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ORIGINAL PAPER

Modeling the Impact of the Type of Cutting and Storage Temperature on the Bioactive Compound Content, Phenylpropanoid Metabolism Enzymes and Quality Attributes of Fresh-Cut Strawberries

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Abstract The aim of this work was to model the effect of the type of cutting (whole without hull, halved, and quartered), storage temperature $(2, 6, \text{ and } 13 \degree C)$ and time on the changes of bioactive compounds, phenylpropanoid metabolism enzyme activities, and quality attributes of fresh-cut strawberries. The effect of increasing the intensity of wounding revealed an activation of the phenylalanine ammonia lyase (PAL) enzyme, with the consequent synthesis of phenolic compounds. Results revealed that quartered strawberries stored at 2 °C for 15 days accumulated up to 22% more phenolics than whole strawberries. The changes on quality parameters (soluble solids, pH, and color), total anthocyanins, and polyphenol oxidase were adequately fitted with zero order kinetic. All rate constants of these attributes, except for anthocyanins, fitted appropriately with Arrhenius equation. Changes on total phenolic contents and on PAL activity were fitted with a consecutive reaction mechanistic kinetic model. The rate constants of phenolics kinetic showed no dependence with temperature. However, rate constants of PAL activity fitted appropriately with Arrhenius equation. This global study offers a better understanding of the effects of processing and storage conditions on general quality, bioactive compounds, and phenylpropanoid metabolism enzymes of freshcut strawberries.

Keywords Abiotic stress . Mechanistic kinetic modeling . Total phenolics . Total anthocyanins . Phenylalanine ammonia lyase . Polyphenol oxidase

Introduction

Strawberry is one of the most commonly consumed fruit due to its attractive color and taste. It is recognized as a very rich source of vitamin C and phenolic compounds with human health benefits due to their antioxidant properties (da Silva Pinto et al. [2008\)](#page-15-0). Vitamin C is found at concentrations between 0.40 to 0.60 g kg^{-1} of fresh weight (Davey et al. [2000\)](#page-15-0). Meanwhile, glycoside derivatives from the anthocyanins, pelargonidin, and cyanidin are the main phenolic compounds found in strawberries with reported concentrations of up to 0.4325 g kg^{-1} of fresh weight (Van de Velde et al. [2016\)](#page-15-0). Anthocyanins are responsible for the red color of strawberries and represent one of the major antioxidant sources in this fruit (Crecente-Campo et al. [2012\)](#page-14-0).

The industrial process to obtain fresh-cut strawberries involves selection, pre-wash, calyx and peduncle elimination, cutting, washing-disinfection, and packaging (Pirovani et al. [2004\)](#page-15-0). Cutting transforms the products into smaller pieces, and essentially is causing the tissue to endure wounding stress (Surjadinata and Cisneros-Zevallos [2012](#page-15-0)). The wounding results in an increased metabolism and respiration rate of freshcut products (consumption of sugars, lipids, and organic acids), promoting changes in color, flavor, texture, and nutritional quality; and a higher production of ethylene, which induces ripening and cause senescence (Soliva-Fortuny and Martin-Belloso [2003](#page-15-0)). Moreover, it is recognized that a stress or an injury to a plant cell trigger two types of responses in phenolic metabolism. The first response is the oxidation of the existing phenolic compounds as a result of rupture of the cell

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membrane, causing the phenolics to combine with the oxidative enzyme systems, mainly polyphenol oxidase (PPO, EC 1.14.18.1), which promote the degradation of the antioxidant. On the other hand, the other response involves the synthesis of monomeric or polymeric phenolics to repair the wounding damage. This second response is caused by changes in phenylalanine ammonia lyase activity (PAL, EC4.3.1.5), since it is the key metabolic enzyme in the phenylpropanoid pathway (Kang and Saltveit [2002\)](#page-15-0), which promote antioxidant synthesis. Surjadinata and Cisneros-Zevallos ([2012\)](#page-15-0) studied the effect of wounding intensity on the phenolic compounds of carrots prepared as slices, pies, and shreds and reported that the most intense wounding (shreds) induced a 2.5-fold increase in the phenolic content after 4 days of storage at 15 °C compared to whole carrots. Authors stated that a more severe cutting process elicited a greater wounding response of tissues, which was consistent with a higher synthesis of antioxidant phenolic compounds. Therefore, according to Cineros-Zevallos [\(2003\)](#page-14-0), the control of the wounding intensity can be used as a simple tool to provide an inexpensive source of phenolic antioxidants for the fresh market such as fresh-cut products.

Several authors studied the changes in bioactive compounds such as phenolics and vitamin C in whole and freshcut strawberries during the storage at different temperatures (Ayala-Zavala et al. [2004;](#page-14-0) Cordenunsi et al. [2005;](#page-14-0) Gil et al. [2006\)](#page-15-0); and under low-oxygen, superatmospheric oxygen, or passive atmospheres (Zheng et al. [2007;](#page-15-0) Odriozola-Serrano et al. [2010\)](#page-15-0). Moreover, regression models have been used to predict the shelf life and quality changes of fresh-cut vegetables throughout the storage time (Riva et al. [2001;](#page-15-0) Piagentini et al. [2005\)](#page-15-0). The use of models is an efficient tool to predict characteristic changes in a biological system affected by various environmental conditions, without the need to assess these conditions in real time. This methodology has been successfully employed in many other sciences areas (Valipour [2012;](#page-15-0) Valipour et al. [2013,](#page-15-0) [2017\)](#page-15-0). For the successful use of a model, it is necessary that the model can accurately represent the desired physical phenomenon. A model requires previous validation, which means that its parameters must have been identified or adjusted based on experimental data (Tijskens and Schouten, [2009\)](#page-15-0).

In relation to bioactive compounds, Odriozola-Serrano et al. ([2009\)](#page-15-0) modeled the changes in vitamin C, total anthocyanins, and antioxidant capacity using kinetics of zero and first order, as well as a model based on Weibull distribution function to evaluate the effects of storage time and temperature of quartered strawberries stored under 80 kPa of oxygen. Additionally, Amodio et al. [\(2014\)](#page-14-0) developed and validated a kinetic model based in a consecutive reaction mechanism able to describe the changes in the phenolic content in fresh-cut produce during storage and explained the effect of different variables on phenolic synthesis and oxidation. Authors reported that the type of cutting of lemon samples affected the increase in the phenolic content likely due to the activity of newly produced PAL induced by wounding. However, authors have not used any regression model to explain the changes on PAL activity of fresh-cut products.

