



Impact of a new postharvest disinfection method based on peracetic acid fogging on the phenolic profile of strawberries



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ABSTRACT

The retentions of fresh strawberry individual phenolic compounds after fogging using an environmentally friendly sanitizer based on peracetic acid (PAA) (mixture of 5% peracetic acid and 20% hydrogen peroxide) were studied and modeled as a function of the concentration (3.4, 20.0, 60.0, 100.0 and 116.6 $\mu\text{L PAA L}^{-1}$ air chamber) and the treatment time (5.7, 15.0, 37.5, 60.0 and 69.3 min), using Response Surface Methodology. Information obtained from high performance liquid chromatography with photodiode array and fluorescence detection in combination with mass spectrometry was used for analyzing and quantifying the phenolics that naturally occur in strawberries (variety 'Camarosa') and to study the effects of PAA on them. Results showed that PAA fogging at certain concentrations and times caused degradation in the phenolic profile of strawberries. Anthocyanins were the most affected of the phenolic compounds, followed by proanthocyanidins with a low degree of polymerization, hydroxycinnamic acid derivatives, and the ellagitannin Sanguin H-6. In general, pelargonidin-based anthocyanins were more susceptible to oxidation than cyanidin-based anthocyanin under the same PAA fogging conditions. In summary, the stability of strawberry individual phenolic compounds after fogging treatments was dependent on the concentration and the exposure time of PAA treatments as well as the chemical nature of them. The models developed herein allow to predict retentions of individual phenolic compounds at different fogging PAA conditions.

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1. Introduction

Epidemiological studies have noted that fruit and vegetable consumption protects against degenerative diseases, including cancer, heart disease and stroke, and can contribute to control of diabetes and obesity. These benefits are linked to the optimal mix of antioxidant and anti-inflammatory phytoactive compounds present in edible produce (Alvarez-Suarez et al., 2014). Strawberry is one of the most commonly consumed fruits due to its attractive color and taste, and recognition as a very rich source of antioxidant compounds including vitamin C and phenolic compounds (da Silva Pinto et al., 2008). Phenolics contribute to both the sensory and organoleptic quality attributes of strawberry, and its health-protective value (Espín and Tomás-Barberán, 2001).

Proanthocyanidins (PAC), also known as condensed tannins, are mixtures of oligomers and polymers composed of flavan-3-ol, and represent one of the main categories of phenolic compounds found in strawberries (Gu et al., 2003). The PAC concentration reported in several varieties of strawberries ranged between 53.9 and 163.2 mg 100 g^{-1} of fresh weight (FW) (Buendía et al., 2010). Glycoside derivatives from the anthocyanins, pelargonidin and cyanidin, are the main flavonoids found in strawberries with reported concentrations of up to 65 mg 100 g^{-1} FW. 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). Another interesting group of phenolic compounds in strawberry is the hydrolysable tannins (ellagitannins) (Clifford and Scalbert, 2000). Other phenolics present in lower concentrations are flavonols (quercetin and kaempferol glycosides), esters of hydroxycinnamic acids (especially of *p*-coumaric acid), ellagic acid and ellagic acid glycosides (Määtä-Riihinen et al., 2004; Buendía et al., 2010).

Like most other fruits, strawberries can be consumed fresh, which can be advantageous to consumers since nutritional losses

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due to processing can be avoided. However, the storage period and the shelf-life of this fruit are very short due to its perishability and susceptibility to the growth of rot-causing pathogens (Vardar et al., 2012). Therefore, the application of decontamination processes that can ensure microbiological safety and shelf-life extension of the product, while retaining quality attributes close to the fresh characteristics, becomes crucial for this kind of fruit (Alexandre et al., 2014). Unfortunately, strawberries can be damaged easily when traditional washing is performed in a processing line, and the drying period delays pre-cooling, which may facilitate pathogen infection (Vardar et al., 2012). Hence, the application of disinfectant agents by fogging can be an effective alternative technology since handling and wetting of the fruit is minimized (Oh et al., 2005; Vardar et al., 2012). This operation has been already used successfully for the decontamination and control of postharvest diseases of strawberries, employing chlorine dioxide, sodium hypochlorite, citric acid and ethanol as disinfectant agents (Vardar et al., 2012); and similarly figs have been treated postharvest with chlorine dioxide (Karabulut et al., 2009).

Fogging with the sanitizer based on peracetic acid (PAA) may be a promising option for controlling the microbial population and extending the storage time for up to 7 days at 2 °C of fresh whole strawberries, employing a disinfectant that is recognized for being environmentally friendly (Van de Velde et al., 2016). The commercially available PAA works with a quaternary equilibrium of acetic acid, hydrogen peroxide, peracetic acid and water, and its decomposition products are only oxygen and acetic acid. Moreover, the advantages of the use of PAA over other agents, such as chlorine, include a lack of or only negligible formation of toxic or carcinogenic compounds, and that its activity is little influenced by the presence of organic material and is not dependent on factors such as pH and temperature (Vandekinderen et al., 2009). The effectiveness of PAA solutions in reducing the initial microbiologic loads was demonstrated in the washing-disinfection of a wide range of fresh-cut fruits and vegetables (Artés et al., 2009; Van de Velde et al., 2013), and its use for fogging of lettuce leaves (Oh et al., 2005). Recently we showed that fogging with PAA was effective for reducing the total mesophilic microbial and yeast and mould loads of whole strawberries for up to 7 days of storage at 2 °C (Van de Velde et al., 2016).

