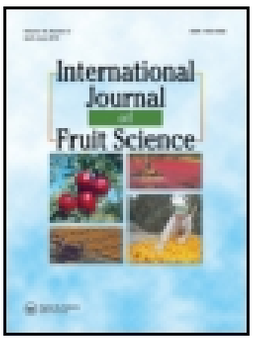

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Spray Washing Disinfection with Peracetic Acid in the Processing of Fresh-Cut Strawberries: An Alternative for Dipping Techniques

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ABSTRACT

The effect of spray washing disinfection with peracetic acid (PAA) on quality attributes of fresh-cut strawberries was studied. The effectiveness on native microflora and experimentally inoculated *Escherichia coli* was also investigated. The responses were evaluated at 0 day and after 7 days of storage (2 °C). PAA concentration (1–240 mg L^{-1}) and spraying time (11–138 seconds) did not affect the retentions of quality attributes. Optimizing the process at days 0 and 7 allowed to obtain significant reductions for total mesophilic microorganism, molds, and yeasts; furthermore, *E. coli* reduction was 3.4 log (day 0). All these results suggest that spraying with PAA could be a good disinfection method.

KEYWORDS

Spraying; disinfection; fresh-cut strawberries; bioactive compounds

Introduction

Strawberry (*Fragaria x ananassa*) is one of the most commonly consumed fruits due to its highly desirable taste, color, and flavor and great amounts of bioactive compounds such as vitamin C (VitC), phenolic compounds, and other antioxidants including flavonoids which are capable of scavenging several different reactive oxygen species and may promote health benefits in liver protection, anticancer, and coronary heart disease (Octavia and Choo, 2017). The quality of strawberry in the market is defined by its visual and internal characteristics such as color, size, firmness, sweetness, acidity, aroma, and the nutritional value, especially the antioxidant content (Shin et al., 2008). Lifestyles of modern consumers, along with the demand for natural, fresh, flavorful, convenient, and high-quality products, with health benefits, have raised the production and consumption of fresh-cut, minimally processed, or ready-to-eat fruits and vegetables. During minimal processing, each step affects quality and microflora of these products (Allende et al., 2006).

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Washing is an important element in minimally processing operations which significantly influences produce quality and safety. There is a global concern on developing alternative disinfection strategies to minimize environmental and public health impacts. The washing-disinfection operation is an essential step to eliminate foreign matter, microorganisms on the surface of the vegetable product and in the wash water, and cellular fluids released by cutting (Pirovani et al., 2006). Sodium hypochlorite (NaClO) is effective for microbiological decontamination, but their reaction with organic matter may result in the formation of carcinogenic halogenated disinfection by-products, such as trihalomethanes and haloacetic acids (Ölmez and Kretzschmar, 2009). Based on these concerns, peracetic acid (PAA) is an interesting alternative, and its effectiveness was demonstrated in a wide range of fresh-cut fruits and vegetables (Vandekinderen et al., 2009; Van de Velde et al., 2016). PAA is a strong oxidant that has been demonstrated to be effective against spoilage and pathogenic microorganisms (Rodgers et al., 2004). PAA is a combination of PAA and hydrogen peroxide and typically commercialized as a liquid. PAA is an interesting alternative to NaClO since its breakdown products, acetic acid, oxygen, and water, make its use completely sustainable and ecofriendly. PAA showed possibilities for extending shelf life without pronounced effects on nutritional content (Artés-Hernández et al., 2013).

Dipping and/or spraying are some of the methods of applying the sanitizer. Most of the research works, nowadays, study the PAA application by dipping (Silveira et al., 2008; Van de Velde et al., 2014). However, the spray systems could be considered a good method for fresh-cut fruits washing disinfection, as it can achieve higher pathogen reduction because of increased physical removal of bacteria in conjunction with antimicrobial efficacy of sanitizers, but it has not been extensively studied yet (Chang and Schneider, 2012). The optimization of each step to minimize the negative consequences of processing in fresh-cut fruits and vegetables will increase the shelf life and maintain the appearance and the nutritional quality of these products (Gil and Allende, 2012). For this reason, the objective of this work was to study and model the effect of spray washing disinfection with PAA on the microbiological, nutritional, and sensory quality of fresh-cut strawberries. Moreover, the effectiveness of this method was evaluated for reducing the population of a model pathogen (*Escherichia coli*) on fresh-cut strawberries.

Materials and Methods

Plant Material

Lots (5 kg each one) of strawberries (*Fragaria x ananassa* Duch.) of *Camarosa* variety were collected during August to December from

Arroyo Leyes, Santa Fe (Argentina), with full ripe stage (90% of the surface showing red color).

PAA

A commercial sanitizer called Oxilac Plus (Indaquim S.A., Santa Fe, Argentina) was used in the spray washing disinfection. This sanitizer is a stabilized mixture of 5% PAA, 20% hydrogen peroxide, 8% acetic acid, and water. Each concentration (% PAA), according to the experimental design, was determined by iodometric titration according to American Public Health Association–American Water Works Association–Water Environment Federation (APHA-AWWA-WEF, 1989).

Minimal Processing

The minimal processing was performed at the pilot plant of the Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral. Strawberries were selected, and the calyxes and peduncles were removed. Fruits were prewashed with flowing tap water (20 °C, 2 min), drained over absorbent paper, and cut longitudinally into quarts. Fresh-cut strawberries were placed on a fixed wire screen and sprayed at different concentration of PAA solution and times according to the experimental design. The spraying was performed using a hand plastic pressure portable sprayer (Pressure Sprayer) with a flow rate of 6.4 mL s^{-1}. The nozzle was placed perpendicular at 0.195 m over the fruits.