Complete studies about modeling the evolution of quality parameters, bioactive compound contents, and phenylpropanoid metabolism enzyme activities of freshcut commodities with different intensity of wounding as a function of storage time and temperature are barely found in literature. Therefore, the aim of this work was to model the effect of the type of cutting, storage temperature and time on the changes of quality attributes, bioactive compound contents, and phenylpropanoid metabolism enzyme activities of fresh-cut strawberries.

Materials and Methods

Reagents

Polyvinylpolypyrrolidone (PVPP), phenylmethylsulfonic fluoride (PMSF), and Folin-Ciocalteu reagent were acquired from Sigma-Aldrich Inc., (St. Louis, MO, USA). L- (.+) ascorbic acid, DL-dithiothreitol (DTT), and metaphosphoric acid were purchased from Merck KGaA (Damnstadt, Germany). Sodium acetate trihydrate and potassium phosphate dibasic were acquired from Cicarelli Reagents S.A. (Santa Fe, Argentina). Methanol HPLC grade was obtained from PanreacQuímica S.L.U. (Barcelona, Spain). Oxilac Plus (Indaquim S.A., Santa Fe, Argentina) was used for the washing-disinfection operation. Oxilac Plus is a stabilized mixture of peracetic acid (PAA) (minimum 5%), hydrogen peroxide (minimum 20%), and water.

Plant Material

Cultivated strawberry fruit (Fragaria x ananassa Duch.) of 'Camarosa' variety was obtained from one planting at Arroyo Leyes (31°27′0″S, 60°40′0″W), Santa Fe, (Argentina). Fruit was harvested at ripe stage (90% of the surface showing red color) and transported 20 km directly from the field to the laboratory of the Instituto de Tecnología de Alimentos, FIQ, UNL, (Argentina).

Minimal Processing

Processing was performed in the pilot plant of the Instituto de Tecnología de Alimentos (FIQ-UNL). Strawberry fruit (20 kg) was sorted, eliminating fruit with signs of damage. Calyxes and peduncles were removed, and then, fruit was prewashed with tap water (20 °C, 2 min) and drained on absorbent paper in a refrigerated room (18 °C). Fruit was hulled and cut manually, using a stainless-steel blade smooth knife. To obtain different types of cutting, producing different intensity of wounding, three treatments were prepared: whole without hull (W), halved (H), and quartered (Q) strawberries.

Subsequently, each lot of fruit was submitted to a washingdisinfection operation by immersion in a PAA solution. The washing-disinfecting conditions were as follows: 20 mg L^{-1} PAA at 18 °C for 52 s, according to Van de Velde et al. [\(2014\)](#page-15-0). A ratio of 3 L of washing solution per kg of fruit was used and washed fruit was drained by gravity to remove the excess of liquid. Finally, samples of about 0.10 kg of W, H, and Q strawberries were put into round containers with lids and stored under refrigeration and passive modified atmosphere and 90% RH. The round containers (Rotlen model, Treviplast S.A., Argentina) are commercially used for the storage of ready-to-eat fruits and vegetables, and are made of polyethylene terephthalate (PET) of 0.42 mm thick, with a surface area of 0.045 m², transmission rates of 4.73 10^{-15} – 9.617 10^{-15} kg m⁻² s⁻¹ Pa⁻¹ for O₂ (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).

Strawberries were studied for 15 days (2 °C), 8 days (6 °C), and 7 days (13 °C) in order to satisfy an acceptable similar loss of quality in all samples (a minimum content of 6–7% soluble solids) and mesophilic aerobic microorganism counts around 107 .

At specific days of the storage, three containers per treatment were taken out of refrigeration units, and then immediately analyzed. Samples stored at 2 °C were analyzed at 0, 1, 3, 6, 8, 10, 13, and 15 days, samples stored at 6 $^{\circ}$ C were analyzed at 0, 1, 3, 6, and 8 days, and samples stored at 13 °C were analyzed at 0, 1, 3, 6, and 7 days.

Gas Composition

Analysis of the headspace gas composition of the round containers was performed by gas chromatography according to Piagentini et al. ([2003](#page-15-0)). Results were expressed in kilopascal of $CO₂, O₂$, and $N₂$.

Total Anthocyanin, Total Phenolic, and Vitamin C Analysis

Extract Preparation

The strawberry content of each one of the three containers per treatment were homogenized using a blender. For total anthocyanin (TA) and total phenolic (TP) analysis, 5 g of homogenized strawberries was added to 75 mL of extraction solvent (80% acetone and 20% water). For vitamin C (VitC) content, 5 g of homogenized strawberries was added to 25 mL of extraction solvent (3% metaphosphoric acid and 8% acetic

acid). The mixtures were homogenized for 1 min, sonicated for 15 min, and then centrifuged at $12,000 \times g$ for 20 min at 4 °C. The supernatants were separated and used for analysis. All extractions were made in triplicate.

Total Anthocyanin Content

TA content was monitored in a Genesis 10S UV–vis spectrophotometer, (Thermo Scientific, Germany) by the pH differential method according to Heo and Lee ([2005](#page-15-0)). The pH of each sample was adjusted to 1.0 with a 0.1 mol L^{-1} hydrochloric acid and 25 mmol L^{-1} potassium chloride solutions; and at pH 4.5 with a 0.4 mol L^{-1} sodium acetate/acetic acid buffer solution. Absorbance was measured at 510 and 700 nm. Results were converted to grams of pelargonidin-3-O-glucoside per kilogram of fresh weight, using a molar extinction coefficient of 2,240,000 L mol⁻¹ m⁻¹, a molecular weight of 433.2 g mol−¹ , and an optical path of 1 cm. The absorbance (A) was calculated by Eq. 1:

$$
A = \left[\left(A_{510} - A_{700} \right)_{\text{pH1}} - \left(A_{510} - A_{700} \right)_{\text{pH4.5}} \right] \tag{1}
$$

where A_{510} and A_{700} are the absorbance measures of samples at pH 1.0 and 4.5.

