Article

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Optimization of strawberry disinfection by fogging of a mixture of peracetic acid and hydrogen peroxide based on microbial reduction, color and phytochemicals retention

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Abstract

The fogging of strawberries using a environmentally friendly sanitizer mixture of peracetic acid (5%) and hydrogen peroxide (20%) was performed in a model chamber and modeled as a function of the concentration (3.4, 20.0, 60.0, 100.0 and 116.6 μL sanitizer L^{-1} air chamber) and the treatment time (5.7, 15.0, 37.5, 60.0 and 69.3 min). The sanitizer fogging was adequate for reducing total mesophilic microbial and yeasts and moulds counts of fruits until seven days of storage at 2°C. However, sanitizer oxidant properties adversely affected the content of total anthocyanins, total phenolics, vitamin C, and antioxidant capacity to various degrees, with some deleterious changes in the fruits color, depending on the fogging conditions. A multiple numeric response optimization was developed based on 2.0 log microbiological reduction, maximum phytochemicals and antioxidant capacity retentions, with no changes in the fruits color, being the optimal fogging conditions achieved: 10.1 μL sanitizer L^{-1} air chamber and 29.6 min. The fogging of strawberries at these conditions may represent a promising postharvest treatment option for extending their shelf-life without affecting their sensory quality and bioactive properties.

Keywords

disinfection, postharvest storage, bioactive compounds, antioxidant capacity

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INTRODUCTION

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 (Vit C) and phenolics compounds (Alexandre et al., 2014). Strawberries are presented in the market as fresh whole fruits or minimally processed, as ready-to-eat fresh-cut fruits. Unfortunately, the storage period and shelf-life of this fruit are very short due to its perishability and susceptibility to the growth of rot-causing pathogens. The most severe postharvest diseases are grey mould (*Botrytis cinerea* Pers. ex. Fr.) and Rhizopus rot (*Rhizopus stolonifer* Ehrenb. Fr.Vuill) (Vardar et al., 2012). The application of decontamination processes that may ensure microbiological safety and shelf-life extension of the product, while retaining quality attributes close to the fresh characteristics

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becomes crucial for this kind of fruit (Alexandre et al., 2014). According to literature, strawberries are soft fruits, and the traditional application of washing with disinfectants would not be recommended because it may be injurious by several factors. The fruit may be damaged easily when washing on a processing line, and the drying period delays pre-cooling that may facilitate pathogen infection (Vardar et al., 2012). Therefore, the application of disinfectant agents by fogging may be a promising technology since handling and wetting of the fruit is minimized (Oh et al., 2005; Vardar et al., 2012). The fogging or aerosolization is the dispersion of a sanitizer agent as a fine mist in air and its application on products. This operation has been already used successfully for the decontamination and controlling of postharvest diseases of strawberries employing chlorine dioxide, sodium hypochlorite, citric acid and ethanol as disinfectant agents (Vardar et al., 2012), and in figs with the use of chlorine dioxide (Karabulut et al., 2009).

The fogging of peracetic acid sanitizer may be a promising option for controlling the microbial population and extending the shelf-life of strawberries. The peracetic acid sanitizer, commercially available, is a mix of a quaternary equilibrium of acetic acid, hydrogen peroxide, peracetic acid and water, and its decomposition products are only oxygen and acetic acid, by which is considered environmentally friendly (Silveira et al., 2008). The advantages of the use of this sanitizer 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). However, information about the application of this sanitizer by fogging is scarce in the literature. Oh et al. (2005) studied the aerosolization of peracetic acid sanitizer over lettuce leaves inoculated with *Escherichia coli* O157:H7, *Salmonella Typhymurium* and *Listeria monocytogenes* for 10 to 60 min. Authors described high reductions (near to 4 log) in the inoculated populations at maximum time, but results about the impact of treatments in the nutrient and phytochemical content of the plant material are not available. Hence, a complete study of fogging application of peracetic acid sanitizer is needed.

The aim of this work was to model and optimize the disinfection operation of fresh strawberries by fogging with a commercial sanitizer based on a mixture of 5% peracetic acid and 20% hydrogen peroxide, reaching good microbiological reductions without affecting their general quality and phytochemical and antioxidant capacity (AC) contents.

MATERIALS AND METHODS

Plant material

Cultivated strawberries (*Fragaria x ananassa* Duch.) of ''Camarosa'' variety were obtained from one planting at Arroyo Leyes (31 27′0″S, 60 40′0″W), Santa Fe, (Argentina). Fruits were harvested by skilled workers at full ripe stage (90% of the surface showing red color) and were transported 20 km directly from the field to the laboratory and stored at 2°C until use. Strawberries characteristics were: 6.2 ± 0.2 Brix, pH 3.35 ± 0.02, and total acidity: 0.8 ± 0.1 mg anhydrous citric acid 100 g^{-1} FW.

Peracetic acid

A commercial sanitizer called Oxilac Plus (Indaquim S.A. Santa Fe, Argentina) was used in the fogging disinfection operation. Oxilac Plus is a stabilized mixture of 5% peracetic acid, 20% hydrogen peroxide and water.