Despite the well documented microbial effectiveness of PAA, these disinfectant solutions are also strong oxidants (the oxidation potentials of peracetic acid and hydrogen peroxide are close to 1.8 eV) (Pechacek et al., 2015), and phytochemicals and other nutritional compounds of strawberries, as well as quality parameters such as color, can be oxidized/affected by the treatment conditions. Özkan et al. (2005) described a degradation of anthocyanins in sour cherry nectar, strawberry and pomegranate juices following hydrogen peroxide treatments. Meanwhile, Van de Velde et al. (2013) reported that the retention of total anthocyanins and vitamin C was adversely affected after a postharvest washing-disinfection of fresh-cut strawberries using PAA solutions. Subsequently, Van de Velde et al. (2016) concluded that the fogging of whole strawberries with PAA reduced the total anthocyanin, total phenolic, and vitamin C contents, the antioxidant capacity, and the color of the fruit. The degree of postharvest loss was conditioned by the concentration and the exposure time of fogging treatments.

Therefore, considering the relevance of different individual phenolic compounds to the color and health-related properties of the fruit, it is important to determine which fogging parameters conditions would be optimal to ensure disinfection, yet minimize damage to phenolic constituents of strawberry. The aim of this work was to study and model the effects of PAA fogging, immediately after the operation, at different concentrations and contact times on the individual naturally occurring phenolic

compounds of strawberries, using the complementary information from high performance liquid chromatography (HPLC) with photodiode array (PDA) and fluorescence (FLD) detection in combination with mass spectrometry (MS).

2. Materials and methods

2.1. Chemicals and reagents

Oxilac Plus, a commercial sanitizer based on peracetic acid (PAA) was obtained from Indaquim S.A. (Santa Fe, Argentina). Oxilac Plus is a stabilized mixture of 5% peracetic acid, 20% hydrogen peroxide and water. Reference compounds procyanidin B2 (PAC-B2) (PubChem CID: 122738), pelargonidin-3-O-glucoside (PubChem CID: 443648), cyanidin-3-O-glucoside (PubChem CID: 441667), ellagic acid (PubChem CID: 5281855), quercetin (PubChem CID: 5280343) and *p*-coumaric acid (PubChem CID: 637542) were purchased from Chromadex (Irvine, CA, USA). All solvents were HPLC grade and obtained from VWR International (Suwanee, GA, USA).

2.2. Plant material

Cultivated strawberries (*Fragaria x ananassa* Duch.) cultivar 'Camarosa' were obtained from one planting at Arroyo Leyes (31° 27' 0" S, 60° 40' 0" W), Santa Fe, Argentina. Fruit was harvested by skilled workers at full ripeness stage (90% of the surface showing red color) and was transported 20 km directly from the field to the laboratory of the Instituto de Tecnología de Alimentos, FIQ, UNL, Argentina, and stored at 2 °C until use. Harvested fruit was selected for uniformity of size, color and absence of defects before use in experiments.

2.3. Fogging system and procedure

Treatments were set up in a 16 L plastic hermetically sealed model chamber specially designed for this experiment. The fogging was performed using an ultrasonic aerosol generator unit (Respirex, Accme, SRL, Córdoba, Argentina) that has a liquid reservoir (30 mL) for holding the liquid to be fogged and produces a fog of droplets between 0.5–8.0 μm in diameter. The small particles are carried away by the airflow and blown inside the chamber. Six round rigid plastic trays (capacity 270 cm³), each containing 150 g of selected strawberry fruit were placed inside the chamber with the lids opened and were fogged at various PAA concentrations (μL of PAA per L of air chamber) and contact times, according to the experimental design (Section 2.4). All the fogging treatments were performed at room temperature (24 °C). The fogging system unit was turned on for nebulization of all the liquid in the reservoir (typically between 5 to 10 minutes), then the fogging system was turned off and the samples were left inside the chamber to complete the contact times according to the experimental design (Section 2.4). Treated samples were taken out of the chamber, frozen at –80 °C before lyophilization in a Flexy-dry freeze dryer (SP Scientific, NY, USA), and then analyzed.

Untreated strawberries (500 g; raw material) were used as the control and were frozen at –80 °C before lyophilization in the same way. The freeze-dried material was weighed and the dry matter content was estimated by difference in weight.

2.4. Experimental design and response modeling

Response Surface Methodology (RSM) using a Central Composite Design (CCD) was used to study the fogging operation. The CCD is the most frequent five levels fractional factorial design used for the construction of a second-order response surface model. The

total number of needed experiments (N) of the CCD design can be determined as shown in Eq. (1):

$$N = 2^k + 2k + N_0 \quad (1)$$

where k is the number of factors and 2^k , $2k$ and N_0 are the cubic, axial and the center point's runs respectively. The center points of CCD are used to calculate the experimental error. The distances of the axial points from the center points are dependent on the number of factors chosen for the experiment (Montgomery, 2001). The PAA fogging concentration and the treatment time were the selected variables in the current study ($k=2$). Therefore, N was calculated using Eq. (1) and was equal to 11 (4 factorial design points + 4 axial points + 3 replicates in the central point). Each of the variables was examined at five different levels ($-\alpha$, -1 , 0 , 1 , and $+\alpha$).

It was assumed that there was a mathematical function for each studied response according to the two variables related to fogging processing (Eq. (2)):

$$Y = f(C, t) \quad (2)$$

where C = PAA fogging concentration (μL of sanitizer per L of air chamber) and t = time of the treatment (min) and the five variable levels were as follows: $C=3.4, 20, 60, 100$ and $116.6 \mu\text{L L}^{-1}$ and $t=5.7, 15, 37.5, 60$ and 69.3 min. The variable limits were determined in previous experiments based on the microbiological efficacy and the retention of the general quality of the fruit (Van de Velde et al., 2016). The studied responses (Y) were the content retentions ($R\%$) of individual phenolic compounds after fogging and were expressed as $(Q/Q_c) \times 100$, where Q and Q_c represent the phenolic content in fogged and control strawberries, respectively.

