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Mild heat treatments before minimal processing reduce browning susceptibility and increase total phenolic content of low-chill apple cultivars

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Abstract

Three low-chill apple cultivars ("Caricia," "Eva," and "Princesa") were subjected to hot water treatments as a postharvest abiotic stress for quality retention. The effects of heating time and temperature, storage time, and apple cultivar were investigated on total phenolic content (TPC), firmness, color and polyphenol oxidase (PPO), and peroxidase (POD) activities. Apples were heat treated in water at 40–50°C for 20–90 min, stored at 2°C during 24 hr, and minimally processed. Samples were analyzed immediately and after 7 days at 2°C. Apple cultivar and storage time significantly affected the evaluated attributes. Heat treatments did not improve the firmness, color, or TPC of "Caricia" and "Princesa." While in "Eva" heat treatments increased TPC by 70%, reduced PPO and POD activities and prevented browning development, after 7 days. In conclusion, mild heat treatments could improve the quality and bioactive compound content of low-chill fresh-cut apples. Nevertheless, the different responses among cultivars should be accounted for.

Practical applications

The three low-chill apple cultivars, "Caricia," "Eva," and "Princesa," showed varying responses toward postharvest heat treatment. The TPC of the three studied cultivars was increased by the heat treatment at the minimal processing day. The application of mild heat treatments before minimal processing proved to be an effective postharvest tool to delay enzymatic browning and increase TPC of fresh-cut "Eva" apples, after 7 days of cold storage.

1 | INTRODUCTION

Argentina is the largest apple producer in South America. However, the production in mild winter areas has been historically limited by the minimum chilling hours required to grow apples (above 700 hr). As a result of breeding work, there are several new cultivars with lower chill requirement (less than 400 hr per year), such as "Caricia," "Eva," and "Princesa." These cultivars allowed the expansion of apple production into warmer areas, as a productive alternative for low-chill regions (Castro, Cerino, Gariglio, & Radice, 2016).

The fresh-cut fruit and vegetable industry is in constant growth due to the increasing consumer demand for healthy and convenient food products (Allende, Tomás-Barberán, & Gil, 2006). Minimal processing could provide an alternative to increase the value of the low-chill apple cultivars. Piagentini and Pirovani (2017) compared the suitability for minimal processing of two important commercial cultivars, "Granny Smith" and "Red Delicious," and three low-chill cultivars, "Caricia," "Eva," and "Princesa." The authors concluded that "Princesa" and "Granny Smith" were the most suitable cultivars, due to their lower browning susceptibility and higher firmness and

juiciness. Thus, even though "Princesa" has shown a potential for this type of processing, strategies need to be developed in order to face the challenges related to the commercial production of fresh-cut apples from low-chill cultivars.

Enzymatic browning, the main cause of quality loss in fresh-cut apples, is known to occur through the enzyme-catalyzed oxidation of ortho-phenols to quinones and the later polymerization of quinones to brown pigments (Huque, Wills, Pristijono, & Golding, 2013). Polyphenol oxidase (PPO) catalyzes the hydroxylation and oxidation of phenols. Peroxidase (POD) is also relevant to enzymatic browning since diphenols can act as reducing substrates, although its activity is limited by the availability of electron acceptor compounds such as hydrogen peroxide (Jang & Moon, 2011).

Hot water treatments have emerged as a safe, free of chemical residues, and effective technique that induces defense responses in the harvested fruit and vegetables (Spadoni, Guidarelli, Phillips, Mari, & Wisniewski, 2015). The application of abiotic stresses, like heat shock, affects the biosynthesis of terpenes, phenolic, and nitrogen compounds, which has a significant effect on the healthy potential of fruit and vegetables (Jacobo-Velázquez & Cisneros-Zevallos, 2018). Hot water treatments can also be used as an enzymatic browning control method, since PPO and POD can be inhibited by temperature.

Treatments by immersion in hot water have been used to prevent quality loss in several fruit and vegetables. Paillart, Otma, and Woltering (2017) reported that a heat shock treatment (45°C, 180 s) significantly reduced pinking in fresh-cut lettuce. Hot water treatment (48°C, 10 min) reduced browning, increased firmness retention, and enhanced antioxidant activity of peaches during cold storage (Huan et al., 2017). Rodoni et al. (2016) found that mild heat treatments (45°C, 3 min) extended the shelf life of organic fresh-cut peppers.

Mild heat treatments outcomes in apples have shown great variability, depending on the cultivar and the treatment conditions (Aguayo, Requejo-Jackman, Stanley, & Woolf, 2015; Kim, Smith, & Lee, 1993; Li, Li, Wang, Jiang, & Ban, 2013; Spadoni et al., 2015). In summary, these treatments could be used as a postharvest tool to increase the suitability for minimal processing of low-chill apple cultivars (increasing firmness and bioactive compounds) and reduce the quality loss during the storage (lower browning development). Nevertheless, to the best of the authors' knowledge, mild heat treatments have not been evaluated yet on the above mentioned cultivars. Therefore, this study aimed to assess the application of mild heat treatments to three low-chill apple cultivars, and to model the effects of heating time and temperature on the firmness, enzymatic browning, and phenolic content of the apples immediately after minimal processing and after 7 days of cold storage.