Total Phenolic Content

TP content was spectrophotometrically monitored using the Folin-Ciocalteu reagent according to Singleton and Rossi ([1965](#page-15-0)). Aliquots of 0.1 mL extracts were added with 0.25 mL of Folin-Ciocalteu reagent, 0.5 mL of 200 g L^{-1} $Na₂CO₃$, and 1.65 mL of distilled water. The mixture was incubated for 25 min at room temperature and then was centrifuged for 5 min at $2000 \times g$. Absorbance was measured at 760 nm. Reagent blanks were prepared by replacing the sample volume by extraction solvent. Quantification was performed through the external standard method with a gallic acid calibration curve (0.02–0.1 g L^{-1}) and results were expressed as grams of gallic acid (GA) per kilogram of fresh weight.

Vitamin C Content

VitC content was determined by HPLC according to Van de Velde et al. ([2012\)](#page-15-0). The analyses were set up on a Konik KNK-500-A HPLC, with UV detector (Konik Instruments, Barcelona, Spain). Separations were achieved in a reversed phase column Phenomenex Gemini 5μ C18 110A attached to a guard column (Phenomenex Inc., CA, USA) at 25 °C. The mobile phase, under isocratic conditions, consisted of a 0.03 mol L^{-1} sodium acetate/acetic acid buffer, 5% methanol (pH = 5.8). The flow rate was 1.15 mL min⁻¹ and the detection was performed at 251 nm. Quantification was performed through the external standard method with VitC calibration curve (0.004–0.020 $g L^{-1}$) and results were expressed as grams of VitC per kilogram of fresh weight.

Soluble Solids and pH Analysis

The analyses of pH and soluble solids were made on homogenized strawberries. The pH values were determined with a pH meter (Horiba B-213 Twin pH meter, Horiba Ltd., Kyoto, Japan) by triplicate. The soluble solids (SS) content was determined by triplicate in the homogenized samples using a hand-held digital refractometer model Pal-alpha (Atago Co Ltd., WA, USA) and results were expressed as percent.

Color Determination

Color was determined using a Minolta 508d spectrophotometer (Minolta Co., LTD., Japan), under the following conditions: illuminant D65, observer angle of 10°, SCE (specular component excluded), evaluating the CIE parameters: $L^*, a^*,$ b^* , C_{ab}^* , and h_{ab} . Five measurements were performed on each sample.

Enzymatic Activity Determinations

Phenylalanine Ammonia Lyase

PAL activity was determined according to Mori et al. ([2000\)](#page-15-0). Five grams of homogenized strawberries was added to 20 mL of extraction solution (phosphate buffer 100 mmol L⁻¹ pH 8, EDTA 2 mmol L⁻¹, PVPP 30 g L⁻¹, DTT 7 mmol L^{-1} , Triton X-100 0.1% v/v). The mixture was homogenized for 30 s and stirred for 1 h at 4 °C. Then, the mixture was centrifuged at $12,000 \times g$ for 15 min at 4 °C and the supernatant was separated and used for activity determination. The reaction mixture consisted of 1060 μL of Tris–HCl 100 mmol L^{-1} pH = 8.8, 530 µL phenylalanine 50 mmol L^{-1} , and 150 μL of enzymatic extract. The reaction mixture was incubated for 1 h at 37 \degree C and the reaction was stopped adding 260 µL of TCA 10 g L^{-1} . Cinnamic acid production was measured in a spectrophotometer at 290 nm. One unit of enzyme activity (U) was expressed as one absorbance increment (in the conditions in which the assay was carried out) per hour and milligram of protein extract.

Polyphenol Oxidase

PPO activity was determined according to Massolo et al. [\(2011](#page-15-0)) with some modifications. Five grams of homogenized strawberries was added to 20 mL of extraction solution (phosphate buffer 100 mmol L^{-1} pH = 6, PVPP 30 g L⁻¹, Triton X-100 0.1% v/v, NaCl 1 mol L⁻¹). The

mixture was homogenized for 30 s and stirred for 1 h at 4 °C. Then the mixture was centrifuged at $12,000 \times g$ for 15 min at 4 °C and the supernatant was separated and used for enzymatic analysis. The reaction mixture consisted of 900 μL of distilled water, 200 μL of pyrocatechol 200 mmol L^{-1} , 150 µL of phosphate buffer 1 mol L^{-1} pH = 6, and 250 µL of extract. The mixture was incubated for 1 h at 37 °C. The oxidation products of pyrocatechol were measured as the change in absorbance at 410 nm. One unit of enzyme activity (U) was expressed as one absorbance increment (in the conditions in which the assay was carried out) per hour and milligram of protein extract.

Protein Determination

Protein determination was performed on the extracts according to Lowry et al. ([1951\)](#page-15-0). Briefly, 200 μL of protein extract is added to 2.5 mL of reagent C (100 parts of 3.0% Na2CO3, 0.4% NaOH, 4% sodium tartrate, and 1 part of reagent B: 2% CuSO₄.5 H₂O), and incubated at room temperature for a minimum of 10 min. Then, 250 μL of Folin-Ciocalteu reagent (diluted $\frac{1}{2}$) is added and the mixture is vigorously shaken and read at 660 nm against a reagent blank. A calibration curve was performed using 1 mg L^{-1} of albumin. Results were expressed as milligram of protein per gram of fresh weight.

Modeling Changes in the Content of TA, VitC, PPO Activity, pH, SS, and Color Parameters in Fresh-Cut **Strawberries**

The changes of each attribute over time were modeled using a general kinetic equation (Eq. 2):

$$
\pm \frac{dQ}{dt} = k \cdot (Q)^n \tag{2}
$$

where Q is the attribute value, t is the time, k is the rate constant, and *n* is the reaction order. The sign $(+)$ of Eq. 2 refers to a response that increases its value with time, and the sign $(-)$ refers to responses whose values decrease with time.

To adjust the changes of the studied attributes of fresh-cut strawberries as a function of time for the different type of cutting, the experimental data were fitted to Eqs. 3 and 4 for order 0 and 1, respectively:

$$
Q = Q_0 \pm k_{(T)} \cdot t \tag{3}
$$

$$
Q = Q_0 \times e^{\pm k_{(T)}t} \tag{4}
$$

where Q_0 is the attribute value at time zero, t is the time, and k is the rate constant of the attribute change at a given temperature (T) .