A second-order polynomial equation was proposed to model Eq. (2) for each response (Eq. (3)):

$$Y = \beta_0 + \beta_C \times C + \beta_t \times t + \beta_{CC} \times C^2 + \beta_{tt} \times t^2 + \beta_{tC} \times t \times C \quad (3)$$

where β_0 , β_C , β_t , β_{CC} , β_{tt} and β_{tC} are the regression coefficients and C and t are the studied variables.

2.5. Soluble solids and pH analyses

The pH values were obtained with a pHmeter (Horiba B-213 TwinpH meter, Horiba Ltd., Kyoto, Japan) in triplicate. The soluble solids content in homogenized samples was determined in triplicate using a hand-held digital refractometer model Pal-alpha (Atago Co Ltd., WA, USA) and results were expressed as °Brix.

2.6. Phytochemical determination

2.6.1. Extract preparation

For the phenolic compound analysis, 0.5 g of freeze-dried strawberries was placed into 15 mL centrifuge vials. Eight mL of 80% methanol: 20% water (0.5% acetic acid) was added and the mixture was sonicated for 10 min. The mixture was then centrifuged at 5000 rpm (Sorvall RC-6 plus, Asheville, NC, USA) for 10 min, and the resulting supernatant was collected into a 25 mL volumetric flask. The extraction of the pellet was repeated two more times and the combined extracts were brought to a final volume of 25 mL with the extraction solvent. All extractions were made in triplicate.

2.6.2. Phenolic determination by HPLC

HPLC analysis for phenolic compounds was conducted using an Agilent 1200HPLC (Agilent Technologies, Santa Clara, CA, USA) with a PDA and an autosampler with Chemstation software as a controller and for data processing.

Anthocyanin separation was performed using a reversed phase Supelcosil-LC-18 column, 25 mm \times 4.6 mm \times 5 μm (Supelco, Bellefonte, PA, USA) at 30 °C. The mobile phase consisted of 5% formic acid in water (A) and 100% methanol (B). The flow rate was constant during HPLC analysis at 1 mL min⁻¹ with a step gradient of 10, 15, 20, 25, 30, 60, 10, and 10% of solvent B at 0, 5, 15, 20, 25, 45, 47, and 60 min, respectively. Quantification of anthocyanins was performed from the peak areas recorded at 520 nm with reference to the external standard calibration curve obtained with pelargonidin-3-O-glucoside (0.125–0.5 mg mL⁻¹) and cyanidin-3-O-glucoside (0.125–0.375 mg mL⁻¹).

HPLC analysis for phenolic compounds was performed using a Synergi 4 μm Hydro-RP 80A column (250 mm \times 4.6 mm \times 5 μm , Phenomenex, Torrance, CA, USA). The mobile phase consisted of 2% acetic acid in water (A) and 0.5% acetic acid in 50% acetonitrile in water (B). The flow rate was set at 1 mL min⁻¹ with a step gradient of 10, 15, 25, 55, 100, and 10% of solvent B at 0, 13, 20, 50, 54, and 60 min, respectively. UV maximum absorption was recorded at 254, 280 and 360 nm. Ellagic acid (0.0125–0.05 mg mL⁻¹), quercetin (0.0078–0.0625 mg mL⁻¹) and *p*-coumaric acid (0.05–1.0 mg mL⁻¹) were used as external standards for the identification and quantification of related phenolic compounds.

Proanthocyanidin separation was performed according to the method of Wallace and Giusti (2010) using a normal phase Develosil Diol column, 250 mm \times 4.6 mm \times 5 μm (Phenomenex). The binary mobile phase consisted of (A) acetonitrile/acetic acid (98:2, v/v) and (B) methanol/water/acetic acid (95:3:2, v/v/v). Separation was accomplished using a linear gradient at 35 °C with 0.8 mL min⁻¹ flow rate as follows: 0–35 min, 0–40% B; 35–40 min, 40–100% B; isocratic 100% B, 45 min; 100–0% B, 50 min; and 0% B to 55 min. The column was re-equilibrated for 5 min between samples. Eluate was monitored by fluorescence detection with excitation at 230 nm and emission at 321 nm as well as at 280 nm with the PDA detector. Proanthocyanidins (PAC) quantification was performed with calibration curves obtained with an external standard of PAC B2 (0.1–0.4 mg mL⁻¹).

LC-MS analysis was used for phenolic compound identification, molecular formula determination and structural interpretation. A Shimadzu LC-ESI-TOF-MSⁿ (liquid chromatography-electrospray ionization ion-trap time-of-flight mass spectrometry) system was used for analysis (Shimadzu Scientific Instruments, Columbia, MD, USA). This LC-ESI-TOF-MSⁿ system was equipped with a Prominence HPLC system and separation was performed using a Shim-pack XR-ODS column (50 mm \times 3 mm \times 2.2 μm) at 40 °C with a binary solvent system comprised of 0.1% formic acid in water (A), and methanol (B). Compounds were eluted into the ion source at a flow rate of 0.35 mL min⁻¹ with a step gradient of B of 5–8% (0–5 min), 8–14% (10 min), 14% (15 min), 20% (25 min), 25% (30 min), 85% (32 min) and back to 5% (40 min). Ionization was performed using an ESI source in the positive and negative modes.

All extracts were analyzed in triplicate, samples were filtered through 0.2 μm nylon filters (Fisher Scientific, Pittsburgh, PA, USA) before being injected onto the HPLC columns and results were expressed as mg phenolic compound per 100 g⁻¹ FW.