2 | MATERIALS AND METHODS

2.1 | Plant material

Fruit from three apple tree cultivars (*Malus domestica* Borkh) with low-chill requirement, "Caricia" (pH 3.5, soluble solids = 13.4%, firmness = 48.1 N), "Eva" (pH = 3.8, soluble solids = 13.8%, firmness = 53.9 N), and "Princesa" (pH = 3.5, soluble solids = 14.2%, firmness = 66.1 N), were used in the study. The apples were harvested on the Experimental Field of Universidad Nacional del Litoral (Santa Fe, Argentina), at optimum maturity (starch index value of 4), transported immediately to our Institute, and stored at 2°C and 90%–95% RH, until used.

2.2 | Sample preparation

Apples of uniform size from each cultivar were heat treated at different time/temperature combinations, according to the experimental design, by dipping them in a temperature controlled water bath with stirring. After the heat treatment, the apples were drained and stored at 2°C and 90%–95% RH. After 24 hr of cold storage, thermally treated (TT) apples together with unheated (U) apples were peeled, cored, and cut into eight wedges using stainless steel sharp knives. Wedges from TT and U apples were packed in polyethylene terephthalate (PET)-sealed containers. Half of TT and U samples were immediately analyzed and the remaining wedges were stored at 2°C for 7 days, to evaluate the effect of the treatments after a storage period.

2.3 | Experimental design

A factorial design was performed to study the effect of apple cultivar, heat treatment, and storage time. The variables and their levels were heating temperature (*T*: 40, 45, 50°C), heating time (*t*: 20, 55, 90 min), storage time (0 and 7 days), and apple cultivar ("Caricia," "Eva," and "Princesa"). A multifactorial ANOVA (factors: *T*, *t*, cultivar, storage time) was performed for each evaluated response. According to the results of the ANOVA, the cultivar and storage time were significant factors; therefore, independent models were developed for these factors. Response surface methodology with a three-level factorial design with two factors (3^2) and two replicates of the central point (11 experimental runs) was used to study the effect of heat treatment on fresh-cut apples for each apple cultivar and storage time. A second-order polynomial equation was proposed to model each response (Equation 1):

$$Y_k = \beta_{k_0} + \sum_{i=1}^{2} \beta_{k_i} X_i + \sum_{i=1}^{2} \beta_{k_{ii}} X_i^2 + \beta_{k_{ij}} X_i X_j \quad (1)$$

where Y_k is the response variable; β_{k_0} is the model constant; β_{k_i} and $\beta_{k_{ii}}$ are the linear and quadratic coefficients, respectively; $\beta_{k_{ij}}$ is the coefficient for the interaction effect; and X_i and X_j are the independent variables (*t*: time and *T*: temperature).

The responses were expressed as a function of the values obtained for the U samples, in order to account only for the effects of thermal treatment, avoiding the effects of minimal processing. The experimental responses (Y_k) studied were the retention of firmness (RF) and total phenolic content (RTPC), the residual activity of PPO and POD enzymes (RPPO and RPOD, respectively) and the relative

changes of color parameters (δL^*, δa^*, δb^*, δC^*_{ab}, and δh_{ab}). RF, RPPO, RPOD, and RTPC were calculated for day *i* according to Equation (2):

$$RA = (A_{TTi}/A_{Ui}) \times 100, \quad (2)$$

where RA represents the retention of the attribute *A* (*A* = *F*, TPC, PPO, POD); A_{TTi} and A_{Ui} represent the attributes measured in TT and U samples, respectively, at day *i* (*i*: 0 or 7 days).

The color parameter relative changes (δQi) were calculated according to Equation (3).

$$\delta Qi\,(\%) = \left[\left(Q_{TTi} - Q_{Ui}\right)/Q_{Ui}\right] \times 100 \quad (3)$$

where Q_{TTi} are the color parameter values of the TT samples (L^*_{TT}, a^*_{TT}, b^*_{TT}, C^*_{abTT}, and h_{abTT}) and Q_{Ui} are the color parameter values of the U samples (L^*_U, a^*_U, b^*_U, C^*_{abU}, and h_{abU}), at the corresponding storage time *i* (0 or 7 days).

2.4 | Firmness

Firmness of the apple wedges was determined through penetration tests. A TA-XT Plus texture analyzer (Stable Micro Systems Ltd., Surrey, UK) with a cylindrical probe of 11-mm diameter and a 1 mm/s penetration and post-run speed (penetration distance: 10 mm, calibration cell load: 50 N) was used. The maximum force was recorded and the results were expressed in Newton (N). Firmness was measured in fifteen wedges per sample.

2.5 | Color

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

2.6 | PPO and POD activities

The enzyme activities were determined on the TT and U samples from the three apple cultivars, after 0 and 7 days of cold storage, according to Soysal (2009).

Extracts were obtained by homogenization of 100 g of apple samples and 150 ml potassium phosphate buffer (20 mM, pH = 7.2) containing Triton X100 (0.1%) and polyvinylpolypyrrolidone (5%), for 3 min at 4°C. The homogenates were centrifuged (12,000x *g*, 20 min, 4°C) and the supernatants were separated and used for enzyme activity determination. The protein content in the enzymatic extracts was determined according to Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as standard, since the value was required for the calculation of the enzyme activity. The protein extract (0.05 ml) was added to 0.95 ml water and 5 ml Lowry reagent (100 parts of 2% Na_2CO_3 in NaOH 0.1 M, 1 part of 1% $CuSO_4$.5 H_2O and 1 part of 2% sodium–potassium tartrate), and incubated at room temperature for 15 min. Then, 0.5 ml Folin–Ciocalteu reagent (diluted 1:3) were added. After 30 min, the absorbance was read at 680 nm against a reagent blank.

