{-1}) and the FRAP results were expressed as μmol Fe^{2+} kg^{-1} FW.

Ascorbic acid and vitamin C quantification

A modification of the method proposed by Van de Velde et al. (2012) was used for the determination of AA and vitamin C. Vitamin C in fruits refers to the total ascorbic acid (ascorbic acid + dehydroascorbic acid). Twenty-five grams of the milled apple samples were added to 50 mL of a metaphosphoric acid (30 g L^{-1}) and acetic acid (80 g L^{-1}) solution. The mixture was homogenized, sonicated, and centrifuged at 12,000 × g at 4 °C. For the AA determination, 1 mL of the supernatant diluted with 1 mL of the mobile phase (0.03 mol L^{-1} sodium acetate/acetic acid buffer, 5% methanol HPLC grade, pH = 5.8) was filtered through a 0.45 μm Millipore membrane and injected in the HPLC system. For the vitamin C quantification, 2 mL of the supernatant with 0.5 mL of DL-dithiothreitol solution (5 mg L^{-1} DTT in 2.6 mol L^{-1} potassium phosphate dibasic) reacted in the darkness for 2 h. Then, 1 mL of mobile phase was added to 1 mL of the reaction mixture, filtered through 0.45 μm Millipore membrane, and injected into a KONIK KNK-500-A Series liquid chromatography, coupled to a variable wavelength detector (Uvis 200, Konik Instruments, Barcelona, Spain). The results were expressed as mg kg^{-1} FW.

Sensory descriptive analysis

Eight panelists trained in sensory descriptive analysis of fresh-cut fruits evaluated the samples using continuous 10-cm scales with two anchor terms located at 1 cm from each edge. The evaluated attributes were general appearance (1 = Bad, 9 = Very good), browning (1 = Slight, 9 = Severe), characteristic flavor, sour taste, astringency, off-flavors and off-odors (1 = Slightly perceptible, 9 = Very perceptible), crispiness and juiciness (1 = Low, 9 = High).

Microbiological analysis

Total mesophilic microorganisms (TAM), psychrotrophic microorganisms (PSYC), yeasts (YST), and molds (MH) were determined according to Rodríguez Arzuaga et al. (2016). Samples (10 g) were aseptically homogenized with 90 mL of 0.1% peptone water. Serial tenfold dilutions were prepared and duplicates of at least three dilutions were plated on the corresponding media. TAM and PSYC were determined in Plate Count Agar (PCA, Merck, USA) incubated at 30 °C for 48 h, and 10 days at 7 °C, respectively. YST and MH were determined using Yeast Extract Glucose Chloramphenicol Agar (YGC, Merck, USA) and plates were incubated at 28 °C for 5 days. The results were expressed as (log CFU g^{-1}).

Microbiological results were fitted to the model proposed by Baranyi and Roberts (1994) using an in-house Excel Add-in package "DMFit" (ComBase) to determine the growth rate (Baranyi and Tamplin 2004). The model can describe growth curves, either with or without the stationary phase and with or without the lag phase (Eq. 1)

$$y(t) = y_0 + \mu_{max}F(t) - \ln(1 + \frac{e^{\mu_{max}F(t)} - 1}{e^{(y_{max} - y_0)}}) \quad (1)$$

where $F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{-h_0} - e^{(-vt-h_0)}\right)$; y(t) = concentration [log CFU g^{-1}] at time t; y_0 = initial concentration [log CFU g^{-1}]; y_{max} = maximum concentration [log CFU g^{-1}]; μ_{max} = maximum specific growth rate [(log CFU g^{-1}) d^{-1}]; υ = rate of increase of the limiting substrate, assumed to be equal to μ_{max}; $h_0 = (\mu_{max}{*}t_{lag})$; and t_{lag} = lag-phase duration [d].

The ability of the models to accurately predict the growth of microbial populations was evaluated by the root mean square error (RMSE) and the coefficient of determination (R^2). RMSE value is the difference between observed and predicted data and values close to zero are desirable as they indicate that predicted values are very close to the observed values. The R^2 value is the proportion of variability in experimental data explained by the model.

Gas composition

Gas composition in the package headspace was determined immediately after removing the samples from cold storage. The O_2 and CO_2 concentrations were determined by gas chromatography with a thermal conductivity detector, according to Piagentini et al. (2003).

Statistical analysis

The effects of chemical treatment, storage time and temperature, and their interactions were evaluated by ANOVA. Statistically significant differences between means were determined using Tukey's Multiple Range Test ($\alpha = 0.05$). The correlation among response variables was measured by Pearson's coefficient (r). All statistical analyses were performed using Statgraphics Centurion XV software (Addinsoft, New York, NY, USA).