Modeling Changes in the Content of TP and PAL Activity in Fresh-Cut Strawberries

A similar kinetic model was used for modeling TP content and PAL activity changes in fresh-cut strawberries as a function of time, for each type of cutting and storage temperature, according to Amodio et al. [\(2014](#page-14-0)) and Van Dijk and Tijskens [\(2000\)](#page-15-0). It was assumed that two consecutive kinetic reactions occurred: (a) formation of phenolics or active enzyme from a precursor (TP_{pre} or PAL_{pre}) governed by a reaction rate constant k_f and (b) transforming of phenolics into oxidized compounds or PAL into an inactive enzyme form (TP_{na} or PAL_{na}) governed by a reaction rate constant k_d . Equations 5 and 6 describe the phenomenon for PAL changes.

$$
PAL_{pre} \xrightarrow{k_f} PAL \tag{5}
$$

$$
PAL \xrightarrow{k_d} PAL_{na}
$$
 (6)

Based on the rules of chemical kinetics, the following set of equations may be obtained:

$$
\frac{d\text{PAL}_{\text{pre}}}{dt} = k_{\text{f}}.\text{PAL}_{\text{pre}}\tag{7}
$$

$$
\frac{d\text{PAL}}{dt} = k_{\text{f}}.\text{PAL}_{\text{pre}} - k_{\text{d}}.\text{PAL}
$$
 (8)

The integration of the differential equations (Eqs. 7 and 8) allowed to obtain the kinetic model (Eq. 9):

$$
PAL_{(t)} = PAL_{pre,0} \cdot k_f \cdot \left(\frac{e^{-k_d \cdot t} - e^{-k_f \cdot t}}{k_f - k_d}\right) + PAL_0 \cdot e^{-k_d \cdot t} \tag{9}
$$

Where $PAL_(t)$ represents the changes of the active form of the enzyme over time, $PAL_{pre,0}$ refers to the enzyme precursor, and PAL₀ is the active form of the enzyme at time 0. k_f is the reaction rate constant for formation of the active form of the enzyme, and k_d is its dissociation rate constant.

A similar set of equations can be obtained for TP changes. Equation 10 shows the kinetic model for TP changes:

$$
TP_{(t)} = TP_{pre,0} \cdot k_f \cdot \left(\frac{e^{-k_d \cdot t} - e^{-k_f \cdot t}}{k_f - k_d}\right) + TP_0 \cdot e^{-k_d \cdot t}
$$
 (10)

where $TP_{(t)}$ represents the changes of the content of phenolic compounds over time, $TP_{pre.0}$ refers to the content of phenolic compound precursors, and TP_0 is the content of phenolic compounds at time $0.$ k_f is the reaction rate constant for formation of phenolics, and k_d is its degradation rate constant.

To describe the changes in the TP content and PAL activity in fresh-cut strawberries as a function of time, $PAL₀$ and $TP₀$ were experimentally measured and k_f , k_d , $PAL_{pre,0}$, and $TP_{pre,0}$ were estimated.

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Modeling the Effect of Temperature on Kinetic Rate Constants

The effect of temperature on any of the reaction rate constants was described using the Arrhenius equation (Eq. 11):

$$
k = k_0 e^{\left(\frac{-\text{E}}{RT}\right)}\tag{11}
$$

where k_0 is the pre-exponential factor, Ea is the activation energy $(J \mod^{-1})$, R is the ideal gas constant $(8.3145 \text{ J K}^{-1} \text{ mol}^{-1})$, and T is the temperature (K).

Statistical Analysis

ORIGIN software (Microcal Software, Northhampton, MA, USA) was used for data analysis through ANOVA and to fit the experimental data. Significant differences among means were determined by Tukey's test at 5% level of significance. Linear and nonlinear regression analysis was used to determine the model parameters. The coefficient of determination $(R²)$ and the residuals analysis were used to assess the goodness of fitting. R^2 is a statistical measure of how close the data are to the fitted regression line. The higher the R^2 , the better the model fits data. Residuals are estimates of experimental error obtained by subtracting the observed responses from the predicted responses. Residuals should satisfy the assumptions of normality, independence, and randomness (Montgomery, [2001\)](#page-15-0).

Results and Discussion

Effect of the Type of Cutting on Bioactive Compound Content, Enzyme Activities, and Quality Attributes of Strawberries

Table [1](#page-7-0) shows the content of TP, TA, VitC, PAL and PPO activities, pH, SS, and color parameters of W, H, and Q 'Camarosa' strawberries, after minimal processing. Results agreed with those reported by other authors for whole or fresh-cut 'Camarosa' strawberries (Cordenunsi et al. [2005;](#page-14-0) Odriozola-Serrano et al. [2009;](#page-15-0) Alexandre et al. [2014\)](#page-14-0).

TP content of W strawberries was 13% higher than Q strawberry content. However, there was no difference in the TP content between W and H strawberries. TA content was 20% higher in W strawberries, and no differences were observed between H and Q strawberry TA contents. Meanwhile, VitC content was higher for W strawberries, decreasing with the intensity of wounding made to the fruit tissue (8 and 14% lower for H and Q strawberries, respectively). According to these results, an increased oxidation of bioactive compounds was found as the wounding intensity increased, after the minimal processing. In agreement, Castro et al. ([2002](#page-14-0))

Media ($n = 3$). L^* , a^* , b^* , C_{ab}^* and h_{ab} color parameters. Values with different letter in the same row are significantly different by Tukey's test $(p \le 0.05)$

TP total phenolics, TA total anthocyanins, VitC vitamin C, W whole without hull strawberries, H halved strawberries, Q quartered strawberries

demonstrated that strawberry hulling almost did not change the content of bioactive compounds. However, the effect of cutting strawberries into small pieces of 3 to 18 mm, after 5 min of contact with air at room temperature, resulted in 50% decrease in the vitamin C content, with no greater losses occurring up to 30 min. Moreover, Castro et al. [\(2002](#page-14-0)) also reported minimal losses in the content of total phenolics and total anthocyanins after 30 min.