The PPO (EC 1.10.3.1) activity was determined by measuring the increase in absorbance at 405 nm with a spectrophotometer Genesys 10S UV–VIS (Thermo Scientific, Germany). The reaction mixture consisted of 0.075 ml the enzyme extract, 0.75 ml sodium acetate (pH = 5.5), 0.62 ml distilled water, and 0.056 ml 800 mM catechol as enzyme substrate.

The POD (EC 1.11.1.7) activity was evaluated by measuring the increase in absorbance at 470 nm. The reaction mixture consisted of 1.2 ml the enzyme extract, 0.4 ml potassium phosphate monobasic (100 mM) with 0.0025% (m/v) guaiacol, and 0.024 ml hydrogen peroxide as enzyme substrate.

For both enzymes, 1 unit of enzyme activity (U) was expressed as one absorbance increment (in the conditions in which the assay was carried out) per minute and milligram of protein extract (1 U = ΔA/min mg^{-1}).

The residual activity for each enzyme was determined by the ratio between the enzyme activity of the heat-treated sample (A_{TT}) and the enzyme activity of the U sample (A_U), expressed as percentage. All determinations were performed in triplicates.

2.7 | Total phenolic content

Two extracts per sample were obtained as described by Rodríguez Arzuaga, Salsi, and Piagentini (2016) and each extract was analyzed in triplicates.

TPC was determined by the Folin–Ciocalteu method modified by Singleton and Rossi (1965). Aliquots of extracts were allowed to react for 30 min at room temperature (ca. 25°C) before absorbance was measured at 760 nm in a Genesis 10S UV–Vis spectrophotometer (Thermo Scientific, Germany). TPC results were expressed as mg of gallic acid equivalents in a kilogram of fresh apple weight (mg kg^{-1}).

2.8 | Statistical analysis

A multifactorial ANOVA was performed for each response variable determining the effect of cultivar, storage time and heat treatment time, and temperature, after running tests to verify the ANOVA assumptions. Second-order polynomial equations were fitted to the experimental data and the coefficients of the equations for the response variables were obtained for each apple cultivar and storage time. The linear stepwise regression procedure was used for the elimination of nonsignificant terms in each model. Lack of fit and coefficient of determination (R^2) were calculated to verify the model adequacy. Significant differences among two samples (heat-treated and U samples or Day 0 and Day 7) were evaluated by *t*-tests ($p < .05$). Data were analyzed using Statgraphics Centurion XV software (Addinsoft, New York, NY, USA).