Results and discussion

Physicochemical attributes

The firmness of the fresh-cut apples was not affected by the antioxidant treatment ($p > 0.05$) and was stable during storage at both temperatures (Table 1). By contrast, Cocci et al. (2006) reported a loss of firmness of about 50% in 'Golden Delicious' fresh-cut apples treated with a solution of 1% AA and 1% CA after only 1 day at 4 °C. Tissue softening of fresh-cut fruits occurs due to enzymatic hydrolysis of cell wall components, as well as water loss, osmotic changes, and other complex mechanisms related to tissue organization (Toivonen and Brummell 2008). Cell walls of apples are made of pectin, hemicellulose, and cellulose, along with some structural proteins. Their composition and structure vary with genetics, stages of development, and growth conditions. The action of pectin methylesterase (PME) and polygalacturonase (PG), among other enzymes, increases the porosity of the cell wall, decreases cell adhesion, and affects the texture of the fruit (Dheilly et al. 2016). Nevertheless, such tissue softening was not observed in the present study for T or C samples, nor in previous studies where the firmness of fresh-cut apples treated with AA and CA or with sucrose, AA and green tea extract remained constant during cold storage (Wu et al. 2012; Rodríguez-Arzuaga et al. 2013; Tappi et al. 2017). Variability of the plant material and storage conditions can probably explain why no significant fruit firmness reduction was observed during the assayed storage time.

According to Piagentini et al. (2012), enzymatic browning of 'Granny Smith' fresh-cut apples could be instrumentally reflected by a reduction of L* over storage (samples became darker) and an increase of a* (loss of green hue and development of reddish hues). Initially, the antioxidant treatment with YM significantly increased L* and reduced a* (Fig. 1). Although L* values of both samples decreased over storage time (samples became darker), T remained lighter (higher L* values) than C, at least until the seventh day at 2 °C and the fifth day at 10 °C (Fig. 1a). An opposite behavior was observed for a* (Fig. 1b), C_{ab}* (Fig. 1c) and b* (data not shown). The values of these parameters increased over storage, remaining lower for T than for C samples until day 7 and 5, at 2 and 10 °C, respectively. During the first 7 days of storage, the color parameters changed faster in the samples stored at 10 °C than at 2 °C (Fig. 1). These results indicate that both, the application of the chemical treatment and the storage at 2 °C significantly delayed browning development of fresh-cut apples. Chen et al. (2016) found that a 0.5% CA dipping treatment increased browning in 'Fuji' apple cubes, which suggests that the lower browning development obtained herein for the treated samples was not due to a pH reduction. In previous work, 'Granny Smith' fresh-cut apples were treated with an aqueous solution of 1% AA, 1% CA, and 1% $CaCl_2$. Initially, the treated apples presented lower a* values than the raw material and the control samples, but no significant differences were found among L* values of treated and control samples (Rodríguez Arzuaga et al. 2013). Thus,

Table 1 Instrumental firmness and sensory attributes of control (C) and treated (T) 'Granny Smith' fresh-cut apples during storage at 2 or 10 °C[a]