2.7. Statistical analysis

STATGRAPHICS Centurion XV 15.2.06 (Statpoint Technologies, Inc., Warrenton, Virginia, USA) was used to perform ANOVA analysis, to fit the second order polynomial equations to the experimental data and to obtain the coefficients of the equations. For verification of the model adequacy, the lack of fit, the coefficient of determination (R^2) and the absolute average deviation (AAD) (Baş and Boyacı, 2007) were calculated. The significance of each term of the models was evaluated and referred

to as the pure error, and the elimination of non-significant terms was done by means of the linear stepwise regression procedure.

3. Results and discussion

3.1. Characterization of phenolic compounds of 'Camarosa' strawberries

Strawberries contain a range of phenolic compounds including flavonoids (anthocyanins and flavonols), phenolic acids (hydroxycinnamic acid derivatives), hydrolysable tannins (ellagitannins) and condensed tannins (proanthocyanidins) (Ignat et al., 2011). Compounds were characterized and identified by their LC retention times, UV-Vis, MS, MS² spectra and by comparison with available references and reported literature. HPLC-PDA-ESI-MS separation for the samples recorded at 520 and 280 nm is shown in Fig. 1a and 1b, respectively. Pelargonidin-3-O-glucoside (peak 6) with a MS [M]⁺ ion at *m/z* 433 and its characteristic MS² fragment ion at *m/z* 271, obtained after the loss of 162 amu (hexose moiety), was the major anthocyanin detected in the samples, in agreement with previous published reports (Kajdžanoska et al., 2010; Aaby et al., 2012). Pelargonidin-3-O-rutinoside (peak 7) was also identified in the samples and confirmed with a MS [M]⁺ at *m/z* 579, and MS² fragments at *m/z* 433 (–146 amu, loss of rhamnose) and 271 (–308 amu, loss of rutinose). A cyanidin based anthocyanin was detected in the samples (peak 5) and the ion at *m/z* 449 and the characteristic MS² fragment at *m/z* 287, obtained after loss of 162 amu (hexose moiety), confirmed the presence of cyanidin-3-O-glucoside (Aaby et al., 2012).

Quercetin-3-O-glucuronide was detected (peak 10) and confirmed in the samples by its MS [M–H][–] ion at *m/z* 477 and a MS² fragment at 301, obtained after loss of glucurone unit (176 amu). This compound was described previously in strawberries and it was reported as the major flavonol in this fruit (Määttä-Riihinen et al., 2004; Kajdžanoska et al., 2010).

A compound with maximum absorption at 314 nm was tentatively identified as a hydroxycinnamic acid derivative (peak 4) and confirmed with an MS [M–H][–] ion at *m/z* 325 and MS² fragment at *m/z* 163 (loss of hexose moiety) as *p*-coumaroyl hexoside (Fig. 1b). Cinnamoyl hexoside was identified in the samples (peak 8) and confirmed by similarity with the MS spectra published by other authors with a MS [M+HCO₂][–] ion at *m/z* 355 and MS² fragment ions at *m/z* 309, 207 and 147 (Lunkenbein et al., 2006; Aaby et al., 2007a, 2012).

A compound of maximum absorption at 243 nm was tentatively identified as an ellagitannin derivative (peak 9). Ellagitannins are hydrolysable tannins since they are esters of hexahydroxydiphenic acid (HHDP: 6,6'-dicarbonyl-2,2',3,3',4,4'-hexahydroxybiphenyl moiety) and a polyol, usually glucose, and in some cases gallic acid (Häkkinen et al., 1999). A double charged ion [M–2H]^{2–} at *m/z* 934, implying a true molecular weight of 1870, with major MS² fragments at 897, 633 and 301 were identified as a dimer of galloyl-bis-HHDP-glucose (sanguin H-6). This compound was also identified in 15 strawberry cultivars from Spain by Buendía et al. (2010) and its presence was also confirmed in 4 strawberry varieties grown in Macedonia by Kajdžanoska et al. (2010).

Normal phase HPLC with fluorescence detection was able to separate proanthocyanidin (PAC) components in the strawberry samples according to their degree of polymerization (Fig. 2). PAC

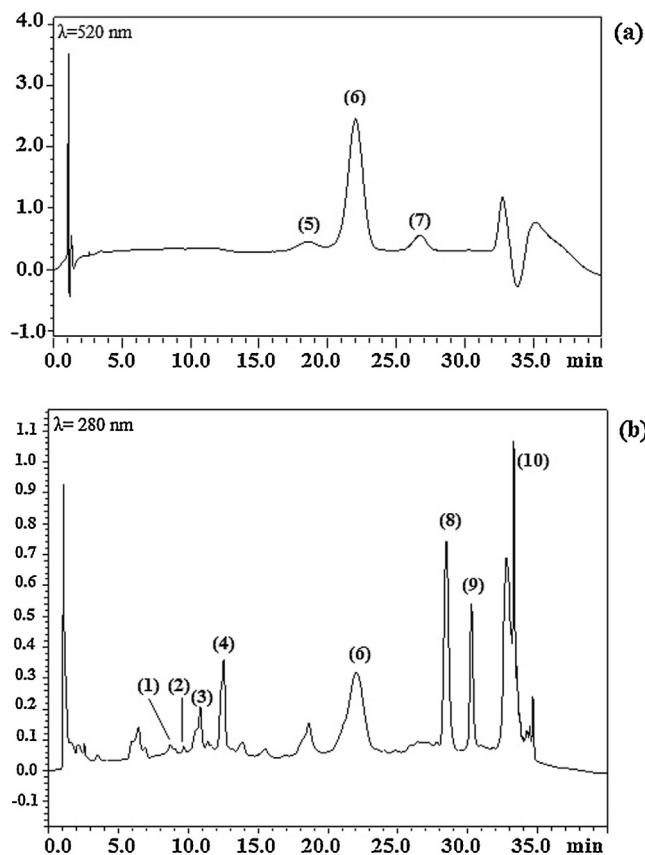


Fig. 1. LC-ESI-TOF-MSⁿ (liquid chromatography–electrospray ionization ion-trap time-of-flight mass spectrometry) chromatograms of strawberries at 520 nm (a) and 280 nm (b). Peak identification: procyanidin B-dimers (1,2), procyanidin trimer (3), *p*-coumaroylhexoside (4), cyanidin-3-O-glucoside (5), pelargonidin-3-O-glucoside (6), pelargonidin-3-O-rutinoside (7), cinnamoylhexoside (8), sanguin H-6 (9), quercetin-3-O-glucuronide (10).