Fogging system and procedure

The treatments were set up in a 16 L plastic hermetic model chamber specially designed for this experiment. The fogging was performed using an ultrasonic aerosol generator unit (Respirex, Accme, SRL, Córdoba, Argentina) that have a liquid reservoir (30 mL) for holding the liquid to be fogged and produces a fog of droplets among 0.5–8 μm in diameter. The small particles are carried away by the airflow and blown inside the chamber. Six round rigid PET trays (capacity 270 cm^3), with 150 g of selected strawberries each one (8–10 fruits) were placed inside the chamber with the lids opened and were fogged at various sanitizer concentrations and treatments times, according to the experimental design. The fogging system unit was turned on for the time needed for fogging of all the liquid in the reservoir (typically between 5 to 10 min), then the system was turned off and the samples were leaved inside the chamber in contact with the fog until the proposed contact time was reached. Then, treated samples were taken out the chamber, tray lids were closed and three of the trays were stored at 2°C for seven days (samples day 7), and the others were analyzed immediately (samples day 0). The samples corresponding to day 0 were used for microbiological, color and phytochemicals analyses. After seven days of cold storage, the corresponding samples were conducted in the same way as for the mentioned analyses.

Untreated strawberries (500 g; raw material) without any previous washing or disinfection process were used as a reference for investigating the microbiological

efficacy of the fogging treatments and the effects on the phytochemicals and AC content and color.

Experimental design and optimization procedure

Response Surface Methodology using a Central Composite Design (CCD) was used to study the fogging operation. The total number of needed experiments (N) of the CCD design was determined using equation (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 centre point's runs, respectively. The center point of CCD is used to calculate the experimental error, and the distance of the axial points from the center points are dependent on the number of factors chosen for the experiment (Montgomery, 2001). The sanitizer fogging concentration and the treatment time were the selected variables in the current study ($k = 2$). Therefore, N was calculated using equation (1) and was equal to 11 (four 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 (equation (2)):

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

where C = sanitizer fogging concentration (μL sanitizer per L of air chamber) and t = time of the treatment (min) and the five levels were as follow: C = 3.4, 20.0, 60.0, 100.0 and 116.6 $\mu L\,L^{-1}$ and t = 5.7, 15.0, 37.5, 60.0 and 69.3 min. The variables limits were determined in previous experiments based on microbiological efficacy and the retention of general quality attributes of fruits. The studied responses (Y) after strawberries fogging were total mesophilic microbial and yeasts and moulds count reductions, total anthocyanins (TA), total phenolics (TC), Vit C and AC content retentions (%) and color parameters changes (%). All responses were evaluated immediately after fogging at day 0, and after seven days of storage at 2°C.

Total mesophilic microbial ($TMCR_i$) and yeast and moulds ($YMCR_i$) count reductions were expressed as $-\log_{10} N_i/N_{ci}$, where N_i is the viable microorganism count of fogged samples at day i, N_{ci} is the viable count of control samples at day i, and i is the analysis day, 0 or 7.

The total anthocyanins (TAR_i), total phenolics (TPR_i), vitamin C ($VitCR_i$) and AC content retentions (ACR_i) were expressed as $(Q_i/Q_{ci}) \times 100$, where Q_i and Q_{ci} represent the attributes measured in fogged and control strawberries at day i (0 or 7), respectively. The color parameter change (δL_i^*, δa_i^*, and δb_i^*) was expressed as a percentage of the difference between fogged strawberry parameter value (L_i^*, a_i^*, and b_i^*) and the control strawberry parameter value (L_{ci}^*, a_{ci}^*, and b_{ci}^*), divided by the last one (equation (3)) at day i (0 or 7).

$$\delta Q_i * (\ \%) = (Q_i * - Q_{ci}*)/Q_{ci} * \times 100 \quad (3)$$

A second-order polynomial equation was proposed to model equation (2) for each response (equation (4)):

$$Y = \beta_o + \beta_C \times C + \beta_t \times t + \beta_{CC} \times C^2 + \beta_{tt} \times t^2 + \beta_{tC} \times t \times C \quad (4)$$

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

Derringer's desirability function was used for multiple response optimization according to Derringer and Suich (1980). The method involves transformation of each predicted response, $\hat{Y}$, to a dimensionless partial desirability function, di. The di values for each response are obtained by specifying the goals, i.e., minimize, maximize or target the response, and boundaries required for each one. Partial desirability functions are combined into a single composite response, the global desirability function D, defined as the geometric mean of the different di-values (equation (5)):

$$D = \left[d_1^{p_1} x d_2^{p_2} x \ldots x d_n^{p_n}\right]^{1/n} \quad (5)$$

where p_1, p_2 and p_n are the assumed relative importance of the responses. A value of D different from zero implies that all responses are in a desirable range simultaneously and, consequently, for a value of D close to 1, the combination of the criteria is globally optimal.