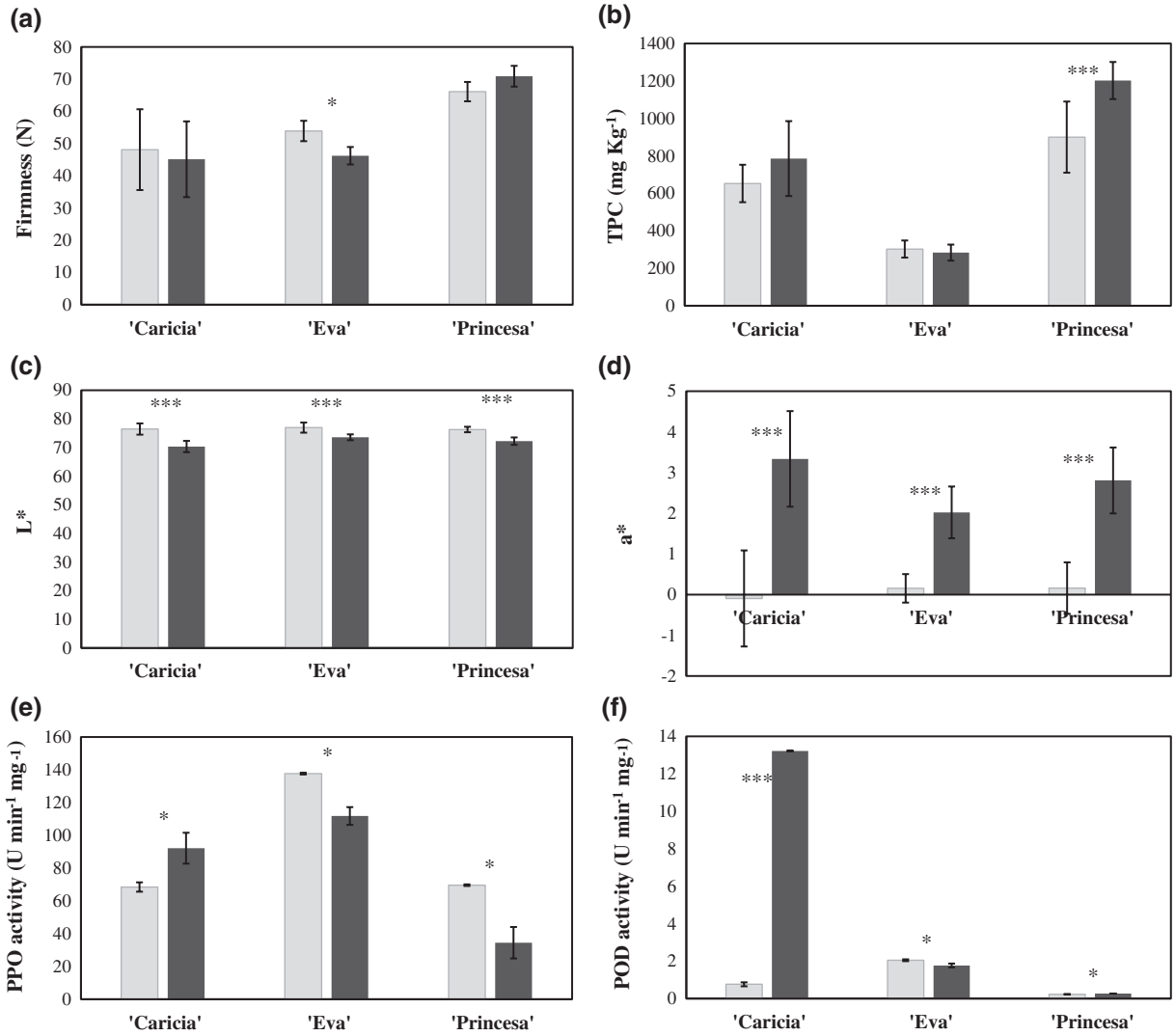

FIGURE 1 Firmness (a), total phenolic content, TPC (b), and instrumental color parameters L^* (c) and a^* (d), polyphenol oxidase, PPO (e), and peroxidase, POD (f) activities of unheated "Caricia," "Eva," and "Princesa" fresh-cut apples, after 0 (▭) and 7 (▬) days of storage at 2°C. *; ***: Significant differences between storage times for each apple cultivar at $p < .05$ and 0.001, respectively. Bars represent standard deviation

3 | RESULTS AND DISCUSSION

3.1 | Effect on firmness

First, the effect of storage time on the firmness of the U samples (U) was evaluated at the minimal processing day and after 7 days at 2°C. Firmness of U "Eva" apples decreased ($p < .05$) by 15% after 7 days. By contrast, firmness of "Caricia" and "Princesa" remained constant ($p > .05$) after 7 days at 2°C (Figure 1a).

The effects of heating time and temperature on the firmness retention were modeled for each apple cultivar and storage time.

The models obtained for the firmness retention of "Caricia" at Day 0 (RF_{C0}) and 7 (RF_{C7}) adequately described the experimental data (Table 1). At Day 0, the interaction between temperature (T) and time (t) was significant (Equation 4). At low T (about 40°C), RF was independent of t, while at higher T, RF decreased over time. After a 40°C–20 min treatment, the firmness of "Caricia" fresh-cut apples at Day 0 was 5.9% higher than the firmness of the untreated apples (RF_{C0} = 105.9%). While RF_{C0} of the apples treated at 50°C for 90 min was 67.9%, the firmness was reduced by about 32% on Day 0. According to the model obtained for the samples stored for 7 days (Equation 5), increasing both T and t reduced RF. Equation (5) showed that the retention of firmness of "Caricia" fresh-cut apples treated at 50°C for 90 min was 72.3% after 7 days of storage, while at Day 0 RF was 67.9%. This indicated that the reduction of firmness of the treated samples was lower after storage. At Day 0, the U samples had a firmness of 48.1 N and after a heat treatment of 50°C during 90 min the firmness was reduced to 32.7 N. After storing both samples for 7 days, the firmness of the treated apples did not change (32.6 N), while the firmness of the U samples decreased to 45.1 N.

$$RF_{C0}(\%) = 51.9 + 1.4T + 1.9t - 0.05Tt \quad (4)$$

TABLE 1 Analyses of variance of regression models for the retention of firmness, retention of total phenolic content, and residual enzyme activities of fresh-cut apples at the minimal processing day (0 day) and after 7 days at 2°C

		"Caricia"				"Eva"				"Princesa"			
Time (d)	Variation source	RF	RTPC	RPPO	RPOD	RF	RTPC	RPPO	RPOD	RF	RTPC	RPPO	RPOD
0	T	**	*	–	–	–	–	–	–	–	–	–	–
	t	*	–	–	–	*	–	–	–	–	–	–	–
	T^2	–	*	–	–	–	–	–	–	–	–	–	–
	$T.t$	*	–	–	–	–	–	–	–	–	–	–	–
	t^2	–	–	–	–	–	–	–	–	–	–	–	–
	$p_{\text{lack of fit}}$	–	–	–	–	–	–	–	–	–	–	–	–
	R^2 (%)	65	74	51	42	89	65	54	52	44	84	48	46
7	T	*	–	–	–	–	–	–	–	–	–	–	–
	t	*	–	–	–	–	–	–	–	–	–	–	–
	T^2	–	–	–	–	–	–	–	–	–	–	–	–
	$T.t$	–	–	–	–	–	–	–	–	–	–	–	–
	t^2	–	–	–	–	–	–	–	–	–	–	–	–
	$p_{\text{lack of fit}}$	–	–	–	–	–	–	–	–	–	–	–	–
	R^2 (%)	65	54	45	41	69	79	65	57	76	80	66	53

Note: RF (%): retention of firmness; RTPC (%): retention of total phenolic content; RPPO (%): residual polyphenol oxidase activity; RPOD (%): residual peroxidase activity. - Non-significant.
*Significant at 0.05 level, **significant at 0.01 level.

$$\text{RF}_{C7}\,(\%) = 233.3 - 2.5T - 0.4t \quad (5)$$

At Day 0, the heating time was the only factor affecting RF of "Eva" (RF_{E0}), which declined linearly with t (Equation 6). The effect of the mild heat treatments on the RF of "Eva" after 7 days of storage could not be modeled since any of the studied factors were significant (Table 1). In average, the treatments reduced by 16% the firmness of "Eva."

$$\text{RF}_{E0}\,(\%) = 110.7 - 0.3t \quad (6)$$

The models for RF in "Princesa" models after 0 and 7 days of storage were not significant (Table 1). The mild heat treatments reduced by 1% the firmness of "Princesa" at Day 0 and by 11% at Day 7.