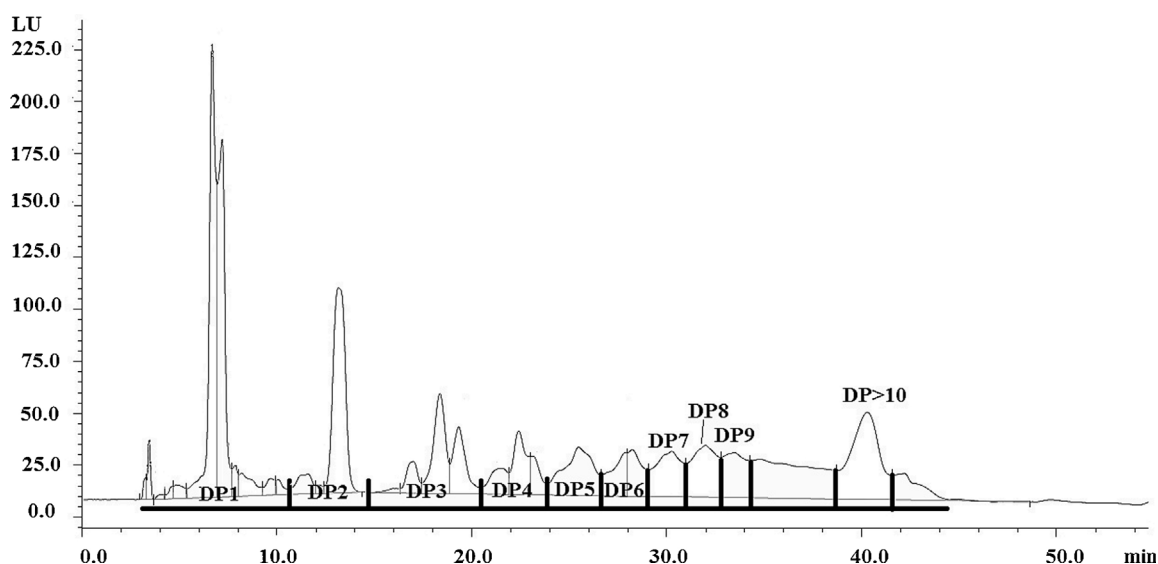


Fig. 2. Normal phase HPLC with fluorescence (FLD) chromatogram (excitation, 230 nm, emission, 320 nm) for proanthocyanidins of strawberries. DP=degree of polymerization.

are mixtures of oligomers and polymers composed of flavan-3-ol units linked mainly through C4–C8 bonds and in minor proportion through C4–C6 bonds (both called B-type); or doubly linked by an additional ether bond between C2–O7 (A-type). Proanthocyanidins with B-type linkages were detected as the only PAC component in strawberries by Gu et al. (2003).

As can be seen in Fig. 2, several peaks corresponding to dimers, trimers, tetramers, and oligomers of B-type proanthocyanidins were distinguished in the extracts. Even though flavan-3-ols have low UV extinction coefficients and reverse phase column cannot be used to separate PAC with a high degree of polymerization, the MS analysis revealed the presence of two proanthocyanidin dimers and one proanthocyanidin trimer (Fig. 1b). The dimers (peaks 1 and 2) were confirmed with an MS fragment $[M-H]^-$ at m/z 577 and MS^2 fragments at 425, 407 and 289 and the trimer (peak 3) was identified with an MS $[M-H]^-$ ion at m/z 865 and MS^2 fragment ions at m/z 801, 577, 407 and 287. The sequences in these oligomers were combinations of (epi) catechins because of the consecutive losses of 288 amu, corresponding to the (epi) catechin unit (Gu et al., 2003; Buendía et al., 2010).

Table 1 shows the average content of the individual phenolic compounds quantified in non-fogged samples of ‘Camarosa’ strawberries (control). Results were in agreement with reported concentrations of phenolic compounds for this cultivar (Buendía et al., 2010). The highest contributions to the sum of all the individual phenolic compounds concentrations in the strawberries samples (Table 1) were represented by the sum of the anthocyanins (43%) and the sum of all the polymerized forms of the proanthocyanidins (40%). Anthocyanins based in the aglycone pelargonidin represented 93% of the total anthocyanins reported, in agreement with other authors (Buendía et al., 2010; Aaby et al., 2012). Individual phenolic content detected and quantified in the control treatment was used as a reference for analyzing the effect of PAA fogging.

3.2. Effects of PAA fogging on quality attributes and phenolic compounds of strawberries

Strawberry characteristics were: 6.2 ± 0.2 Brix (soluble solids; SS) and pH 3.35 ± 0.02 . The PAA fogging treatments did not produce any significant change on several general quality attributes in strawberries, as presented in Table A1

(Appendix A). As shown, pH and SS of the sanitized strawberries were not different with respect to pH and SS of untreated fruit, except when fruit was treated with a PAA concentration of $116 \mu L^{-1}$ for a duration of 37.5 min. In this case, the pH value of treated strawberries was slightly lower than the pH of untreated fruit.