T (°C)	t (d)	Firmness (N)		Characteristic flavor		Sour taste[b]		Off-flavors		Off-odors	
		C	T	C	T	C	T	C	T	C	T
2	0	$63.8 \pm 4.2^{a,A}$	$62.7 \pm 2.3^{a,A}$	4.8 ± 0.9^{aA}	4.0 ± 0.7^{bA}	4.2 ± 0.9^{aA}	5.6 ± 0.6^{abB}	0.1 ± 0.2^{aA}	2.9 ± 1.1^{bB}	0.6 ± 0.6^{aA}	2.2 ± 0.6^{bB}
	1	$60.7 \pm 5.8^{a,A}$	$66.0 \pm 4.5^{a,A}$	4.9 ± 1.1^{aA}	4.1 ± 1.0^{bA}	5.2 ± 1.0^{aA}	6.1 ± 1.1^{abA}	0.5 ± 0.6^{aA}	1.0 ± 1.0^{aA}	0.2 ± 0.4^{aA}	0.3 ± 0.5^{aA}
	2	$64.8 \pm 3.5^{a,A}$	$62.2 \pm 3.1^{a,A}$	4.6 ± 0.9^{aA}	3.4 ± 1.0^{abA}	5.5 ± 0.9^{abA}	6.7 ± 0.8^{bA}	0.6 ± 0.9^{aA}	0.7 ± 0.6^{aA}	1.3 ± 0.8^{aA}	0.7 ± 0.7^{abA}
	4	$65.8 \pm 7.2^{a,A}$	$62.2 \pm 3.4^{a,A}$	3.9 ± 1.1^{aA}	3.5 ± 1.5^{abA}	4.1 ± 0.1^{aA}	4.9 ± 1.8^{abA}	0.8 ± 1.1^{aA}	1.3 ± 0.4^{abA}	1.0 ± 1.4^{aA}	0.8 ± 1.1^{abA}
	7	$62.7 \pm 3.5^{a,A}$	$60.8 \pm 10.0^{a,A}$	5.0 ± 0.9^{aA}	4.9 ± 0.8^{bA}	6.9 ± 0.9^{bB}	4.6 ± 1.0^{aA}	0.4 ± 0.5^{aA}	1.2 ± 1.0^{aA}	0.7 ± 0.8^{aA}	0.8 ± 0.8^{abA}
	10	$66.5 \pm 3.9^{a,A}$	$65.6 \pm 7.0^{a,A}$	4.4 ± 0.8^{aA}	4.5 ± 0.9^{bA}	5.5 ± 1.1^{abA}	4.8 ± 1.0^{aA}	0.2 ± 0.4^{aA}	0.5 ± 0.8^{aA}	0.4 ± 0.6^{aA}	1.3 ± 1.1^{abB}
	15	$66.8 \pm 2.7^{a,A}$	$63.0 \pm 2.2^{a,A}$	4.9 ± 1.3^{aB}	3.5 ± 1.4^{abA}	6.8 ± 1.4^{bB}	5.0 ± 1.6^{abA}	1.0 ± 1.3^{aA}	3.2 ± 0.9^{bB}	1.8 ± 1.7^{aA}	1.6 ± 1.1^{abA}
	18	$65.0 \pm 3.9^{a,A}$	$63.3 \pm 6.3^{a,A}$	5.0 ± 1.3^{aB}	2.2 ± 1.0^{aA}	6.9 ± 1.0^{bB}	5.5 ± 1.5^{abA}	0.8 ± 1.1^{aA}	2.9 ± 1.8^{bB}	1.5 ± 1.4^{aA}	1.8 ± 1.4^{abA}
10	0	$67.3 \pm 5.1^{a,A}$	$59.1 \pm 3.5^{a,A}$	4.9 ± 0.9^{aB}	3.5 ± 0.2^{abA}	–	–	0.6 ± 0.8^{aA}	3.1 ± 1.1^{bB}	0.5 ± 0.7^{aA}	2.3 ± 0.8^{abcdB}
	1	$59.8 \pm 4.3^{a,A}$	$63.2 \pm 8.8^{a,A}$	5.2 ± 1.0^{aA}	4.5 ± 1.0^{abA}	–	–	1.8 ± 0.8^{abA}	1.1 ± 1.0^{aA}	1.9 ± 0.7^{abcA}	1.4 ± 0.4^{abA}
	2	$65.3 \pm 4.5^{a,A}$	$59.8 \pm 1.9^{a,A}$	3.8 ± 1.0^{aA}	4.4 ± 0.8^{abA}	–	–	2.3 ± 1.0^{bA}	2.7 ± 1.0^{abA}	2.5 ± 1.1^{bcA}	1.7 ± 1.0^{abcA}
	5	$64.6 \pm 2.4^{a,A}$	$61.9 \pm 7.6^{a,A}$	5.0 ± 0.9^{aA}	4.9 ± 1.1^{bA}	–	–	2.5 ± 0.7^{bA}	2.6 ± 0.8^{abA}	2.7 ± 1.0^{cA}	3.0 ± 1.1^{cdA}
	7	$64.7 \pm 5.3^{a,A}$	$61.5 \pm 4.8^{a,A}$	4.1 ± 1.1^{aA}	3.3 ± 1.1^{aA}	–	–	2.3 ± 0.9^{bA}	3.8 ± 1.1^{bB}	2.7 ± 0.4^{cA}	2.6 ± 0.6^{bcdA}
	9	$66.0 \pm 4.8^{a,A}$	$64.8 \pm 6.3^{a,A}$	–	–	–	–	–	–	1.0 ± 1.0^{abA}	1.1 ± 1.1^{aA}
	12	$61.8 \pm 5.3^{a,A}$	$59.1 \pm 4.1^{a,A}$	–	–	–	–	–	–	1.2 ± 1.1^{abA}	3.2 ± 0.3^{dB}
	15	$61.4 \pm 4.8^{a,A}$	$61.0 \pm 5.1^{a,A}$	–	–	–	–	–	–	2.3 ± 0.5^{bcA}	1.2 ± 1.1^{abA}

Mean ± standard deviation. Different capital letters and lowercase letters indicate significant differences ($p < 0.05$) by Tukey's test, among samples (T and C) and storage times, respectively

[a]The flavor attributes of the samples stored for more than 7 days at 10 °C were not evaluated by the sensory panel

[b]Sour taste of samples stored at 10 °C was 5.3 ± 1.0 in average and constant ($p > 0.05$) for both samples throughout storage