Microbiological analysis

Total mesophilic microorganisms and yeasts and moulds were quantified in strawberries. Raw material (10 g), or fruits after each fogging treatment, were aseptically homogenized in a stomacher using 90 mL of peptone water 0.1% for 2 min. Decimal dilutions were carried out in peptone water 0.1%. Enumeration of total mesophilic microorganisms was assessed, in duplicate, using Plate Count Agar (PCA, Merck, USA). The

plates were incubated at 30°C for 48 h, and the results were expressed as log of colony forming unit (CFU) g^{-1} strawberries. Yeasts and moulds enumeration was assessed, in duplicate, using Yeast Extract Glucose Chloramphenicol Agar (YGC, Merck, USA). Plates were incubated at 28°C for five days. Results were expressed as log CFU g^{-1} strawberries.

Total anthocyanins, total phenolics, vitamin C and antioxidant capacity content analysis

Extract preparation. For TA, TP and AC content, the extraction consisted in 5 g of homogenized strawberries that were added to 75 mL of extraction solvent (80% acetone and 20% water). For VitC content, samples of 5 g homogenized strawberries were added to 25 mL of extraction solvent (3% metaphosphoric acid and 8% acetic acid). Both mixtures were homogenized for 1 min, sonicated for 15 min and then centrifuged at 12,000 *g* for 20 min at 4°C. Supernatants were separated and used for analyses (triplicates).

Total anthocyanins content. TA content was monitored in a Genesis 10 S UV-Vis spectrophotometer, (Thermo Scientific, Germany) by the pH differential method according to Heo and Lee (2005). Results were converted as mg pelargonidin-3-glucoside 100 g^{-1} of fresh weight (FW).

Total phenolics content. TP content was monitored spectrophotometrically using the Folin-Ciocalteu reagent according to Singleton and Rossi (1965). Results were expressed as mg gallic acid equivalents 100 g^{-1} FW.

Vitamin C content. VitC content was determined by HPLC according to the protocol developed by Van de Velde et al. (2012). Results were expressed as mgVitC 100 g^{-1} FW.

Antioxidant capacity. AC content of the samples was estimated spectrophotometrically by determining the free-radical scavenging capacity evaluated with the stable radical DPPH, according to Sánchez Moreno et al. (2003). The antioxidant capacity of strawberry extracts was expressed as milligram ascorbic acid (AA) equivalent 100 g^{-1} FW.

Color measurement

Color was determined using a Minolta 508 d spectrophotometer (Minolta Co., LTD., Japan), evaluating the CIE system parameters: L*, a* and b*. Five measurements were performed per sample.

Statistical analysis

STATGRAPHICS Centurion XV 15.2.06 (Statpoint Technologies, Inc., Warrenton, Virginia, USA) was used to perform ANOVA, to fit the second-order polynomial equations to the experimental data and to obtain the coefficients of the equations. The significance of each term of the models was evaluated referred to the pure error and the elimination of non-significant terms was done by means of the linear stepwise regression procedure. For verification of the model adequacy, the lack of fit, the coefficient of determination (R^2) and the absolute average deviation (AAD) were calculated (Baş and Boyaci, 2007). STATGRAPHICS was used as well for the numerical optimization procedure through the Derringer's desirability function.

RESULTS AND DISCUSSION

Effect of sanitizer fogging on the microbial load reduction

The initial total mesophilic microbial count of fresh strawberries (control samples) was in average 4.5 log CFU g^{-1}. After seven days at 2°C, total mesophilic microbial counts of these samples did not change, indicating that total bacterial load did not rise during the refrigerated storage period. The yeasts and moulds counts at day 0 and 7 were 3.4 and 5.0 log CFU g^{-1}, respectively. Results showed a 1.6 log increase in this microbial population after cold storage time in control samples (without a sanitizing treatment). Yeast and moulds are responsible for the most severe diseases in the postharvest life of strawberries (Vardar et al., 2012). So, it is important to reduce their occurrence in strawberries.

According to $TMCR_i$ and $YMCR_i$ results, the higher the sanitizer fogging concentration and treatment time, the higher the reduction in total mesophilic microbial and yeasts and moulds counts (Table 1). The ANOVAs of the corresponding models of the microbiological reductions presented not significant lack of fit ($P > 0.05$), and the R^2 and AAD values were acceptable (Table 2). The reduced models obtained through the stepwise regression procedure are shown in equations (6)–(9).

$$TMCR_0 = 0.61 - 0.019C + 0.017t + 0.0003C^2 \quad (6)$$

$$TMCR_7 = 1.5 - 0.027C + 0.0003C^2 \quad (7)$$

$$YMCR_0 = 0.24 + 0.009C + 0.014t \quad (8)$$

$$YMCR_7 = -0.34 + 0.015C + 0.054t \quad (9)$$

Table 1. Experimental results of the microbiological count reductions of fogged strawberries with a sanitizer based on a mixture of 5% peracetic acid and 20% hydrogen peroxide at day 0 and until 7 days at 2°C