PAA fogging treatment elicited an oxidation effect on the individual phenolic compounds of the fruit to various degrees, depending on the fogging conditions. The flavonoid retention (R%) under different fogging conditions is presented in Table 2. As PAA fogging concentration and treatment time increased, anthocyanin and quercetin-3-O-glucuronide contents were decreased. The

Table 1
Phenolic content in ‘Camarosa’ strawberries.

Phenolic compounds	mg 100 g ⁻¹ FW
Flavonoids	
Anthocyanins	
Pelargonidin-3-O-glucoside	37.8 ± 0.4
Pelargonidin-3-O-rutinoside	2.4 ± 0.2
Cyanidin-3-O-glucoside	3.25 ± 0.02
Flavonols	
Quercetin-3-O-glucuronide	10.6 ± 0.6
Phenolic acids	
Hydroxycinnamic acid derivatives	
p-Coumaroyl hexoside	2.4 ± 0.2
Cinnamoyl hexoside	3.6 ± 0.1
Hydrolysable tannins	
Ellagitannins	
Sanguin H-6 (dimer of galloyl-bis-HHDP-glucose)	1.7 ± 0.1
Condensed tannins	
Proanthocyanidins	
DP ₁	8.4 ± 0.4
DP ₂	5.2 ± 0.1
DP ₃	4.9 ± 0.5
DP ₄	3.6 ± 0.3
DP ₅	3.1 ± 0.2
DP ₆	2.9 ± 0.2
DP ₇	2.8 ± 0.1
DP ₈	2.7 ± 0.2
DP ₉	2.3 ± 0.1
DP > 10	4.2 ± 0.4
Total phenolics	101.9

DP: degree of polymerization.

Table 2
Results of flavonoids (anthocyanins and flavonol) compounds retentions (R%) of strawberries after peracetic acid (PAA) fogging disinfection operation.

Fogging conditions			Anthocyanins			Flavonol
Run	PAA ($\mu\text{L L}^{-1}$) ^a	Time (min)	Cyanidin-3-O-glucoside (R %)	Pelargonidin-3-O-glucoside (R %)	Pelargonidin-3-O-rutinoside (R %)	Quercetin-3-O-glucuronide (R%)
1	100	15.0	76.6	63.0	37.5	72.1
2	20	15.0	83.1	100.9	130.2	80.2
3	20	60.0	81.4	87.4	98.2	72.6
4	60	5.7	103.9	96.5	99.7	102.0
5	60	37.5	70.4	75.3	69.6	78.0
6	60	37.5	79.1	89.7	68.8	73.7
7	3.4	37.5	115.5	102.6	99.8	122.3
8	60	37.5	68.5	87.8	83.2	82.4
9	60	69.3	56.1	51.2	17.1	62.9
10	100	60.0	35.9	19.7	43.8	75.3
11	116.6	37.5	6.6	15.0	43.6	80.2

^a $\mu\text{L PAA per L of air chamber}$.

differences observed between the chromatograms of fogged and control strawberries indicated important losses of individual flavonoids after the disinfection operation, especially when high PAA concentrations and long treatment times were used (data not shown). On the contrary, results obtained after nebulization of strawberries, for instance, in run 7 (Table 2) when the lowest PAA concentration was used ($3.4 \mu\text{L L}^{-1}$), for 37.5 min, indicated almost no oxidation of flavonoids.

RSM was used to model the effect of the PAA disinfection by fogging as a function of the concentration and the contact time on the phenolic compound profile of the fruit.

The ANOVA for the corresponding flavonoid models described the experimental data adequately. The lack of fit was not significant ($P > 0.05$), and the coefficients of determination (R^2) and AAD (%) were acceptable (Table A.2, Appendix A). The reduced models obtained through the stepwise regression procedure are shown in Eqs. (4)–(7) below:

$$\text{Cyanidin-3-O-glucoside (R\%)} = 132.2 - 0.6C - 0.6t \quad (4)$$

$$\text{Pelargonidin-3-O-glucoside (R\%)} = 139.9 - 0.7C - 0.7t \quad (5)$$

$$\text{Pelargonidin-3-O-rutinoside (R\%)} = 144.1 - 0.7C - 0.8t \quad (6)$$

$$\text{Quercetin-3-O-glucuronide (R\%)} = 106.6 - 0.2C - 0.3t \quad (7)$$

Flavonoid models were affected both by PAA fogging concentration and time through their linear factors ($P \leq 0.05$). Fig. 3 shows the decrease in the retention of pelargonidin-3-O-glucoside (R%) as PAA fogging concentration and treatment time increase. Anthocyanin predicted retentions at the most severe PAA concentration and treatment time ($116.6 \mu\text{L L}^{-1}$ and 69.3 min) are 20.7%, 9.8%,

and 7.0% for cyanidin-3-O-glucoside, pelargonidin-3-O-glucoside, and pelargonidin-3-O-rutinoside, respectively. Meanwhile, quercetin-3-O-glucuronide retention prediction at these processing conditions was 62.5%.

As it can be seen from experimental results and predicted values, anthocyanins, which are known to be highly unstable, were the most susceptible to degradation after PAA fogging-disinfection. Anthocyanins are subject to degradation even while they are in the fresh tissue or they may be destroyed during the processing and storage of the product (Castañeda-Ovando et al., 2009). The oxidizing effects of the PAA, which contains peracetic acid and hydrogen peroxide, on anthocyanins, and its association with the color of the fruit, are well documented in the literature (Özkan et al., 2005; Van de Velde et al., 2013; Alexandre et al., 2014). Apparently, hydrogen peroxide or its decomposition products cleave the neighboring carbon-carbon bond at the C-2 and C-3 positions of anthocyanins to form colorless malvones (Özkan et al., 2005). Some deleterious changes in color were recently observed after PAA fogging of strawberries (Van de Velde et al., 2016). For instance, lighter, less red and less yellow fruit was obtained when they were treated with PAA at $20 \mu\text{L L}^{-1}$ both at short (15 min) and long times (60 min) (Van de Velde et al., 2016). The oxidation of anthocyanins due to fogging conditions was most likely responsible for some observed color loss in treated fruit. As shown in Table 2, in general, pelargonidin-based anthocyanins were more oxidized than the cyanidin-based anthocyanin, suggesting higher susceptibility to the PAA. In agreement, Özkan et al. (2005) described an apparently higher susceptibility of strawberry anthocyanins, among other fruits such as pomegranates and sour cherries, to hydrogen peroxide treatment. As pelargonidin is the principal anthocyanin aglycone found in strawberries, its retention should be especially prioritized, taking into account that anthocyanins contribute significantly to the color, and are the group of polyphenolic compounds with the highest contribution to the total