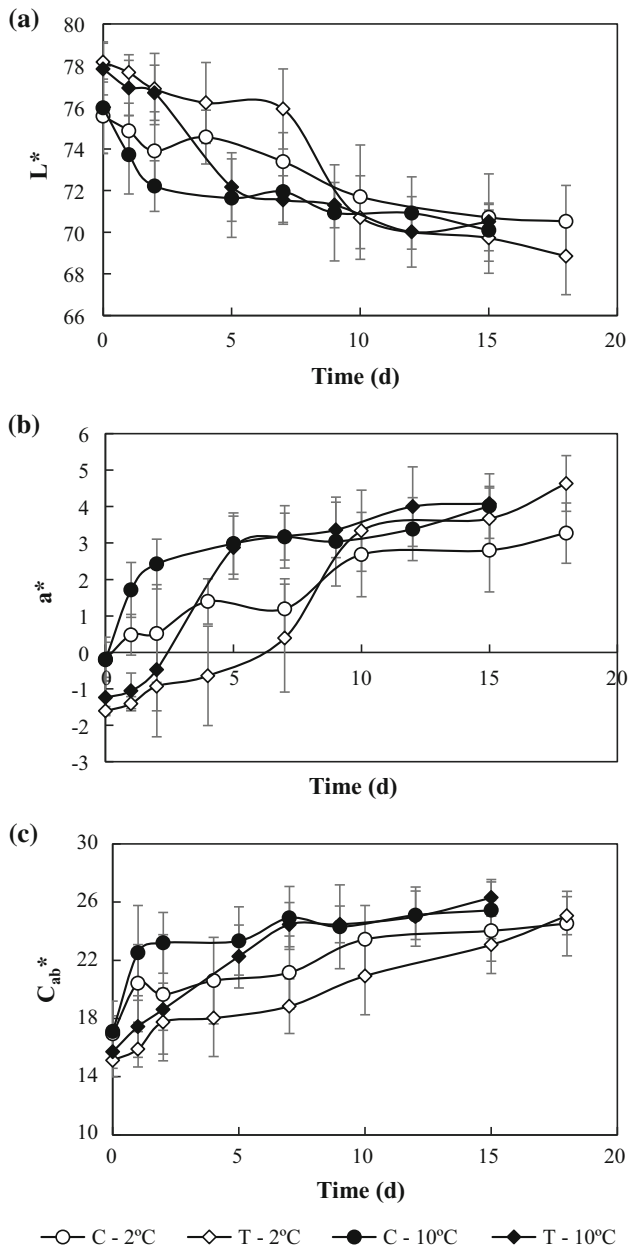

Fig. 1 L* (**a**), a* (**b**) and C_{ab}*(**c**) instrumental color parameters of treated (T) and control (C) 'Granny Smith' fresh-cut apples, during storage at 2 and 10 °C. Bars represent standard deviation

considering that the CA and AA concentrations were similar to the ones used in the current study, the better initial color values and the reduction in the quality loss obtained herein could be attributed to the action of YM. Pirovani et al. (2015) reported the inhibitory effect of YM infusion, alone or in combination with CA and/or AA, on polyphenol oxidase extracts from 'Princesa' apples. The inhibitory activity of YM and other natural substances such as honey, green tea, and onion extract, can be explained by their high concentration of phenolic compounds, which act as natural antioxidants (Pirovani et al. 2015).

Bioactive compounds and antioxidant capacity

At day 0, the TPC of T was 59% higher than C (557 mg kg^{-1}). The initial TPC obtained for the control samples agreed with previously published values (Tappi et al. 2017; Chen et al. 2016). The phenolic compounds of YM, incorporated into the apples during the chemical treatment, could explain the differences between samples. The TPC of T was greater than C until the end of storage at both

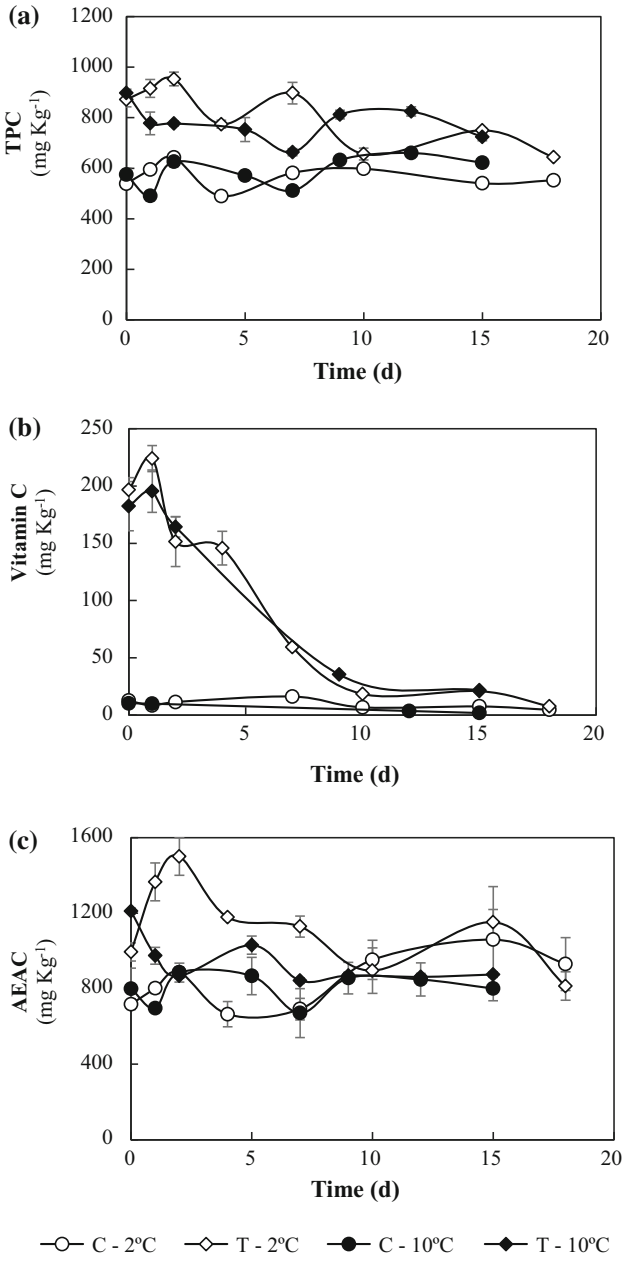

Fig. 2 Total phenolic content (TPC) (**a**), vitamin C content (**b**) and antioxidant capacity by DPPH* method (AEAC) (**c**) of treated (T) and control (C) 'Granny Smith' fresh-cut apples, during storage at 2 and 10 °C. Bars represent standard deviation