At Day 0, firmness of "Caricia," declined with the heating time at high temperatures, while in "Eva" apples a reduction over heating time was observed at any temperature (by 3%–8% for times of 20–50 min). However, the "Eva" samples heated at 40–45°C for up to 30 min were firmer than the U ones.

By contrast, the treatments did not affect the firmness of "Princesa" cultivar. After 7 days of cold storage, the heat treatments had reduced the firmness of "Eva" by 16% and of "Princesa" by 11%, while the firmness of "Caricia" decreased linearly with both the temperature and time of heat treatment. Previous studies reported that the heat treatment effect on the fruit texture depended not only on the cultivar but also in the treatment conditions. Kim et al. (1993) found that the firmness of apples from nine different cultivars treated at 50°C decreased with the treatment duration. Aguayo et al. (2015) reported that a hot water treatment (48 or 55°C for 2 min) did not affect significantly the sensory texture of "Mahana Red" fresh-cut apples, while Li et al. (2013) reported that "Red Fuji" and "Golden Delicious" heat-treated apples were firmer than the U ones, after cold storage. Firmness increase by heat treatments has been explained by the activation of pectin methylesterase. The enzyme demethyls the metoxyl groups from galacturonic acid residues of pectic substances, making the pectin carboxyl groups available for complexing with endogenous calcium, forming Ca-pectates, and increasing the rigidity of the cell wall and middle lamella (Koukounaras, Diamantidis, & Sfakiotakis, 2008).

3.2 | Effect on browning development

Enzymatic browning in apples is instrumentally reflected by a decrease in L^* and h_{ab}, and an increase in a^*, b^*, and C^*_{ab} values (Piagentini et al., 2012). During storage of the U fresh-cut apples, L^* decreased (Figure 1c) and a^* increased (Figure 1d), for the three studied cultivars. These results indicate that enzymatic browning occurred on the U samples from the three cultivars, as expected. "Caricia" developed the highest level of browning (L^* decreased by 8%), in agreement with the results reported by Piagentini and Pirovani (2017). The activity of the enzymes involved in the browning reactions could explain this result. "Caricia" was the only cultivar that showed a significant (p = .014) increase in the PPO activity (Figure 1e) and was the cultivar with the highest POD activity increase (Figure 1f). The increase in PPO and POD activities during cold storage of fruit and vegetables has been previously reported (Lo'ay & EL-Khateeb, 2018; Min et al., 2017; Zhao, Lv, Fan, & Li, 2018).

The effects of the treatments on the apples' color were also evaluated. The lack of fit of δL^* and δh_{ab} models for "Caricia" at Day 0 were significant (Table 2). Nevertheless, δa^*, δb^*, and δC^*_{ab} could be modeled (Equations 7–9).

$$\partial a^*_{C0}(\%) = 841.8 - 50.1t \quad (7)$$

$$\partial b^*_{C0}(\%) = -67.3 + 1.7T + 2.3t - 0.05Tt \quad (8)$$

$$\partial C^*_{abC0}(\%) = -65.4 + 1.6T + 2.2t - 0.05Tt \quad (9)$$

According to the model, δa^* declined linearly with t. However, the low R^2 value obtained for this model (below 50%, Table 2), suggests that it did not fully explain the variability of the data. Probably, some other factors like heterogeneity of the raw material contributed to the δa^* variability as well. The a^* value for the U (a^*_U) "Caricia" samples at Day 0 was negative (Figure 1d), therefore $\delta a^* > 0$ when $a^*_{TT} < a^*_U$. Taking this into account, the heating time required to avoid browning development ($\delta a^* \geq 0$) may be below 16 min, lower than the shortest experimental time assayed (20 min). TT "Caricia" samples presented reddish hues, indicating browning development ($\delta a^* < 0$, $a^*_{TT} > a^*_U$).

The interaction term between T and t was significant ($p < .05$) for δb^* and δC^*_{ab} (Equations 8 and 9). For both color parameters, at 40°C, the differences with the U samples were higher for longer heating times (more browning development), while at 50°C, the impact of t on these color parameters was lower.

None of the models obtained for the relative changes in the color parameters of "Caricia" at Day 7 had any significant factors. Apples treated at 40–50°C for 20–90 min, showed absolute mean values of δL^*, δC^*_{ab} and δh_{ab} lower than 1%, suggesting that the mild heat treatment applied did not affect the color of "Caricia" fresh-cut apples after 7 days of storage.

The models for color parameters in "Eva" at Day 0, were not significant (Table 2). In average, the mild heat treatments produced samples with higher luminosity (L^*_{TT0} = 78.52 ± 0.44; L^*_{U0} = 76.96 ± 1.77).

δL^* of the "Eva" samples at Day 7 decreased as t increased (Equation 10; Figure 2). The luminosity was always higher in the heat-treated samples ($\delta L^* > 0$).

$$\partial L^*_{E7}(\%) = 4.7 - 0.03t \quad (10)$$

None of the models obtained for δa^*, δb^*, δC^*_{ab}, and δh_{ab} of "Eva" presented significant terms (Table 2). After 7 days at 2°C, a^*, b^*, and C^*_{ab} were lower in the TT samples than in U samples (22%, 4%, and 4%, respectively), while h_{ab} was 1% higher for TT. Along storage, TT "Eva" samples developed less browning than U samples, since the flesh color was less reddish and intense (higher L^* and h_{ab}, and lower C^*_{ab}).