Run	Sanitizer ($\mu g\ L^{-1}$)	Time (min)	i (day)	$TMCR_i$ ($-\log_{10} N_i/N_{ci}$)	$YMCR_i$ ($-\log_{10} N_i/N_{ci}$)
1	100	15.0	0	1.1	1.1
			7	0.9	1.2
2	20	15.0	0	1.0	1.2
			7	1.0	1.4
3	20	60.0	0	1.8	1.5
			7	2.4	4.1
4	60	5.7	0	0.8	0.7
			7	1.7	0.4
5	60	37.5	0	1.0	1.6
			7	0.4	3.4
6	60	37.5	0	1.3	1.7
			7	1.0	3.7
7	3.4	37.5	0	0.7	0.3
			7	0.6	0.4
8	60	37.5	0	1.0	1.3
			7	0.6	2.9
9	60	69.3	0	1.2	0.8
			7	0.9	3.2
10	100	60.0	0	2.7	3.1
			7	2.6	4.1
11	116.6	37.5	0	3.0	1.3
			7	3.0	4.0

$TMCR_i$: total mesophilic microbial count reduction at day i; $YMCR_i$: yeast and moulds count reduction at day i; N_i is the viable microorganism count of fogged samples at day i. N_{ci} is the viable count of control samples at day i. i is the analysis day, 0 or 7.

Table 2. ANOVA of microbiological count reductions of fogged strawberries with a sanitizer based on a mixture of 5% peracetic acid and 20% hydrogen peroxide at day 0 and until 7 days at 2°C

			Sum of squares	
Source	df	i (day)	$TMCR_i$ ($-\log_{10} N_i/N_{ci}$)	$YMCR_i$ ($-\log_{10} N_i/N_{ci}$)
X_1 (C)	1	0	2.4*	1.1*
		7	1.2	3.1*
X_2 (t)	1	0	1.2*	0.8*
		7	0.5	11.7*
X_1^2	1	0	1.1*	0.06
		7	2.1*	0.8
X_1X_2	1	0	0.1	0.7
		7	0.01	0.008
X_2^2	1	0	0.001	0.1
		7	0.7	1.9
Lack of fit	3	0	1.2	2.3
		7	3.7	4.9
Pure error	2	0	0.08	0.08
		7	0.2	0.3
R^2		0	0.8	0.5
		7	0.5	0.8
AAD (%)		0	14.4	13.4
		7	13.2	15.0

*Significant terms at $p \leq 0.05$.
AAD: absolute average deviation; C: sanitizer fogging concentration (μL sanitizer per L of air chamber); t = time of the treatment (min); $TMCR_i$: total mesophilic microbial count reduction at day i; $YMCR_i$: yeast and moulds count reduction at day i; N_i is the viable microorganism count of fogged samples at day i, N_{ci} is the viable count of control samples at day i, and i is the analysis day, 0 or 7.

$TMCR_i$ models were affected by sanitizer concentration and time through the lineal terms and by sanitizer concentration through the quadratic term at day 0 ($P \leq 0.05$) and by lineal and quadratic terms in concentration at day 7 ($P \leq 0.05$). $YMCR_i$ models were affected by sanitizer concentration and time through the lineal terms ($P \leq 0.05$) both at zero and seven days.

The predicted highest $TMCR_i$, using the reduced models, are 3.6 and 2.4 log at day 0 and 7, respectively, obtained at the maximum studied design fogging conditions (116.6 μL sanitizer L^{-1} and 69.3 min). As it can be seen, the TMCR is lower after seven days of storage, remaining at good reduction levels. Meanwhile, the predictions for $YMCR_i$ at the same conditions are 2.3 and 5.2 log at day 0 and 7, respectively. The latter results showed higher reductions on yeasts and moulds after seven days of storage, indicating a residual action of fogged sanitizer because control samples did not show any microbial count reduction during cold storage.

The fogging of strawberries at 0, 750, 1000, 1500 and 2000 μL L^{-1} of chlorine dioxide, hydrogen peroxide (50%), sodium hypochlorite (5%) and citric acid; and various concentrations (20%, 30%, 40%) of ethanol for 60 min at room temperature was studied by Vardar et al. (2012). Treatments significantly reduced total microorganisms, fungal and bacterial population on the fruit surface immediately after disinfectant fogging. The application of 50% hydrogen peroxide at 2000 μL L^{-1} achieved approximately 2 log reduction on the fruit surface of total microorganism population (Vardar et al., 2012). As it was mentioned, the sanitizer used herein is based on a mixture of 5% peracetic acid and 20% hydrogen peroxide. Our prediction in $TMCR_0$ at the highest design fogging sanitizer concentration (116.6 μL L^{-1}) and 60 min is 3.5 log, 1.5 log

higher than the reduction obtained by fogging 50% hydrogen peroxide at 2000 $\mu L\,L^{-1}$ for 60 min reported by Vardar et al. (2012). According to Baldry (1983), peracetic acid is an excellent bactericide and hydrogen peroxide by itself is more effective as a sporicide than as a bactericide. Therefore, it could explain the higher reductions that can be reached in the total microbial load using the peracetic acid/hydrogen peroxide mixture as disinfectant agent instead of employing higher concentrations of hydrogen peroxide only.