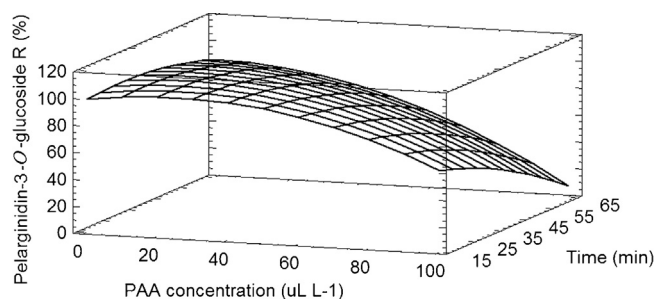


Fig. 3. Surface response plot of pelargonidin-3-O-glucoside (R %) as a function of the peracetic acid (PAA) fogging concentration and the treatment time.

antioxidant capacity of strawberries (Aaby et al., 2007a, 2007b; da Silva Pinto et al., 2008; Tulipani et al., 2008).

The hydroxycinnamic acid derivatives and ellagitannin experimental retentions (R%) at designed fogging conditions are presented in Table 3. The ANOVA for the corresponding models described the experimental data adequately, the lack of fit was not significant ($P > 0.05$), and the coefficients of determination (R^2) and AAD (%) were acceptable (Table A.3, Appendix A).

The reduced models for hydroxycinnamic acid derivatives retentions are presented in Eqs. (8) and (9) below:

$$p\text{-Coumaroyl hexoside (R\%)} = 103.6 - 0.2C - 1.2t - 0.01 t^2 \quad (8)$$

$$\text{Cinnamoyl hexoside (R\%)} = 101.8 - 1.0C - 0.006C^2 \quad (9)$$

The retention model of *p*-coumaroyl hexoside was affected by PAA fogging concentration through its linear term and by time through both linear and quadratic terms ($P \leq 0.05$). Meanwhile, the retention model of cinnamoyl hexoside was affected only by PAA fogging concentration through both linear and quadratic terms ($P \leq 0.05$). The predicted lowest retentions at the maximum design fogging conditions ($116.6 \mu\text{L L}^{-1}$ PAA and 69.3 min) are 45.1 and 66.8%, for *p*-coumaroyl hexoside and cinnamoyl hexoside, respectively. According to the experimental results and predicted values, the retention of *p*-coumaroyl hexoside after fogging treatments was, in general, lower than the retention of cinnamoyl hexoside, suggesting that *p*-coumaroyl hexoside would be more sensitive to oxidation by the sanitizer. Therefore, among hydroxycinnamic acid derivatives, any postharvest disinfection treatment should take particular care to retain *p*-coumaroyl hexoside content.

Eq. (10) presents the reduced model obtained for the sanguin H-6 retention after PAA fogging operation.

$$\text{Sanguin H-6 (R\%)} = 122.8 - 0.2C - 0.6t \quad (10)$$

The ellagitannin's retention was affected by the PAA fogging concentration and treatment time through its linear terms ($P \leq 0.05$). In this case, the retention prediction at the maximum fogging condition ($116.6 \mu\text{L L}^{-1}$ PAA, 69.3 min) is 57.9%. Therefore, retention of ellagitannin close to 60% can be expected even under the most adverse PAA fogging conditions. Ellagitannins retention is particularly relevant because it represents the second most important phenolic compound group that contributes to the total antioxidant capacity of strawberries (Aaby et al., 2007b).

Proanthocyanidin retention results are shown in Table 4. The ANOVA results for the corresponding models described the experimental data adequately, the lack of fit was not significant

($P > 0.05$), and the coefficients of determination (R^2) and AAD (%) were acceptable (Table A.4, Appendix A).

In general, PAC retention decreased as PAA fogging concentration and time increased. The differences between the chromatograms of fogged strawberries in comparison with the control were based on the lower amounts of each degree of polymerization (DP) PAC found after PAA oxidation.

The reduced models for PAC at the different DP are presented in Eqs. (11)–(19).