temperatures (Fig. 2a). Similarly, Tappi et al. (2017) explained that the higher concentration of TPC on fresh-cut apples vacuum impregnated with sucrose, AA and green tea extract was due to the action of AA against the oxidation of the native phenolics of apples and green tea extract. The TPC of both samples increased during the first 2 days of storage at 2 °C. Amodio et al. (2014) also obtained an increase in the TPC of 'Stark Red' apples in the first 2 days of storage at 5 °C. Often, fresh-cut fruits and vegetables increase their TPC in the first days of storage, due to several abiotic stresses that occur during minimal processing (peeling, decoring, cutting). These stresses promote the activity of the phenylalanine ammonia-lyase, responsible for the first step in the phenylpropanoid metabolism (Saltveit 2000). After 1 day at 10 °C, the TPC of C and T decreased by 15 and 13%, respectively. After the initial decline, the TPC of both samples remained constant. By the end of storage at 10 °C (day 15), T had a 16% higher TPC than C. The initial differences found in the TPC of C and T were lower by the end of storage at both temperatures, although the phenolic concentration was always higher in T than in C (Fig. 2a).

The changes in AA (data not shown) and vitamin C (Fig. 2b) contents followed similar patterns for both samples and storage temperatures. T had higher ($p < 0.05$) AA and vitamin C concentrations than C until day 15 at both temperatures. The AA content of C decreased from 10 to 2 mg kg^{-1} throughout storage at both temperatures, while the vitamin C content was between 16 and 5 mg kg^{-1} at 2 °C and between 10 and 2 mg kg^{-1} at 10 °C (Fig. 2b). The initial AA and vitamin C contents of T were 182 and 197 mg kg^{-1}, respectively. At both temperatures, the AA and vitamin C contents of T samples increased on day 1 and then decreased until the end of storage. Cocci et al. (2006) reported that fresh-cut apples treated with a 1% AA solution had AA values 20-fold higher than the controls, due to the acid uptake, but they observed a 60–80% reduction after only one day of storage.

The initial antioxidant capacity determined by the DPPH* method (AEAC) was 759 mg kg^{-1} for C and 1066 mg kg^{-1} for T. The AEAC of C samples remained constant throughout storage at both temperatures ($p > 0.05$). On the other hand, T samples showed an increase of AEAC during the first 2 days at 2 °C and a reduction during the same period at 10 °C (Fig. 2c). This behavior was consistent with the one obtained for TPC and AA and vitamin C contents (Fig. 2a, b). From day 2, AEAC in T remained constant at both temperatures. At 2 °C, T had higher AEAC ($p < 0.05$) than C until day 7, while there were no significant differences among samples between days 10 and 18. In previous work, a solution of 1% AA + 1% CA applied to 'Granny Smith' fresh-cut apples did not increase significantly the antioxidant capacity by the DPPH* method (Seipel et al. 2009). This result indicates that the addition of YM played a significant role in the increment of the antioxidant activity of the samples.

According to the FRAP results, the antioxidant capacity of both samples (2161 ± 223 and 2816 ± 410 µmol Fe^{2+} kg^{-1}, for C and T, respectively) remained constant ($p > 0.05$) for 15 days at 10 °C, although T was always higher than C. On the other hand, the FRAP antioxidant capacity of T and C declined after 1 day at 2 °C and then remained constant. After 15 d at 2 or 10 °C, FRAP of T samples was 20 and 26% higher than C, respectively.

Significant and positive Pearson's correlation coefficients (r, $p < 0.01$) were obtained between the antioxidant capacity of the fresh-cut apples and their bioactive compounds concentration.

Descriptive sensory analysis

The visual appearance of fresh-cut fruits strongly affects the purchase decision, since it is the most immediately obvious attribute to the consumer. As shown in Fig. 3,