Another probable cause of the loss of bioactive compounds, indirectly linked to the type of cutting, is the leaching and/or oxidation of them during the washing-disinfection. All strawberry samples were submitted to a treatment with PAA at optimized conditions where the retention of bioactive compounds was prioritized, with an acceptable microbiological reduction as it was determined by Van de Velde et al. [\(2014\)](#page-15-0). Therefore, the greater exposed areas of H and Q strawberries to the sanitizer solution resulted in an increase of the oxidation and/or leaching of bioactive compounds in the fresh-cut strawberries.

Interestingly, H and Q samples presented more intensive color than W samples (higher C_{ab}^* values) (Table 1), and this can be attributed to the low pH value of the PAA solution $(pH = 4.20)$ that could have intensified the anthocyanin red color in the fruit surface with more exposed area to the sanitizer. Nevertheless, pH values of W, H, and Q strawberries, measured in the entire sample, were not significantly different ($p > 0.05$).

The content of SS in fresh-cut strawberries decreased as the wounding intensity increased (Table 1), likely due to a higher lixiviation caused by the washing-disinfection after the cutting operation, or due to a higher sugar oxidation as a result of the higher wounding intensity of the tissues.

Finally, PAL and PPO activities did not show any difference among strawberries with different intensity of wounding, after the minimal processing.

Impact of Storage at Different Temperatures of W, H, and Q Strawberries on Bioactive Compound Content, Enzyme Activities, and Quality Attributes

Total Phenolics

Figure [1](#page-8-0) shows the changes in the TP content of W, H, and Q strawberries stored at 2, 6, and 13 °C. In general, the TP content of W, H, and Q strawberries was similar at the beginning and at the end of the storage time, for all studied temperatures. However, significant transient increases, resulted of the de novo synthesis of phenolics as a response to the wounding stress, were recorded at specific periods of the storage time for each type of cutting and storage temperature (Fig. [1\)](#page-8-0).

An increase of 13% on TP content was observed from day 8 in W strawberries at 2 °C. At 6 °C, W strawberries experienced a slight transient increase in TP content from day 6 of storage. Meanwhile, at 13 °C, the TP content of W fruit showed a slight decrease over time (Fig. [1a](#page-8-0)).

Minimal changes on TP content in H strawberries at 2 and 6 °C could be noticed in the analyzed days. At 13 °C, a transient increase (22%) was experimented by H strawberries at day 6 (Fig. [1](#page-8-0)b).

Q strawberries (Fig. [1](#page-8-0)c) at 2 °C presented a progressive increase in the TP content until day 6 of storage (22% higher than the initial TP content). Subsequently, even though a decrease in the TP content was observed for these samples, TP content remained even higher than the initial value at the end of the storage. At 6 °C, this fruit experienced 25% increase in TP content at day 3. Meanwhile, a transient increase in the TP content occurred at day 3 (24%) at 13 °C.

As observed, the highest increase in the TP content occurred in Q strawberries, fruit with the highest wounding intensity. In agreement, Surjadinata and Cisneros-Zevallos [\(2012\)](#page-15-0) stated that the biosynthesis of phenolic compounds in carrot tissues, prepared as slices, pies, and shreds, increased with the wounding intensity. The most intense wounding (shreds) induced an approximately 2.5-fold increase in the phenolic content after 4 days at 15 °C compared to whole carrots. Meanwhile, Odriozola-Serrano et al. [\(2010\)](#page-15-0) reported that quartered strawberries stored in passive atmosphere at 4 °C, experimented 38% quercetin content increase at day 21.

A consecutive reaction kinetic model (Eq. [10\)](#page-6-0) was used for modeling TP content in fresh-cut strawberries as a function of time, for each type of cutting, stored at 2, 6, and 13 °C. Table [2](#page-8-0) presents the kinetic parameters obtained for TP modeling. In general, R^2 values were higher than 0.80, and residuals were

Fig. 1 Experimental and predicted total phenolic content retention (TP R $\%$) of whole without hull (W) (a), halved (H) (b), and quartered (Q) (c) strawberries stored at 2, 6, and 13 °C. Experimental values (circle for 2 °C, square for 6 °C, triangle for 13 °C); models (for 2 °C, for 6 °C, \rightarrow \rightarrow for 13 °C). Vertical bars indicate the standard error of the means $(n = 3)$

normal and independently distributed (data not shown). As observed for a given temperature, k_f values increased with the wounding intensity $(W < H < Q)$, indicating a stimulation of the phenylpropanoid metabolism. For instance, k_f values at 2 °C were 0.04, 0.30, and 0.50 for W, H, and Q strawberries, respectively (Table 2). However, k_d values did not increase with the wounding intensity at a given temperature, indicating no dependence of the phenolic oxidation with wounding intensity. For example, k_d values at 2 °C were 0.050, 0.012, and 0.030 for W, H, and Q strawberries (Table 2). In agreement, Amodio et al. ([2014](#page-14-0)), who developed the

Table 2 Estimated parameters of proposed model of total phenolics changes on W, H, and Q strawberries (Eq. [10\)](#page-6-0)

Sample	Temperature $(^{\circ}C)$	$TP_{pre,0} (\%)$	$k_f(R^2)$	$k_d(R^2)$
W	2	190.52	0.04(0.35)	0.050(0.35)
	6	144.39	0.08(0.41)	0.070(0.41)
	13	1.80	0.04(0.86)	0.040(0.86)
H	2	4.84	0.30(0.82)	0.012(0.82)
	6	40.83	0.20(0.86)	0.004(0.86)
	13	77.65	0.20(0.69)	0.090(0.69)
Q	2	21.19	0.50(0.92)	0.030(0.92)
	6	40.69	0.40(0.79)	0.020(0.79)
	13	33.91	0.30(0.82)	0.012(0.82)

 $TP_{pre,0}$ predicted constant of phenolic compound precursors (%), W whole without hull strawberries, H halved strawberries, Q quartered strawberries, k_f (day⁻¹) reaction rate constant for formation of phenolics. k_d (day⁻¹) degradation rate constant of phenolics

consecutive reaction kinetic model used herein, observed a higher k_f value in half-slice lemons than in slice ones, suggesting the phenylpropanoid metabolism activation with wounding intensity. Moreover, authors could not find differences between k_d of slice and half-slice lemons, indicating that the cut type did not have a significant effect on the phenolic oxidation.