$$\text{PAC DP}_1 \text{ (R\%)} = 91.0 + 0.1C + 0.2t - 0.01Ct \quad (11)$$

$$\text{PAC DP}_2 \text{ (R\%)} = 89.9 + 0.1C + 0.4t - 0.01Ct \quad (12)$$

$$\text{PAC DP}_3 \text{ (R\%)} = 95.1 + 0.04C + 0.4t - 0.01Ct \quad (13)$$

$$\text{PAC DP}_4 \text{ (R\%)} = 109.9 - 0.08C + 0.3t - 0.01Ct \quad (14)$$

$$\text{PAC DP}_5 \text{ (R\%)} = 91.1 + 0.2C + 0.6t - 0.01Ct \quad (15)$$

$$\text{PAC DP}_6 \text{ (R\%)} = 110.5 - 0.3C \quad (16)$$

$$\text{PAC DP}_7 \text{ (R\%)} = 104.1 - 0.3C \quad (17)$$

$$\text{PAC DP}_8 \text{ (R\%)} = 99.6 - 0.3C \quad (18)$$

$$\text{PAC DP}_9 \text{ (R\%)} = 101.8 - 0.2C \quad (19)$$

PAC reduced models for degree of polymerization 1–5 were affected by PAA fogging concentration and time through their linear terms ($P \leq 0.05$). Moreover, the interaction between concentration and time was also significant ($P \leq 0.05$). Higher degree of polymerization PAC models (DP 6, 7, 8, and 9) were only affected by PAA fogging concentration through its linear term ($P \leq 0.05$). The oxidizing effects of PAA on PAC with high DP were less than the effect observed on PAC with lower DP. The predicted retention of PAC at the maximum experimental fogging conditions ($116.6 \mu\text{L L}^{-1}$ PAA, 69.3 min) are 31.0, 48.5, 46.7, 40.6, 75.2, 75.5, 69.1, 64.6, and 78.5%, for DP1, DP2, DP3, DP4, DP5, DP6, DP7, DP8

Table 3

Results of hydroxycinnamic acid derivatives and ellagitannin compounds retentions (R%) of strawberries after peracetic acid (PAA) fogging disinfection operation.

Fogging conditions			Hydroxycinnamic acid derivatives		Ellagitannins
Run	PAA ($\mu\text{L L}^{-1}$) ^a	Time (min)	<i>p</i> -coumaroyl hexoside (R%)	Cinnamoyl hexoside (R%)	Sanguin H-6 (R%)
1	100	15.0	83.0	55.0	86.5
2	20	15.0	79.1	74.2	97.2
3	20	60.0	62.4	77.3	70.0
4	60	5.7	85.1	100.5	131.2
5	60	37.5	61.2	64.1	83.0
6	60	37.5	58.5	55.6	90.0
7	3.4	37.5	83.3	106.6	108.0
8	60	37.5	65.0	55.3	82.1
9	60	69.3	63.9	61.6	79.9
10	100	60.0	52.9	54.9	71.2
11	116.6	37.5	53.0	79.6	76.3

^a μL PAA per L of air chamber.

Table 4
Results of proanthocyanidins retentions (R%) of strawberries after peracetic acid (PAA) fogging disinfection operation.

Fogging conditions			Proanthocyanidins									
Run	PAA (μLL^{-1}) ^a	Time (min)	DP ₁ (R%)	DP ₂ (R%)	DP ₃ (R%)	DP ₄ (R%)	DP ₅ (R%)	DP ₆ (R%)	DP ₇ (R%)	DP ₈ (R%)	DP ₉ (R%)	DP _{>10} (R%)
1	100	15.0	94.9	95.8	90.5	93.3	102.3	93.7	89.9	81.3	88.8	73.1
2	20	15.0	93.1	97.0	102.8	110.4	117.8	107	105.0	101.4	100.7	63.9
3	20	60.0	84.9	93.9	101.8	104.0	121.8	106	100.5	94.1	103.0	94.5
4	60	5.7	88.9	91.6	96.1	101.8	91.8	88.3	89.3	91.5	91.6	72.4
5	60	37.5	78.1	81.7	83.5	84.6	84.0	92	92.8	77.1	84.7	76.0
6	60	37.5	83.0	89.3	90.4	90.4	93.0	100	84.1	77.6	85.2	82.8
7	3.4	37.5	97.4	103.3	105.3	125.1	100.2	97.8	94.7	94.4	94.2	79.7
8	60	37.5	82.9	88.4	84.1	91.2	92.7	104.4	91.4	87.7	93.1	93.9
9	60	69.3	76.8	86.5	91.9	93.2	93.9	105.4	92.3	86.4	93.6	96.4
10	100	60.0	50.2	53.0	48.3	50.6	55.0	48.6	47.1	44.8	53.3	46.3
11	116.6	37.5	47.3	67.0	63	71.5	79.2	82.7	83.2	83.1	88.6	55.8

DP: degree of polymerization.

^a μL PAA per L of air chamber.

and DP₉, respectively. As noted, the higher the DP of the PAC, the more the resistant to oxidation the flavan-3-ol polymer was to be during the fogging process. Retention of PAC with DP > 10 was not affected by the processing variables (Table 4).

It has been reported that PAC possess, directly and indirectly, antioxidant, antimicrobial, antiallergic, and antihypertensive properties and inhibit the activities of some physiologic enzymes and receptors (Santos-Buelga and Scalbert, 2000; Giampieri et al., 2012). Moreover, previous studies have suggested that only proanthocyanidin oligomers with DP < 4, and metabolites formed in the colon after PAC fermentation could be absorbed and bioavailable in the gastrointestinal tract, and able to exert antioxidant capacity (Scalbert et al., 2000). In these tests, the retention of PAC with DP ≤ 5 was shown to be more susceptible to degradation as PAA fogging concentrations and durations were increased. Therefore, the retention of the PAC with low degree of polymerization should be prioritized, considering their particular susceptibility to oxidizing conditions and the relevance of their consumption to human health.

5. Conclusions

The fogging of PAA at different concentrations and contact times affected the phenolic composition of strawberries, to various degrees depending on the fogging conditions and the chemical structure of the phenolic compound examined. The models developed herein allow to predict retentions of individual phenolic compounds at different fogging PAA conditions. Phenolic constituents differed in their observed retention after postharvest fogging. Anthocyanins were the most vulnerable to oxidation during PAA fogging, followed by PAC with a low DP, the hydroxycinnamic acid derivatives, and the ellagitannin Sanguin H-6. Therefore, the optimum condition for PAA postharvest fogging of strawberries must take into account both adequate microbiological load reductions, and the retention of quality attributes including health-protective polyphenolic compounds that shown to be more susceptible to PAA oxidation.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.postharvbio.2016.03.005>.

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