Effect of sanitizer fogging on general quality attributes and the retention of bioactive compounds

Strawberries characteristics were 6.2 ± 0.2 Brix, pH 3.35 ± 0.02, and total acidity: 0.8 ± 0.1 mg anhydrous citric acid $100\,g^{-1}$ FW. The sanitizer fogging treatments did not produce any significant change in these general quality attributes (data not shown).

The contents of TA, TP, VitC and AC of control strawberries at day 0 were 39 ± 1, 187 ± 11, 72 ± 2 and 431 ± 3 mg $100\,g^{-1}$ FW, respectively. The corresponding contents of TA, TP, VitC and AC of control strawberries at day 7 were 37 ± 1, 199 ± 5, 66.1 ± 0.5 and 419 ± 3 mg $100\,g^{-1}$ FW, respectively. These results are in agreement with those reported for whole 'Camarosa' strawberries (Da Silva-Pinto et al., 2008). According to the results, TA content decreased slightly (5%) after seven days of storage at 2°C. Despite of the fact that anthocyanins have phenolic nature, the TP content was in the same range from the beginning to the end of the storage. Meanwhile, VitC content decreased slightly (less than 10%) after one week of refrigerated storage. AC decreased 3% at day 7, probably because of the concomitant decrease observed in both TA and VitC contents.

The experimental results of TAR_i (%), TPR_i (%), $VitCR_i$ (%) and ACR_i (%) were affected by both sanitizer fogging concentration and treatment time at day 0 and 7 (Table 3). The increase in the magnitude of both experimental variables resulted in lower bioactive compounds retentions. The obtained models described the experimental data adequately. The lack of fit was not significant ($P > 0.05$), and R^2 and AAD values were acceptable (Table 4). The reduced models for TAR_i

Table 3. Experimental results of bioactive compounds retentions of fogged strawberries with a sanitizer based on a mixture of 5% peracetic acid and 20% hydrogen peroxide at day 0 and until 7 days at 2°C

Run	Sanitizer ($\mu g\,L^{-1}$)	Time (min)	*i* (day)	TAR_i (%)	TPR_i (%)	$VitCR_i$ (%)	ACR_i (%)
1	100	15.0	0	72.5	84.6	84.0	96.1
			7	73.7	82.1	68.6	82.8
2	20	15.0	0	94.1	92.0	88.0	90.0
			7	85.7	93.6	78.0	88.8
3	20	60.0	0	80.1	90.0	78.9	85.5
			7	76.9	90.0	70.5	83.5
4	60	5.7	0	92.7	101.0	94.7	90.8
			7	92.5	99.6	94.8	100.9
5	60	37.5	0	68.2	92.1	87.9	92.7
			7	65.0	88.2	75.6	79.8
6	60	37.5	0	60.8	91.0	83.9	88.3
			7	57.9	91.9	72.5	82.5
7	3.4	37.5	0	91.7	99.8	94.4	100.4
			7	89.5	98.3	90.1	97.3
8	60	37.5	0	62.2	88.0	85.6	90.4
			7	72.1	91.6	71.1	75.2
9	60	69.3	0	53.3	84.9	83.8	84.9
			7	54.2	85.9	73.1	75.7
10	100	60.0	0	58.0	85.3	76.8	80.8
			7	60.2	82.9	78.6	70.9
11	116.6	37.5	0	31.6	82.6	70.8	78.5
			7	29.1	75.3	72.6	72.6

TAR_i (%): total anthocyanins retention at day *i*; TPR_i (%): total phenolics retention at day *i*; $VitCR_i$ (%): vitamin C retention at day *i*; ACR_i (%): antioxidant capacity retention at day *i*. *i* is the analysis day, 0 or 7.

Table 4. ANOVA of bioactive compounds retentions of fogged strawberries with a sanitizer based on a mixture of 5% peracetic acid and 20% hydrogen peroxide at day 0 and until 7 days at 2°C

			Sum of squares			
Source	df	*i* (day)	TAR_i (%)	TPR_i (%)	$VitCR_i$ (%)	ACR_i (%)
X_1 (C)	1	0	2069.8*	164.8*	197.0*	109.4*
		7	1630.2*	328.8*	85.3	356.4*
X_2 (t)	1	0	889.0*	72.6	125.5*	98.7*
		7	732.4*	61.6	99.6*	349.7*
X_1^2	1	0	7.6	2.0	38.1	2.2
		7	4.4	36.7	21.7	14.7
X_1X_2	1	0	0.06	1.8	0.9	29.2
		7	5.5	4.8	77.5	11.1
X_2^2	1	0	263.8	0.4	2.8	11.7
		7	214.2	1.0	60.2	61.1
Lack of fit	3	0	461.2	109.7	124.4	148.4
		7	650.6	71.5	3601	128.8
Pure error	2	0	31.1	9.0	7.9	10.1
		7	100.8	8.2	10.7	27.3
R^2		0	0.9	0.7	0.7	0.6
		7	0.9	0.8	0.5	0.8
AAD (%)		0	10.0	2.9	3.4	3.7
		7	11.7	2.6	5.5	3.6

*Significant terms at $p \leq 0.05$.
AAD: absolute average deviation; C: sanitizer fogging concentration (μL sanitizer per L of air chamber); t = time of the treatment (min); TAR_i (%): total anthocyanins retention at day *i*; TPR_i (%): total phenolics retention at day *i*; $VitCR_i$ (%): vitamin C retention at day *i*; ACR_i (%): antioxidant capacity retention at day *i*. *i* is the analysis day, 0 or 7.