The analysis of the effect of temperature on both k_f and k_d , for each type of strawberry cutting, revealed that the reaction constant values did not increase with increasing temperatures, confirming the independence with temperature of both the de novo synthesis of phenolics induced by wounding and the phenolic compound oxidation. Therefore, activation energies predictions using the Arrhenius equation were not able to obtain. In agreement, Reyes and Cisneros-Zevallos [\(2003](#page-15-0)) reported that blue potatoes showed no significant differences in the accumulation of anthocyanins or phenolics after storage at different temperatures (2, 10, and 20 °C), suggesting that temperature would not affect the phenylpropanoid metabolism in purple-flesh potatoes. In the same way, Amodio et al. [\(2014\)](#page-14-0) reported that for fresh-cut purslane samples, k_f values at 0 and 5 °C were not different between each other, indicating the non-dependence of phenylpropanoid metabolism activation with temperature. However, these authors reported that samples stored at 5 °C shown a k_d value 1.4-fold higher than the value observed at 0 °C, suggesting a dependence of phenolic oxidation in fresh-cut purslane with temperature.

Total Anthocyanins

Anthocyanins are highly unstable and very susceptible to degradation. Its stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, and the presence of enzymes, proteins, and metallic ions (Castañeda-Ovando et al. [2009](#page-14-0)).

Despite of the transient increase observed in TP contents as response to abiotic stress produced by wounding, the TA contents of fresh-cut strawberries decreased during the storage time for all the types of cutting, at all the studied temperatures (Fig. 2). The highest losses of TA were 35.0, 16.5, and 25.8% in W, H, and Q strawberries stored at 2 °C, at the end of the storage time (day 15) (Fig. 2).

The degradation of anthocyanins could be occasioned by the progressive accumulation of $CO₂$ inside the round containers during the storage. Specifically, W, H, and Q strawberries stored at 2 \degree C showed an increase in CO₂ concentrations that was accompanied by a decrease in the concentrations of O_2 , remaining practically constant from day 1 to the end of storage. W samples reached equilibrium values between 2 and 4 kPa of $CO₂$, and H and Q samples showed equilibrium values between 5 and 9 kPa of $CO₂$. Equilibrium values in $O₂$ content ranged from 12 to 16 kPa in these samples.

The accumulation of $CO₂$ has been shown to promote anthocyanin losses in stored pomegranate arils (Holcroft et al. [1998\)](#page-15-0), and in fresh-cut strawberries (Gil et al. [1997\)](#page-15-0). As indicated before, the stability of anthocyanins is influenced by pH. Equilibrium exists among four anthocyanin species, including quinonoidal base, the flavylium cation (red color), the pseudobase or carbitol (colorless), and the chalcones. The atmospheres enriched with $CO₂$ could affect the organic acid metabolism, increasing the pH of the tissues and thus reversing equilibrium of anthocyanins toward the formation of the pseudobase or carbitol (Odriozola-Serrano et al. [2010\)](#page-15-0).

As it can be seen in Fig. 2, the content of TA in Q strawberries, those with the highest wounding intensity, resulted in intermediate values between W and H strawberries, indicating that these samples experimented lower TA losses during storage, presumably due to a higher anthocyanin synthesis via wounding stress PAL activation.

Moreover, Zheng et al. ([2007](#page-15-0)) and Odriozola-Serrano et al. [\(2010\)](#page-15-0) reported that the storage of fresh-cut strawberries at atmospheres with \leq 21 kPa O₂, as presented in passive atmospheres, could also increase the anthocyanin content, presumably due to PAL activation. This would mean an additional phenomenon to the de novo synthesis of phenolic compounds as a response to the abiotic stress induced by wounding. Therefore, the result of the concentration of anthocyanins would be determined by the degradation produced by the $CO₂$ accumulation or other factors, and the synthesis in response to PAL activation caused by wounding and the modified gas composition.

Zero order kinetic model (Eq. [3\)](#page-5-0) was adopted for describing the changes in TA content as a function of time and type of cutting at each storage temperature, based on the better fitting parameters obtained (data not shown). The ANOVA indicated that linear model was significant ($P < 0.05$), R^2 values were, in general, higher than 0.80 (Table [3\)](#page-10-0), and residuals satisfied the

Fig. 2 Experimental and predicted total anthocyanin content retention (TA R $%$) content of whole without hull (W) (a), halved (H) (b), and quartered (Q) (c) strawberries stored at 2, 6, and 13 °C. Experimental values (circle for 2 °C, square for 6 °C, triangle for 13 °C); models $($for 2 °C, $-$ for 6 °C, $-$ - for 13 °C). Vertical bars indicate the standard error of the means $(n = 3)$

assumptions of normality, independence, and randomness (data not shown). As shown in Table [3,](#page-10-0) the reaction rate constants (k) of Q strawberries calculated at 2, 6, and 13 $^{\circ}$ C were intermediate among values of W and H strawberries $(W > Q > H)$. For instance, k values at 2 \degree C were 1.0, 0.37, and 0.65 for W, H, and Q strawberries, respectively. These results would explain that even though the general trend of anthocyanins throughout the storage was to be oxidized by $CO₂$ accumulation and other factors, the activation of the phenylpropanoid metabolism due to the wounding stress and the modified atmosphere could limit this decrease through the anthocyanin synthesis.

The analysis of the effect of temperature on k for each type of strawberry cutting revealed that the degradation constant Table 3 Estimated rate reaction constants (k) for the zero order model proposed (Eq. [3](#page-5-0)) for total anthocyanin changes on W, H, and Q strawberries

W whole without hull strawberries, H halved strawberries, O quartered strawberries

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values did not increase with increasing temperatures. Therefore, prediction of activation energies could not be estimated, suggesting that the kinetic of change of TA content seems not to be dependent on temperature in this range (2 to 13 °C), as seen on Fig. [2.](#page-9-0) In agreement, Odriozola-Serrano et al. [\(2009\)](#page-15-0) who modeled the changes in anthocyanins of quartered fresh-cut strawberries stored under 80 kPa $O₂$ reported that anthocyanins content in samples stored at 5 °C was degraded during the 1st days of storage, but then the content was almost maintained over time. On the contrary, authors stated that anthocyanins in samples stored between 10 and 20 °C were increasingly destroyed over time and degradation rate constants were temperature dependent.