The heating time was the only significant factor affecting δL^*, δb^*, and δC^*_{ab} of "Princesa" at Day 0 (Table 2). While δL^* decreased linearly with t, δb^* and δC^*_{ab} increased with t showing browning development at longer heating times (Equations 11–13).

$$\partial L_{P0}(\%) = 2.4 - 0.1t \quad (11)$$

$$\partial b^*_{P0}(\%) = -7.8 + 0.2t \quad (12)$$

$$\partial C^*_{abP0}(\%) = -8.0 + 0.2t \quad (13)$$

TABLE 2 Analyses of variance of regression models for the relative changes in color parameters of fresh-cut apples at the minimal processing day (0 day) and after 7 days at 2°C

		"Caricia"					"Eva"					"Princesa"				
Time (d)	Variation source	δL^*	δa^*	δb^*	δC^*_{ab}	δh_{ab}	δL^*	δa^*	δb^*	δC^*_{ab}	δh_{ab}	δL^*	δa^*	δb^*	δC^*_{ab}	δh_{ab}
0	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	t	*	*	–	–	*	–	–	–	–	–	*	–	*	*	–
	T^2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	$T.t$	–	–	*	*	–	–	–	–	–	–	–	–	–	–	–
	t^2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	$p_{\text{lack of fit}}$	*	–	–	–	*	–	–	–	–	–	–	–	–	–	–
	R^2 (%)	46	49	62	58	48	81	50	40	40	51	89	67	67	69	66
7	T	–	–	–	–	–	–	–	–	–	–	–	*	–	–	*
	t	–	–	–	–	–	*	–	–	–	–	*	–	*	*	–
	T^2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	$T.t$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	t^2	–	–	–	–	–	–	–	–	–	–	*	–	*	*	–
	$p_{\text{lack of fit}}$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	R^2 (%)	38	34	61	53	37	88	85	79	80	85	90	90	77	80	87

Note: $\delta Qi(\%) = \frac{(Q_{TTi} - Q_{Ui})}{Q_{Ui}} \times 100$ (Q_{TTi}:L^*_{TT}, a^*_{TT}, b^*_{TT},C^*_{abTT}, h_{abTT}; and Q_{Ui}: L^*_U, a^*_U, b^*_U,C^*_{abU}, h_{abU}, at i = 0 or 7 days). - Non-significant.
*Significant at 0.05 level.

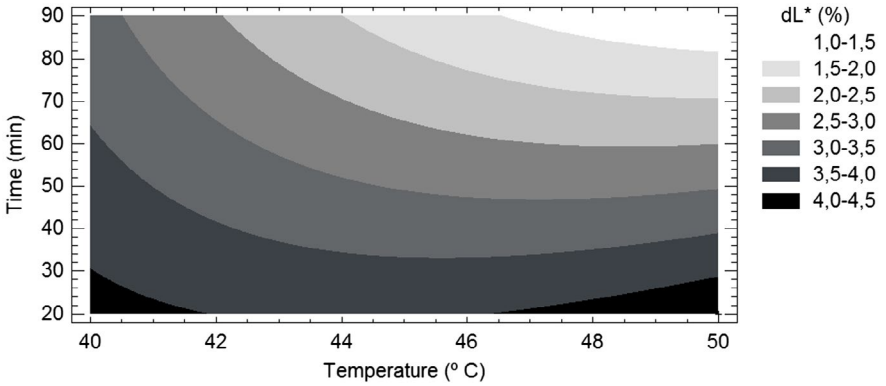

FIGURE 2 Contour plot for ∂L^* of fresh-cut "Eva" apples after 7 days of storage at 2°C

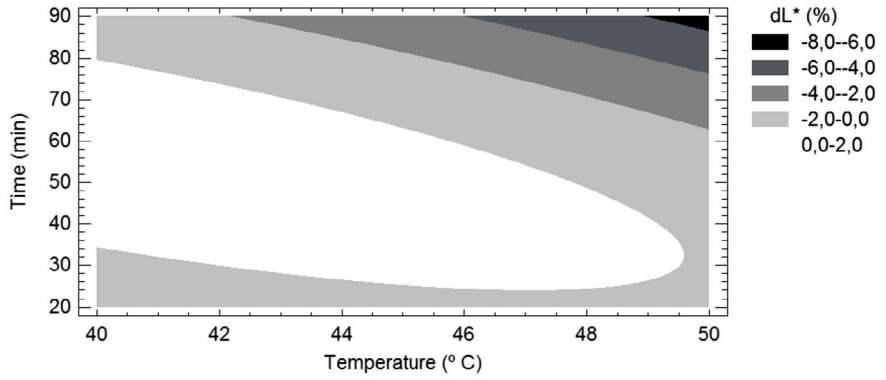

FIGURE 3 Contour plot for ∂L^* of fresh-cut "Princesa" apples after 7 days of storage at 2°C

The models obtained for δa^* and δh_{ab} of "Princesa" were not significant. The a^* mean value for the U samples was 0.16 ± 0.63, while the TT samples had an a^* mean value of 2.22 ± 1.24. This result suggested that the heat treatment increased the red hue in the apple flesh. Furthermore, at Day 0, the mild heat treatment reduced the hue angle of "Princesa" apples toward reddish hues (h_{abU0} = 89.73 ± 1.32, and h_{abTT0} = 85.88 ± 2.67).