(%) are shown in equations (10) and (11) at day 0 and 7, respectively.

$$TAR_0 = 111.3 - 0.40C - 0.47t \quad (10)$$

$$TAR_7 = 107.1 - 0.36C - 0.43t \quad (11)$$

TAR*i* models were affected by sanitizer concentration and time through their lineal factors.

Equations (12) and (13) show the reduced models obtained for TPR_i (%) at day 0 and 7, respectively.

$$TPR_0 = 96.9 - 0.11C \quad (12)$$

$$TPR_7 = 98 - 0.16C \quad (13)$$

In this case, only sanitizer concentration through its linear factor affected the response.

The reduced models for $VitCR_i$ (%) are presented in equations (14) and (15) at day 0 and 7, respectively.

$$VitCR_0 = 98.5 - 0.12C - 0.18t \quad (14)$$

$$VitCR_7 = 82.7 - 0.16t \quad (15)$$

VitCR model at day 0 was affected by sanitizer fogging concentration and treatment time through their linear factors. Otherwise, the model at day 7 was only affected by processing time through its lineal factor.

Finally, ACR_i (%) reduced models are presented in equations (16) and (17) at day 0 and 7, respectively.

$$ACR_0 = 100.4 - 0.09C - 0.16t \quad (16)$$

$$ACR_7 = 103.7 - 0.16C - 0.29t \quad (17)$$

Similarly to the most models, ACR_i were affected by sanitizer concentration and treatment time by their lineal factors.

The predicted phytochemicals retentions working at maximum sanitizer fogging concentration ($116.6\,\mu L\,L^{-1}$) and treatment time (69.3 min) are 32.1% and 18.4% for TAR*i*, 84.1% and 79.3% for TPR_i, 72.0% and 71.6% for $VitCR_i$ and 78.8% and 65.0% for ACR_i at day 0 and 7, respectively. According to the results, working at these processing conditions may be achieved retentions higher than 65% in TP, VitC and AC, until seven days at 2°C. However, latter fogging conditions may cause about 70% losses in the TA content of strawberries. Anthocyanins are highly instable and very susceptible to degradation during the processing and storage of fruits (Castañeda-Ovando et al., 2009). The oxidizing effects of peracetic acid and hydrogen peroxide on anthocyanins and other bioactive compounds of berries are well documented in literature (Alexandre et al. 2014; Özkan et al., 2005). Additionally, there is an apparent higher susceptibility of strawberry anthocyanins (mainly pelargonidin-based anthocyanins) to hydrogen peroxide among other fruits as pomegranate and sour cherry (Özkan et al., 2005). Therefore, taking into account, the high susceptibility of anthocyanins, their relevance on the strawberries color and their contribution to the total antioxidant capacity of the fruits, the retention of anthocyanins should be prioritized.

The sanitizer fog had a less oxidizing effect on VitC content than on TA content in strawberries. L-ascorbic acid is the main biologically active form of VitC, being reversible oxidized to form L-dehydroascorbic acid. Further oxidation generates diketogulonic acid, which has no biological function, and the reaction is no longer

reversible (Hernández et al., 2006), meaning a loss of VitC. Therefore, the fogging of strawberries at maximum processing conditions (116.6 μL L^{-1} and 69.3 min) plus seven days of storage at 2°C could decrease the initial VitC content with biological activity to approximately 30% (predicted $VitCR_7 = 71.6\%$).

Effect of sanitizer fogging on the color parameters

The L_{c0}*, a_{c0}* and b_{c0}* (color parameters of control strawberries at day 0) were 28.5 ± 0.6, 29.3 ± 0.5 and 13.1 ± 0.4, respectively. In the same way, L_{c7}*, a_{c7}* and b_{c7}* (color parameters of control strawberries at day 7) were 29.5 ± 0.4, 28.0 ± 0.3 and 12.2 ± 0.3, respectively. According to the results, the effect of the storage of strawberries at 2°C for seven days induced slight but significant changes in color attributes. The increase of the L* value and the decrease in both a* and b* values after seven days indicate that control fruits were lighter, less red, and less yellow, respectively. These slight changes can be related with the own fruit senescence after one week of refrigerated storage and with the consequent slight decrease in the anthocyanins observed in the fruits as shown before. According to Castañeda-Ovando et al. (2009), anthocyanins are pigments responsible for the brilliant color of fresh strawberries.