Vitamin C

The VitC content of fresh-cut strawberries of all types of cutting remained almost constant (2 and 6 $^{\circ}$ C) for 15 and 8 days, respectively, or with slight losses (less than 10%) at 13 °C for 7 days (data not shown). Therefore, it can be inferred that storage temperature (2 to 13 °C) in fresh-cut strawberries did not produce important losses in the VitC content, and then, these results could not be modeled. In agreement, Gil et al. [\(2006\)](#page-15-0) reported that the VitC content remained constant in quartered strawberries variety 'Seascape' after 9 days of storage at 5 °C. However, Odriozola-Serrano et al. ([2010](#page-15-0)) reported 40% losses in the VitC content of quartered strawberries after 21 days of storage at 4 °C. The increase in the VitC catabolism reported by authors was attributed to the high accumulation of $CO₂$ inside the round containers due to product respiration for 21 days.

Phenylalanine Ammonia Lyase

The relative PAL activity changes (%) during the storage of W, H, and Q strawberries stored at 2, 6, and 13 °C are shown in Fig. 3. According to the results, an increase in PAL activity was observed as the intensity of wounding increased. It is recognized that wounding promotes an increase in PAL activity, which catalyzes the first step of phenylpropanoid metabolism (Murata et al. [2004\)](#page-15-0). Therefore, the effect of the intensity of wounding results to be proportional to the PAL activity and the TP content (Cantos et al. [2002](#page-14-0)).

Fig. 3 Experimental and predicted relative phenylalanine ammonia lyase (PAL $\%$) activity of whole without hull (W) (a), halved (H) (b), and quartered (Q) (c) strawberries stored at 2, 6, and 13 °C. Experimental values (circle for 2 °C, square for 6 °C, triangle for 13 °C); models (........for 2 °C, $-$ for 6 °C, $-$ for 13 °C). Vertical bars indicate the standard error of the means $(n = 3)$

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Table 4 Estimated parameters of proposed model of relative PAL activity (%) (Eq. [9](#page-6-0)) and activation energies (Eq. [11](#page-6-0)) of W, H, and Q strawberries

PAL phenylalanine ammonia lyase, W whole without hull strawberries, H halved strawberries, Q quartered strawberries, Ea_f (J mol⁻¹) activation energy of enzyme formation, Ea_d (J mol⁻¹) activation energy of enzyme inactivation, $PAL_{pre,0}$ predicted constant of enzyme precursor, W whole without hull strawberries, H halved strawberries, Q quartered strawberries, k_f (day^{−1}) reaction rate constant for formation of the active form of the enzyme, k_d (day⁻¹) dissociation rate constant of the active form of the enzyme

Moreover, analysis of the results revealed that the higher the temperature of storage for each type of cutting, the higher the PAL activity (Fig. [3](#page-10-0)). Ardila et al. ([2007\)](#page-14-0) reported increases in PAL activity with raising temperatures, having determined 37 °C as the optimum enzyme temperature. However, as discussed above, it was demonstrated that the increase in the storage temperature for a given type of strawberry cutting did not influence the phenolic compound synthesis as a response to the abiotic stress induced by wounding. However, the losses in the total anthocyanins contents observed in all samples at all temperatures would be the reason by which we could not notice an increased in the total phenolic contents, even when higher PAL activities were obtained as temperature increased.

A kinetic model (Eq. [9](#page-6-0)) was used for modeling PAL activity in fresh-cut strawberries as a function of time, for each type of cutting, stored at 2, 6, and 13 °C. Table 4 presents the kinetic parameters obtained for PAL modeling. R^2 values ranged since 0.71 to 1.00, and residuals satisfied the assumptions of normality, independence, and randomness (data not shown). As observed, in general, for a given temperature, the greater the intensity of wounding, the higher the values of formation constants (k_f) of active enzyme (W < H < Q). For instance, at 2 °C , k_f values were 0.3, 1.1, and 2.1 for W, H, and Q strawberries (Table 4). However, this behavior was not followed by the dissociation constants (k_d) , whose values did not present differences among type of cuttings at a given temperature. For example, at 2 $\mathrm{^{\circ}C}, k_{\mathrm{d}}$ values were 0.04, 0.02, and 0.03 for W, H, and Q strawberries (Table 4).

The Arrhenius equation allowed analyzing the effect of temperature on k_f and k_d for each type of strawberry cutting. Regarding, W and H strawberries, it was observed that both rate constants (k_f and k_d) increased with temperature, favoring initially the formation of the active PAL, and then its inactivation. However, the higher energy activation (Ea) values calculated for k_f parameters in W strawberries indicate a higher dependence of this type of cutting with temperature. In the case of Q strawberries, they showed similar k_f values at all temperatures, and these values were higher than those observed for W and H strawberries (Table 4), suggesting higher functionality of k_f with abiotic stress produced by wounding rather than with temperature. However, k_d values showed functionality with temperature for Q strawberries; thus, the inactivation of PAL was favored as temperature increased (Table 4).

Polyphenoloxidase

Figure [4](#page-12-0) shows the relative PPO activity changes (%) during the storage of W, H, and Q strawberries stored at 2, 6, and 13 °C. As shown, an increase on PPO activity was observed during the storage of some of the samples. However, other samples did not experience an increase on enzyme activity during storage time. The highest PPO activities were quantified for W strawberries, the least wounded samples, at all temperatures (Fig. [4\)](#page-12-0). These results highlight an apparent independence of PPO activity with abiotic stress produced by wounding, differing from the behavior observed for PAL activity.

Phenolic compounds and PPO are in different compartments in plant cells, thus they cannot interact with each other. By peeling and/or cutting of the fruit, cells are disrupted, and so, the enzyme can contact its substrate (phenolic compounds) (Cantos et al. [2002\)](#page-14-0). Therefore, it is probably that PPO is better protected in its cellular compartment and remained in better conditions for the enzymatic reaction after extraction in W and H samples than in Q ones.