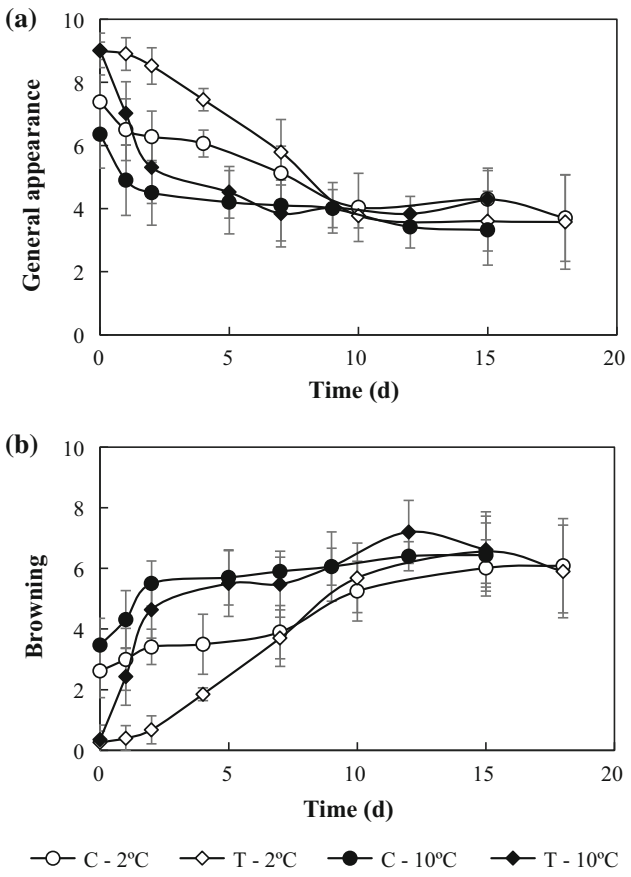

Fig. 3 General appearance (**a**) and browning (**b**) of treated (T) and control (C) 'Granny Smith' fresh-cut apples, during storage at 2 and 10 °C. Bars represent standard deviation

general appearance decreased, while browning increased with storage time. At day 0, C developed more browning and scored lower for general appearance than T ($p < 0.05$). These differences between samples were significant until the seventh day at 2 °C and the fifth at 10 °C. From those days through the end of storage, no differences ($p > 0.05$) were found among samples in any of these visual sensory attributes, in agreement with the instrumental color results (Fig. 1). General appearance and browning correlated significantly ($p < 0.001$) with the color parameters ($| r | \geq 0.87$), evidencing that the instrumental color measurement accurately reflects enzymatic browning in fresh-cut apples.

A highly significant ($p < 0.001$) negative correlation coefficient (r = -0.95) was found between the attributes general appearance and browning. This suggests that inhibiting browning would improve the general appearance of the fresh-cut apples. Moreover, the differences obtained for both samples and temperatures indicate that the chemical treatment had a beneficial effect on the appearance of the fresh-cut apples during storage and that its application together with the use of lower temperatures would prolong their shelf life. A general appearance score of 5 has been selected in previous studies as a limit below which the appearance of fresh-cut fruits and vegetables is no longer acceptable (Piagentini et al. 2005). Considering this, the YM treatment increased the time to reach the limit value of the acceptable general appearance of fresh-cut 'Granny Smith' apples by 2–5 d compared to control samples, at both storage temperatures (Fig. 3).

Although consumers make their first purchase based mainly on appearance, repeat purchases also depend on sensory attributes related to flavor, odor, and texture (Putnik et al. 2017b). In the current study, the characteristic flavor was not affected ($p > 0.05$) by storage for 18 days at 2 °C and 7 days 10 °C. The chemical treatment did not affect ($p > 0.05$) the characteristic flavor of the apples for 10 days at 2 °C (Table 1). Initially, the dipping solution increased the sour taste, off-flavors, and off-odors, but after only 1 day at 2 °C, the judges were not able to detect differences ($p > 0.05$) among samples (Table 1). For the apples stored at 10 °C, the chemical treatment and storage time did not affect ($p > 0.05$) the sour taste, which was 5.3 ± 1.0.

The crispiness is a desirable sensory attribute that suggests freshness, and, together with juiciness, is an important characteristic affecting consumer acceptability of apples (Cortellino et al., 2017). Crispiness and juiciness only depended on the storage temperature and were independent ($p > 0.05$) from the chemical treatment and storage time. The fresh-cut apples scored 8.0 ± 0.8 and 7.4 ± 1.0 for crispiness, and 7.7 ± 0.9 and 7.1 ± 1.0 for juiciness, at 2 °C and 10 °C, respectively. Therefore, the samples stored at 2 °C kept higher crispiness and juiciness. Astringency (1.5 ± 0.8) was independent of the three studied factors (temperature, time, and chemical treatment).

Microbiological analysis

The changes in TAM, PSYC, MH, and YST populations were accurately described by the Baranyi and Roberts (1994) model, with $R^2 > 0.88$ and RMSE < 0.22, for C and T samples stored at 2 and 10 °C.

TAM and PSYC showed similar growing models for both samples, although there were significant differences between storage temperatures (Fig. 4). The TAM and PSYC populations followed no asymptotic models for C and T samples at 2 °C. On the other hand, at 10 °C these microbial populations fitted a linear model for C and a no lag-phase model for T.