The experimental results of color parameters changes of fogged strawberries at day 0 and after 7 days at 2°C are presented in Table 5. The ANOVAs of the corresponding models of the changes in the color parameters (δL_i*, δa_i* and δb_i*) presented not significant lack of fit ($P > 0.05$), and the R^2 and AAD values were acceptable (Table 6).

The reduced models for δL_i* are presented in equations (18) and (19) at day 0 and 7, respectively.

$$\delta L_0* = 3.6 - 0.06C \quad (18)$$

$$\delta L_7* = 13.1 - 0.14C - 0.7t - 0.008t^2 \quad (19)$$

Both models were affected by sanitizer concentration through its lineal factor but the model at day 7 was also affected by treatment time through its lineal and quadratic term.

For δa_i*, the obtained reduced models are shown in equations (20) and (21) at day 0 and 7, respectively.

$$\delta a_0* = -2.9 - 0.03C + 0.06t \quad (20)$$

$$\delta a_7* = 20.8 - 0.13C - 0.9t + 0.01t^2 \quad (21)$$

δa_i* models were affected by sanitizer concentration and time through their lineal factors at both zero and seven days. Moreover, the corresponding model at day 7 was affected by the time quadratic term as well. Equations (22) and (23) show correspondingly the reduced models at day 0 and 7 for δb_i*.

$$\delta b_0* = 2.4 - 0.3C + 0.002C^2 \quad (22)$$

$$\delta b_7* = 26.4 - 1.8t + 0.02t^2 \quad (23)$$

δb_0* model was affected by sanitizer concentration through its lineal and quadratic term and δb_7* model was affected by time through its lineal and quadratic term.

Depending on treatment conditions, positive and negative values in δL_i* (Table 5) were obtained, indicating that the fogged strawberries were lighter or darker that untreated samples, respectively. Lighter fruits were obtained when they were treated with

Table 5. Experimental results of color parameters changes of fogged strawberries with a sanitizer based on a mixture of 5% peracetic acid and 20% hydrogen peroxide at day 0 and until 7 days at 2°C

Run	Sanitizer (μg L^{-1})	Time (min)	*i* (day)	δL_i*	δa_i*	δb_i*
1	100	15.0	0	−1.5	−6.9	7.1
			7	−17.7	−3.2	−3.6
2	20	15.0	0	3.5	−3.9	1.6
			7	3.2	17.7	28.1
3	20	60.0	0	9.2	0.6	−3.3
			7	1.1	12.7	10.9
4	60	5.7	0	−0.7	−3.5	−7.2
			7	5.8	3.6	9.9
5	60	37.5	0	−1.0	−2.4	−8.3
			7	−5.5	−8.5	−13.6
6	60	37.5	0	−3.5	−1.7	−9.6
			7	−9.0	−5.0	−10.2
7	3.4	37.5	0	−0.8	−0.6	0.3
			7	−6.8	−6.5	−20.7
8	60	37.5	0	−1.2	−2.8	−7.4
			7	−6.2	−10.2	−18.4
9	60	69.3	0	0.2	−0.7	−4.5
			7	−8.0	−4.4	−10.4
10	100	60.0	0	−0.3	−3.9	−5.6
			7	−6.8	−2.7	0.1
11	116.6	37.5	0	−4.2	−3.0	−6.0
			7	−19.0	−9.3	−12.8

δL_i*: change in L* color parameter at day *i*; δa_i*: change in a* color parameter at day *i*; δb_i*: change in b* color parameter at day *i*; *i* is the analysis day, 0 or 7.

Table 6. ANOVA of the color parameters changes of fogged strawberries with a sanitizer based on a mixture of 5% peracetic acid and 20% hydrogen peroxide at day 0 and until 7 days at 2°C

			Sum of squares		
Source	df	Day	δL_i*	δa_i*	δb_i*
X_1 (C)	1	0	45.8*	15.4*	49.9*
		7	264.6*	203.1*	122.9
X_2 (t)	1	0	8.4	16.4*	0.1
		7	14.6	30.8	223.8
X_1^2	1	0	2.9	0.1	49.8*
		7	35.5	37.2	52.2
X_1X_2	1	0	5.0	0.5	10.2
		7	42.3	7.7	109.3
X_2^2	1	0	18.9	0.5	12.2
		7	65.3*	225.7*	718.2*
Lack of fit	3	0	49.3	8.6	8.1
		7	122.8	345.7	991.4
Pure error	2	0	3.9	0.6	2.4
		7	6.8	14.2	35.7
R^2		0	0.6	0.8	0.9
		7	0.8	0.6	0.5
AAD (%)		0	3.0	3.5	0.6
		7	9.6	4.9	4.0

*Significant terms at $p \leq 0.05$.
AAD: absolute average deviation; C: sanitizer fogging concentration (μL sanitizer per L of air chamber); t = time of the treatment (min); δL_i*: change in L* color parameter at day *i*; δa_i*: change in a* color parameter at day *i*; δb_i*: change in b* color parameter at day *i*; *i* is the analysis day, 0 or 7.