A zero order kinetic model (Eq. [3](#page-5-0)) was adopted for describing the changes (%) in relative PPO activity rather than first order model (data not shown). Figure [4](#page-12-0) shows

Fig. 4 Experimental and predicted relative polyphenoloxidase (PPO %) activity of whole without hull (W) (a), halved (H) (b), and quartered (Q) (c) strawberries stored at 2, 6, and 13 °C. Experimental values (circle for 2 °C, square for 6 °C, triangle for 13 °C); models (\dots for 2 °C, $$ for 6 °C, \bullet \bullet \bullet \bullet for 13 °C). Vertical bars indicate the standard error of the means $(n = 3)$

changes (%) in PPO activity of fresh-cut strawberries during storage at all temperatures adjusted to the proposed model, when it was possible to perform. H strawberries showed no changes at 2 and 6 °C. Table 5 presents kinetic parameters for PPO activity in fresh-cut strawberries obtained using this model, when it was possible to calculate. Even though R^2 values were not acceptable in some cases. the reaction constants (k) of W strawberries were higher than Q values at all studied temperatures, confirming the higher PPO activity in W strawberries.

In general, the increase in the storage temperatures from 2 to 13 °C for each type of cutting elicited higher PPO activities (higher reaction rate constants). For instance, k values for Q strawberries were 0.40, 3.67, and 12.26 at 2, 6, and 13 °C (Table 5). In agreement, Gasull and Becerra ([2006](#page-15-0)) observed that PPO activity of apples and pears increased with an increase in temperature, having been higher between 30 and 40 °C. However, the activation energy results for W and H strawberries were similar and lower than the value obtained for Q strawberries (Table 5), suggesting that in the last case (more wounded tissue) PPO activity rate constants are more sensitive with the temperature changes.

pH and Soluble Solids

In general, the SS contents of W, H, and Q strawberries stored at 2, 6, and 13 °C decreased during storage (data not shown). Q strawberries stored at 6 and 13 °C presented the highest losses of SS, 10 and 15%, respectively. Higher depletion of SS at this temperature could be explained by higher respiratory activities of these fruits (Ayala-Zavala et al. [2004](#page-14-0)).

Meanwhile, the pH values of samples tended to progressively increase during storage or they were maintained near to their initial values (data not shown). Although high $CO₂$ atmospheres, reached especially in the samples stored at 13 °C (higher than 20 kPa from day 3 of storage), may have decreased the pH of the tissues by dissolution of $CO₂$ gas, the concomitant oxidation of organic acids during product respiration produced no changes or slight increases in pH values at the end of the storage.

SS and pH changes could be better modeled with zero order kinetics (Table [6\)](#page-13-0). The activation energy obtained for

PPO polyphenol oxidase, W whole without hull strawberries, H halved strawberries, Q quartered strawberries, Ea activation energy

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constants (k) for model (Eq. 3)

changes of W. strawberries

SS soluble solids, W whole without hull strawberries, H halved strawberries, Q quartered strawberries

the SS changes of W and H strawberries was similar between each other and lower than the value calculated for Q strawberries (Table 6). Latter results suggest more dependence of SS change with temperature in the most wounded samples. In the case of pH change, according to the activation energy values obtained, temperature affected similarly the increase of this attribute for all the type of cutting (Table 6). Similarly, no differences in pH values were found during the storage of whole strawberries at 0, 5, and 10 $^{\circ}$ C for up to 13 days by Ayala-Zavala et al. [\(2004\)](#page-14-0).

Color

A decrease in L^* parameter for all samples at all temperatures was observed. This decrease (darkening of the fruit) was faster in samples stored at high temperatures (13 °C) (data not shown). Similarly, Wright and Kader [\(1997](#page-15-0)) reported a decrease in L^* parameter with storage time in whole and quartered strawberries variety 'Selva'stored at 5 °C for 7 days. In the same way, h_{ab} ^{*} values of samples studied herein decreased progressively during storage and with the increase of

temperature. Smaller values of h_{ab} ^{*} near to 0° mean an increase in the red hue of the fruit color with the storage time. Meanwhile, C_{ab}^* values (color chromaticity) decreased over time as the temperature increased for all types of cutting (data not shown).

Color parameters could be better modeled with zero order kinetics (Table [6](#page-13-0)). The reaction rate constants obtained for L^* parameter, for all types of cutting, confirm edits more pronounced decrease with the increase of temperature from 2 to 13 °C (Table [6](#page-13-0)). Moreover, according to the predicted activation energies, there would be a higher sensibility to tissue browning at higher temperatures, as the intensity of wounding increase. Additionally, h_{ab} ^{*} rate constant changes were higher as temperature increased for a given type of cutting, and the predicted activation energies had similar values for the three types of cutting, showing no dependence of this color parameter with the intensity of wounding of the plant tissue (Table [6](#page-13-0)).

Similarly, Fadda et al. ([2015](#page-15-0)) reported a decrease in color parameters with storage time of whole strawberries stored at 0 °C for 12 days. Authors stated that the decrease in L^* was probably caused by enzymatic browning and changes in the color were only due to a decrease in saturation, as it was also demonstrated by the concomitant decrease in the chroma values.

Conclusions

The results obtained in this global study revealed that it is possible to prepare quartered strawberries with high quality up to 15 days at 2 °C and with higher content of phenolic compounds (up to 22% more than whole strawberries). The effect of increasing the intensity of wounding of fresh-cut strawberries revealed an activation of the phenylpropanoid metabolism, which resulted in higher synthesis of phenolic compounds. The proposed models described the changes on bioactive compounds, on the enzymes of the phenylpropanoid metabolism and the evolution of the quality attributes as a function of time and temperature in fresh-cut strawberries with different types of cutting. The changes on quality parameters (soluble solids, pH, and color), anthocyanin content retention, as well as relative PPO activity of whole without hull, halved, and quartered strawberries were adequately fitted with zero order kinetic. All the rate constants of these attributes, except for anthocyanins, fitted appropriately with Arrhenius equation. The changes on total phenolic content retention and on the relative PAL activity were adequately fitted with a consecutive reaction mechanistic kinetic model for each type of cutting. Better fit was obtained for quartered strawberries (with the higher wounding stress). The rate constants of phenolic kinetic showed no dependence with

temperature. However, rate constants of PAL activity fitted appropriately with Arrhenius equation, with different activation energies depending on the type of cutting. This global study offers a better understanding of the effects of processing and storage conditions on general quality, bioactive compounds, and phenylpropanoid metabolism enzymes of fresh-cut strawberries.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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