At 2 °C, TAM counts remained constant (1.27–1.57 log CFU g^{-1}) from day 0 to 12 and 14 (t_{lag}) for C and T apples, respectively. Then, the counts increased in both samples through the end of storage, reaching similar values (4.25 log CFU g^{-1}), and being the μ_{max} significantly higher for T (0.973 log CFU g^{-1} d^{-1}) than C (0.485 log CFU g^{-1} d^{-1}) at 2 °C. At 10 °C, there were no lag phases, and TAM increased faster on T samples for 5 days and then remained constant at 4.25 log CFU g^{-1} until the end of storage. Although TAM in C apples had a lower μ_{max} value, the counts obtained after 15 days at 10 °C were similar to the counts obtained in T (Fig. 4). Wu et al. (2012) analyzed fresh-cut 'Fuji' apples treated with 0.5% AA, 0.5% CA and 0.5% $CaCl_2$ during storage at 4 °C and reported similar initial TAM values (1.2 log CFU g^{-1}) but higher counts (5.6 log CFU g^{-1}) after 14 d.

PSYC showed the same trends as TAM for all samples and storage temperatures (Fig. 4). At 2 °C, t_{lag} and μ_{max} values were higher for T (14.18 d and 0.840 log CFU g^{-1} d^{-1}) than C samples (8.07 d and 0.307 log CFU g^{-1} d^{-1}). It should be noticed that at 2 °C, TAM and PSYC in T samples had similar t_{lag} (14.78 and 14.18 d, respectively), but in C samples PSYC had lower t_{lag}. At 10 °C, PSYC in T samples increased faster during the first 9 days and remained constant between days 9 and 15 (y_{max} = 3.95 log CFU g^{-1}). PSYC population of C increased during the 15 days of storage at lower μ_{max} but reaching the same final count than T.

At 2 and 10 °C, MH of C and T samples followed the no asymptotic model. No differences ($p > 0.05$) were found in the MH counts of the samples, until the tenth day of storage at both temperatures. The maximum MH count (2.2 log CFU g^{-1}) was obtained for T after 18 days at 2 °C.

Similarly, YST counts followed no asymptotic models for C and T samples at 2 °C. On the other hand, YST

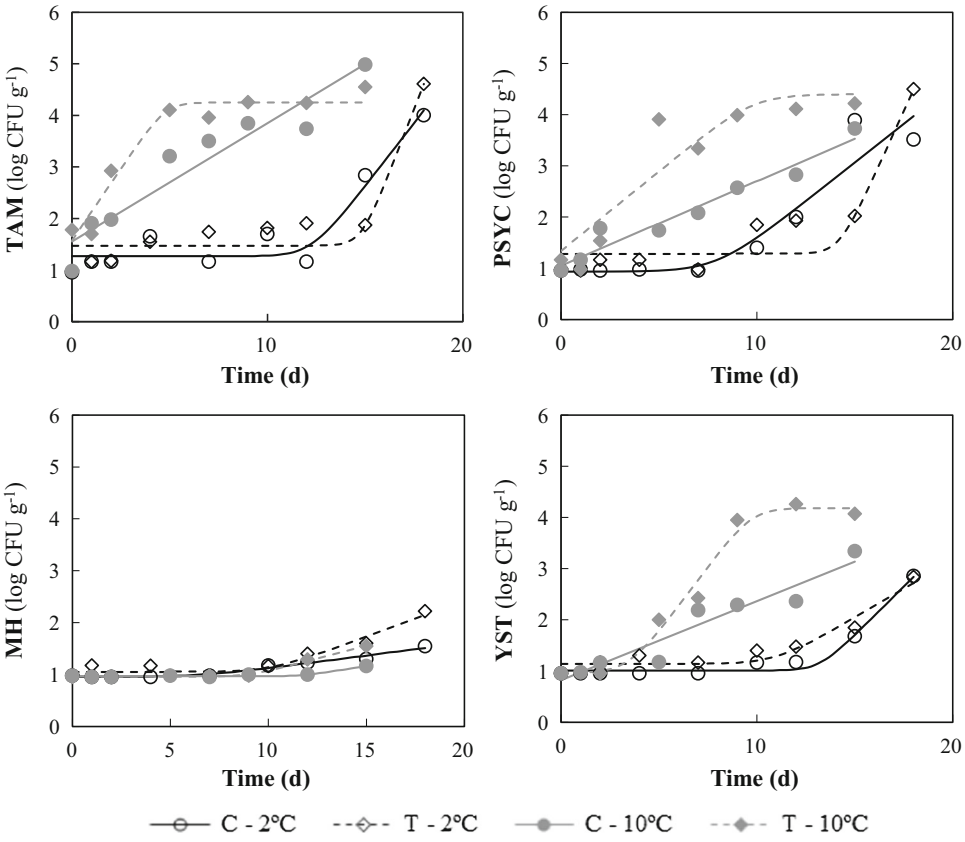

Fig. 4 Total mesophilic (TAM) and psychrotrophic (PSYC), molds (MH) and yeasts (YST) growth curves of treated (T) and control (C) 'Granny Smith' fresh-cut apples, during storage at 2 and 10 °C. Symbols represent the experimental values and lines represent the growth curves predicted by the Baranyi and Roberts (1994) model

populations fitted the linear model for C, and a complete model, with a t_{lag} of 3.5 days, for T, at 10 °C. At 2 °C, YST remained constant until day 13 and 11 in C and T samples, respectively. From those t_{lag}, YST counts increased (faster for T samples) reaching the same value for both samples (2.8 log CFU g^{-1}) on day 18.