Table 7. Responses for strawberries fogged with a sanitizer based on a mixture of 5% peracetic acid and 20% hydrogen peroxide in optimized conditions (10.1 μL L^{-1}, 29.6 min) at Day 0 and until 7 days at 2°C

Response	Day 0	Day 7
$TMCR_i$	1.0	1.3
$YMCR_i$	0.7	1.4
TAR_i (%)	93.3	73.8
TPR_i (%)	95.8	96.4
$VitCR_i$ (%)	92.0	78.0
ACR_i (%)	94.8	93.5
δL_i*	3.0	−2.0
δa_i*	−1.4	1.6
δb_i*	−0.4	−9.3

$TMCR_i$: total mesophilic microbial count reduction at day *i*; $YMCR_i$: yeast and moulds count reduction at day *i*; TAR_i (%): total anthocyanins retention at day *i*; TPR_i (%): total phenolics retention at day *i*; $VitCR_i$ (%): vitamin C retention at day *i*; ACR_i (%): antioxidant capacity retention at day *i*; δL_i*: change in L* color parameter at day *i*; δa_i*: change in a* color parameter at day *i*; δb_i*: change in b* color parameter at day *i*; *i* is the analysis day, 0 or 7.

sanitizer at low fogging concentration (20 μL L^{-1}) both at short (15 min) and long times (60 min). The oxidation of anthocyanins observed after the application of sanitizer by fogging could justify the lighter fruits obtained in this experimental domain. On the other side, when fruits were fogged with high-sanitizer concentrations (higher than 20 μL L^{-1}) the strawberries turned darker (δL_i*<0) than no treated fruits, despite of the fact that anthocyanins continued decreasing in this experimental domain. This situation was probably caused because of a browning phenomenon that took place in the fruit surface after fogging with sanitizer at very high levels.

The δL_i* predictions using the reduced models at the maximum sanitizer fogging concentration (116.6 μL L^{-1}) and time (69.3 min) are −3.4 and −13.3 at day 0 and 7, respectively, indicating an ulterior browning of the strawberries treated in these conditions after the storage period.

Regarding to the changes in a* and b*, the negative values obtained in δa_0* and δb_0* (Table 5) in the entire experimental domain after treatments indicate less red and less yellow fruits. Later results correlate with the higher losses observed in TA as sanitizer fogging concentration increased. The δ_{ai}* predictions using the reduced models at the maximum processing variables levels (116.6 μL L^{-1} and 69.3 min) are −2.2 and −8.7 at day 0 and 7, respectively. For the case of δ_{bi}*, the predictions are −0.4 and −2.3 at day 0 and 7, respectively. As it can be seen, the lost in the red and yellow colors would be intensified on the storage of fruits fogged in these conditions.

Fogging optimization

A multiple response optimization of fogging disinfection of strawberries was performed using the Derringer's desirability function. For the optimization procedure, all the studied responses that were significantly affected by processing variables at day 0 were included. Optimization criteria were to maximize ($P = 5$) the bioactive compounds retentions (TAR_0%, TPR_0%, $VitCR_0$% and ACR_0%) with not differences in color change (δL_0*, δa_0* and δb_0* target values = 0; $P = 3$), and with an acceptable microbiological reduction ($TMRC_0$ and $YMRC_0$) target of 2 log UFC g^{-1} ($P = 1$). The global desirability function value at day 0 was 0.8, and the obtained optimal conditions were 10.1 μL L^{-1} sanitizer fogging concentration and 29.6 min treatment time. In Table 7 are presented the

responses using the reduced models at day 0 and 7 when fogging is done at optimal conditions. As it can be seen, fogging strawberries at these conditions can achieve good microbial reductions with more than 92% of bioactive compounds retention and almost no changes in the color attributes. Moreover, it is possible to obtain strawberries until seven days of storage at 2°C with appropriate remaining microbiological level reductions, good bioactive compounds retentions and not important changes in the color of the fruits.

CONCLUSIONS

The fogging application of an environmentally friendly sanitizer based on a mixture of 5% peracetic acid and 20% hydrogen peroxide, as a postharvest treatment of strawberries in a model chamber demonstrated to be adequate for reducing the native microbiological load and preserving the quality of the fruits until seven days of storage at 2°C. The oxidant properties of the sanitizer mix were proven to be deleterious in some extent for the bioactive compounds, especially over the anthocyanins which are the most sensitive phytochemicals compounds with high contributions to the antioxidant capacity of strawberries. Moreover, the color of the strawberries was compromised by the fogging treatments. An optimization of the fogging disinfection of strawberries was developed herein based on adequate microbiological reductions, maximum retentions of bioactive compounds and minimal changes in color parameters. All studied responses of fogged strawberries at optimal conditions showed to be appropriate both at processing day and until seven days of storage at 2°C. There are several issues to arrange and study before the operation of fogging disinfection can be scaled up to commercial purposes. The process at the optimal conditions would need to be proven efficient in a commercial chamber and workers need to be trained in safety use of the sanitizer fog.

DECLARATION OF CONFLICTING INTERESTS

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