The δL^* model for "Princesa" at Day 7 showed a quadratic dependence with t (Equation 14). The highest luminosity was obtained for t between 20 and 60 min, at any T within the studied range (Figure 3). Moreover, at short heating times, δL^* was independent of T, while at times above 50 min, δL^* declined with T (Figure 3). Almost every δL^* obtained values were negative ($L^*_{TT7} < L^*_{U7}$), therefore, TT samples were darker than U samples, at Day 7.

$$\partial L^*_{P7}(\%) = -28.4 + 0.6T + 0.6t - 0.01Tt - 0.002t^2 \quad (14)$$

At Day 7, it was found that heat treatments at higher T produced "Princesa" samples with higher δa^* (more reddish), as shown in Equation (15).

$$\partial a^*_{P7}(\%) = -230.8 + 6.2T \quad (15)$$

The predicted models for δb^* and δC^*_{ab} showed a quadratic dependence with t (Equations 16 and 17, respectively). For both color parameters, a minimum value was obtained at times between 20 and 60 min, in the experimental region at which the higher L^* values were found (Figure 3).

$$\partial b^*_{P7}(\%) = 7.7 - 0.4t + 0.004t^2 \quad (16)$$

$$\partial C^*_{abP7}(\%) = 8.5 - 0.4t + 0.005t^2 \quad (17)$$

δh_{ab} decreased linearly with T (Equation 18), developing reddish hues in the samples ($h_{abTT7} < h_{abU7}$).

$$\partial h_{abP7}(\%) = 12.4 - 0.3T \quad (18)$$

The mild heat treatments were effective in reducing the enzymatic browning development on "Eva." At Day 7, TT "Eva" samples had higher luminosity and a less red and intense color than U samples. On the other hand, the heat treatment did not affect the color of "Caricia" after 7 days of storage and increased browning on "Princesa." Kim et al. (1993) reported differences in the susceptibility of apple cultivars toward browning. Aguayo et al. (2015) obtained a slight reduction in L^* and h_{ab} values and increased chroma when treating "Mahana Red" apples at 55°C for 2 min, which is consistent with the increased browning development observed in "Princesa."

3.3 | Effect on PPO and POD enzyme activities

Different apple cultivars present varying PPO and POD enzyme activities (Tappi et al., 2019). In order to understand the different responses of browning toward heat treatment obtained among cultivars, the PPO and POD activities were determined. The enzymatic

activities could not be modeled since the factors studied (T and t) were not significant ($p > .05$) for any cultivar and storage time (Table 1).

The mild heat treatments did not reduce the PPO activity (RPPO > 100%) in "Caricia" and "Princesa" apples (Figure 4a). However, the PPO activity in "Eva" was reduced by the heat treatments, obtaining residual activities of 2.5% and 4.2% at Days 0 and 7, respectively. The POD activity showed a different response toward heat treatment (Figure 4b), but significantly increased in the "Princesa" TT samples at both storage days.

PPO role in the enzymatic browning reaction is to oxidize phenolic compounds to *o*-quinones in presence of oxygen, with the later polymerization of quinones to brown pigments. POD contribution to enzymatic browning is lesser, since it acts in presence of hydrogen peroxide which concentration is low in apples (Mishra, Gautam, & Sharma, 2013).

The PPO residual activity results are consistent with the results obtained for the instrumental color parameters. This suggests that the enzymatic browning reduction observed in "Eva" is due to the PPO inhibition caused by the mild heat treatments. The effect of the mild heat treatments on fruit and vegetable enzymes has been previously reported. Martín-Diana et al. (2005) found that, during cold storage of minimally processed lettuce washed at 50°C, both the activities of PPO and POD were significantly lower than the enzymatic activities of the samples washed at 4 or 25°C. Alegria et al. (2012) reported that POD activity was reduced by 30% in heat-treated (100°C, 45 s) fresh-cut carrots in comparison to U ones.

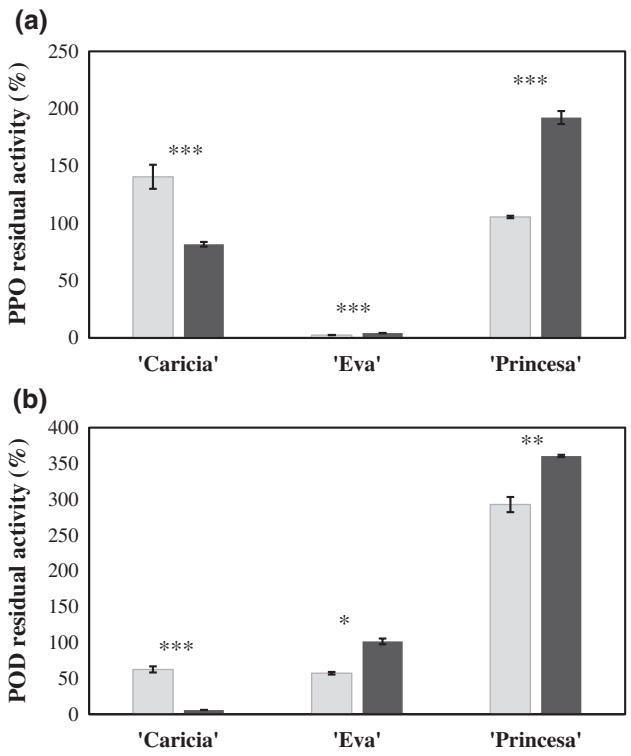

FIGURE 4 Polyphenol oxidase, PPO (a) and peroxidase, POD (b) residual activities of heat-treated "Caricia," "Eva," and "Princesa" fresh-cut apples, after 0 (□) and 7 (■) days of storage at 2°C. *, **, ***: Significant differences between storage times for each apple cultivar at $p < .05$, $p < .01$ and $p < .001$, respectively. Bars represent standard deviation