Gas composition

Initially, the O_2 concentration in the packages of both samples was 21.1% (O_2 concentration in air). At 2 °C, the O_2 concentration declined in both samples ($p > 0.05$) until day 2 (Fig. 5). From that storage time, packages containing T samples kept a lower O_2 concentration. The same behavior was found for the O_2 concentration of samples stored at 10 °C. By the end of storage, the O_2 concentration in C was 18.6% at 2 °C and 15.6% at 10 °C, and in T was 17.7% and 13.7% for samples stored at 2 °C and 10 °C, respectively.

The initial CO_2 concentration in the packages was the one in the air (0.032%) for both samples. Until day 1, the CO_2 concentration was similar for both samples at both temperatures (Fig. 5). At 2 °C the CO_2 concentration increased slower than at 10 °C for both samples, being the CO_2 concentration always higher in T. By the end of

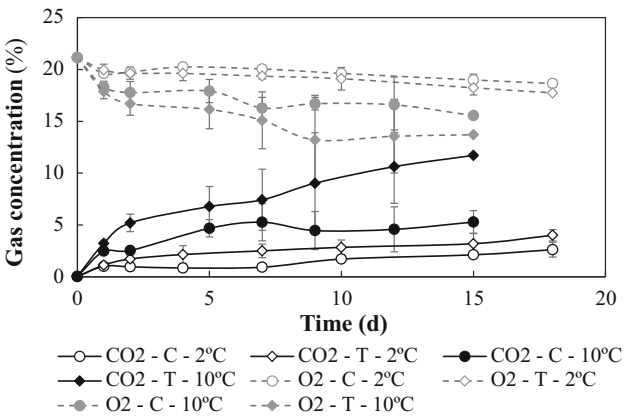

Fig. 5 O_2 and CO_2 concentrations in the packaging headspace of treated (T) and control (C) 'Granny Smith' fresh-cut apples, during storage at 2 and 10 °C. Bars represent standard deviation

storage, C had 2.6% of CO_2 at 2 °C and 5.3% at 10 °C, while T had 4.0 and 11.7% at 2 and 10 °C, respectively.

As expected, at 10 °C, the final concentrations of O_2 were lower and of CO_2 higher than at 2 °C, for both samples. These differences could be due to a predominant effect of temperature on the fresh-cut apple's respiration rate over packaging permeability (Fig. 5).

Moreover, T showed higher concentrations of CO_2 and lower concentrations of O_2 than C, at the end of storage

(Fig. 5). These results could be explained by an increase in the respiration rate as a consequence of the treatment, and/or by the oxygen consumption in the AA oxidation. Yan et al. (2017) obtained a higher O_2 decline and CO_2 increase, on fresh-cut apples treated with 1% AA than on samples washed with water, during storage at 5 °C. Also, Rojas-Graü et al. (2007) reported that 'Fuji' slices treated with 1% AA consumed the O_2 in the headspace faster that the slices treated with 1% N-acetylcysteine, during storage at 4 °C. Gil et al. (1998) also determined that an AA treatment increased the respiration rate of 'Fuji' slices packed in air and decreased the respiration rate of samples packed in an O_2 free atmosphere. By contrast, Tappi et al. (2017), who treated minimally processed apples with solutions containing green tea extract, sucrose, and AA by vacuum impregnation, obtained a reduction in the CO_2 accumulation and the O_2 consumption within the headspaces, in comparison with untreated samples, during storage at 10 °C.

Conclusion

The 'Granny Smith' fresh-cut apples dipped in the proposed antioxidant solution containing YM, showed a slower browning development (as determined by the sensory analysis and color measurement), an enhanced content of the bioactive compounds, and a higher antioxidant capacity than the control samples for at least 7 days at 2 °C and 5 days at 10 °C.

The dipping solution did not affect the characteristic flavor of the apples. The instrumental firmness of fresh-cut apples was neither affected by the treatment, or the storage time and temperature. Reducing the storage temperature from 10 °C to 2 °C improved the crispiness and juiciness of the fresh-cut apples, regardless of the applied treatment. The maximum microbial counts reached by the end of storage at both temperatures were lower than 4.25 log CFU g^{-1}, indicating that the treatment with YM maintains the microbial stability of the fresh-cut apples.

As expected, storing the samples at the lower temperature delayed the loss of quality of fresh-cut apples (a slower enzymatic browning development and microbial growth, and a slower reduction of the bioactive compounds concentration and sensory quality). These results remark the importance of temperature control throughout the cold chain.

The use of YM combined with ascorbic and citric acids can provide 'Granny Smith' fresh-cut apples with good storage stability for at least 7 days a 2 °C, improving their antioxidant capacity and the content of healthy compounds, throughout the storage at 2 °C and 10 °C.

Further studies should focus on economic viability as well as on the application of YM to different fresh-cut fruits and vegetables to fully understand its potential to maintain the quality of minimally processed produce.

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