3.4 | Effect on phenolic content

Initially, the total phenolic contents obtained for the untreated cultivars were 653, 303 and 900 mg kg^{-1} for "Caricia," "Eva," and "Princesa," respectively. TPC increased by 34% during storage ($p < .001$) in "Princesa" U samples, while remained constant ($p > .05$) for "Caricia" and "Eva" (Figure 1b). At Day 0, TPC retention in "Caricia" ($RTPC_{C0}$) showed a quadratic dependence with T (Equation 19). The greatest TPC increase (between 4.7% and 6.1%) was obtained at T above 43°C (maximum $RTPC_{C0}$ = 106% at T = 46.5°C).

$$RTPC_{C0}(\%) = -146.2 + 10.9T - 0.1T^2 \quad (19)$$

RTPC models of "Eva" and "Princesa" at Day 0 were not significant (Table 1). In average, TT "Eva" and "Princesa" sampes had a TPC 30 and 13% higher than U, respectively.

At Day 7, none of the RTPC models were significant (Table 1). TT "Caricia" and "Princesa" samples showed a TPC lower than U, while the mild heat treatments increased TPC of "Eva" by 70% in average (Figure 5). Moreover, the TPC of TT "Eva" samples was 23% higher at Day 7 than at Day 0.

The heat treatments increased the TPC of "Eva" and "Princesa" 24 hr after their application (Day 0), and showed a maximum for "Caricia" samples treated at approximately 46.5°C. Heat-treated "Eva" samples also showed higher TPC levels after 7 days of storage. Similarly, Alegria et al. (2012) obtained higher polyphenol contents in carrots treated at 100°C for 45 s than in untreated ones, after 7 days of storage at 5°C.

Abiotic stresses, such as peeling, decoring, cutting, and heat treatment, produce signals that induce the synthesis of specific proteins, some of which are enzymes of phenolic metabolism (phenylalanine ammonia-lyase). The increased activity of these enzymes leads to the accumulation of phenolic compounds (Saltveit, 2000). Aguayo et al. (2015) obtained different responses

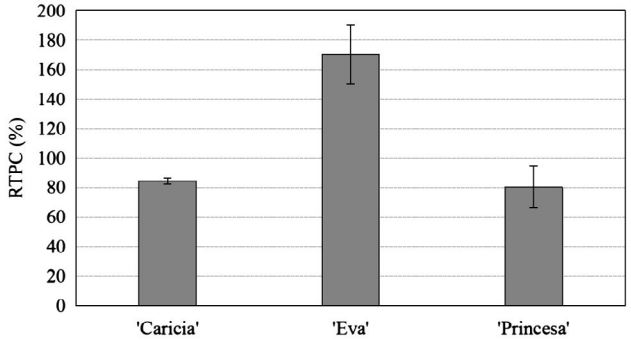

FIGURE 5 Retention of total phenolic content, RTPC (%) of heat-treated "Caricia," "Eva," and "Princesa" fresh-cut apples, after 7 days of storage at 2°C. Bars represent standard deviation

to thermal treatment in phenolic compounds (while levels of quercetin and phloridzin increased, coumaric and procyanidins decreased) of "Mahana Red" apples, which was attributed to differences in the metabolism of the phenolic compounds. Ceymann, Arrigoni, Schärer, Bozzi Nising, and Hurrell (2012) quantified 12 phenolic compounds in 104 apple cultivars and found significant differences among the polyphenol composition of the cultivars. Differences in the phenolic composition of "Caricia," "Eva," and "Princesa" could explain the diverse responses to heat treatment. Phenolic compounds are substrates of PPO in the enzymatic browning reaction, which might lead to an increase in the browning development. However, the increase of TPC on "Eva" did not lead to browning development, probably due to a reduction in the PPO activity during heat treatment (Figure 4a).

4 | CONCLUSIONS

The three low-chill apple cultivars showed different responses toward heat treatment, at both the minimal processing day (0 day) and after 7 days at 2°C.

At Day 0, the firmness of "Caricia" fresh-cut apples treated at temperatures above 45°C decreased with heating time. On the other hand, "Princesa" firmness was not affected by mild heat treatment. The TPC of the three cultivars was increased by the heat treatment at Day 0. After 7 days of cold storage, heat-treated "Caricia" and "Princesa" apples did not show an increase in firmness or TPC nor a decline in the enzymatic browning development.

Mild heat treatments between 40 and 50°C for 20–90 min successfully increased the TPC of "Eva" (70% in average), and reduced browning development, but decreased its firmness. The highest values of RF, RTPC, and ∂L^*, as well as the lowest ∂a^*, in "Eva" after 7 days of cold storage, were obtained for apples treated at high temperatures (approximately 50°C) during intermediate heating times (30–40 min).

In conclusion, applying mild heat treatments before minimal processing could be an effective postharvest tool to delay enzymatic browning development, through the reduction of PPO and POD activities, and to increase the TPC of the fresh-cut "Eva" low-chill apple cultivar.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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