Finally, washed fresh-cut strawberries were drained, and subsamples (30 g) were used for analyses immediately after processing, and the rest (aliquots of 0.1 kg) were packaged within round containers with lids and stored at 2 °C for analyses at 7 d. The containers were made of polyethylene terephthalate (PET) of 0.42 mm thick, with a surface area of 0.045 m^2, transmission rates of 4.73×10^{-15}–9.617×10^{-15} kg m^{-2} s^{-1} Pa^{-1} for O_2 (at 23 °C and 0% relative humidity (RH)) and 1.3×10^{-7}–2.08×10^{-7} kg m^{-2} s^{-1} for water vapor (at 38 °C and 90% RH).

Each experimental run had six control samples, which were whole strawberries (without calyxes and peduncles) packed in the same containers as treated samples. The half of the control samples were analyzed at the processing day (0 d) and, the rest, after 7 d of storage at 2 °C.

Experimental Design, Optimization, and Validation

Response surface methodology using a *central composite design (CCD)* was used to study the operation of spray washing disinfection. The total number of experiments (n) of the CCD design was determined using 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, and the distance of the axial points from the center point is dependent on the number of factors chosen for the experiment (Montgomery, 2001).

The sanitizer concentration and the treatment time were the selected variables in this study (k = 2). Therefore, n was calculated using Eq. [1] and was equal to n = 11 (n_0 = 3). Each of the variables was examined at five different levels. It was assumed that there was a mathematical function for each studied response according to the two variables related to spraying processing (Eq. [2]):

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

where C is the sanitizer concentration (mg L^{-1} of PAA) and t is the time of spraying (s), and the five levels were as follows: C = 1, 36, 120.5, 205, and 240 mg L^{-1} and t = 11, 30, 75, 119, and 138 s (Table 2). The design was replicated twice.

The studied responses (Y) were microbiological counts reduction: total mesophilic microorganisms (TMCR), yeasts (YCR), molds (MCR), and *E. coli* (EcoliCR) count reductions; bioactive compound retentions: total anthocyanin (TAR), total phenolics (TPR), ascorbic acid (AAR), vitamin C (VitCR), antioxidant capacity (ACR) retentions (%), and general quality parameters: soluble solids (SSR) and pH (pHR) retentions (%); and color parameter changes (luminosity (δL_i^*), chroma value (δC_{abi}^*), and hue angle (δh_{abi}) changes (%)). All responses were evaluated immediately after washing (day 0) and after 7 d of storage at 2 °C.

The microbial count reductions were expressed as Eq. [3]

$$NRi = -\log N_i / N_{ci} \quad (3)$$

where NR_i is $TMCR_i$, YCR_i, MCR_i, or $EcoliCR_i$; N_i is the viable microorganism count of sprayed 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 bioactive compound and general quality parameter retentions were expressed as Eq. [4]:

$$QRi = (Q_i / Q_{ci}) \times 100 \quad (4)$$

where QR_i is TAR_i, TPR_i, AAR_i, $VitCR_i$, ACR_i, SSR_i, or pHR_i; Q_i and Q_{ci} represent the sample attributes measured after spray washing and control strawberries at day i (0 or 7), respectively.

The color parameter changes (δL_i^*, δC_{abi}^*, and δh_{abi}) were expressed as Eq. [5]:

$$\delta Qi(\%) = (Q_i^* - Q_{ci}^*)/Q_{ci}^* \times 100 \quad (5)$$

where δQ_i is the percentage of the difference between sprayed strawberry parameter value Q_i^* (L_i^*, C_{abi}^*, and h_{abi}) and the control strawberry parameter value Q_{ci}^* (L_{ci}^*, C_{abci}^*, and h_{abci}), divided by the last one (Eq. [3]) at day i (0 or 7).

A second-order polynomial equation was used to model Eq. [2] for each response (Eq. [6]), according to the experimental design:

$$Y(C,t) = a + b \times C + c \times t + d \times C \times t + e \times C^2 + f \times t^2 \quad (6)$$

where *a, b, c, d, e*, and *f* are the regression coefficients and *C* and *t* are the studied variables.

Each complete model (Eq. [6]) was reduced through the stepwise regression procedure, obtaining an equation with only significant terms.

Furthermore, the multiple response optimization procedure based on the Derringer's desirability function was employed (Derringer and Suich, 1980). The response variables to be optimized (maximized) were $TMCR_0$, YCR_0, MCR_0, and YCR_7. Finally, the validation of the developed models was carried out with an additional experimental run at the optimal time and concentration (three replicates). Strawberries were processed as it was previously explained in section "Minimal Processing." Microbiological analyses were determined on treated samples as well as on control samples immediately after processing and after 7 d of storage at 2 °C.

Bacterial Strain and Preparation of Inoculum

A strain of *E. coli* ATCC 25922 maintained at −80 °C in 15% glycerol was used. The procedure for preparation of inoculum was adapted according to Martínez-Hernández et al. (2015). Cells were grown overnight at 37 °C in brain heart infusion broth (BHI; Merck, St Louis, MO, USA). Another overnight culture was carried out by transferring 20 µL of the previous overnight culture to a sterile nutrient broth (NB; Merck). Then, 1.8 mL of the last overnight culture was transferred to 200 mL of sterile tryptic soy broth (TSB; Merck) and was incubated 24 h at 37 °C. After incubation, culture was prepared by diluting in 800 mL of phosphate-buffered saline

(PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2.0 mM KH_2PO_4; pH 7.4) reaching approximately a final concentration of 10^8 CFU mL^{-1}.

Fresh-cut strawberries were immersed in the inoculum solution and kept under agitation (10 min). The inoculated strawberries were then dried in a laminar flow model BSC-1300IIA2-X (Biobase, Jinan, China) for 1 h to promote the microbiological adherence. These samples were processed as it was previously explained in section "Minimal Processing".

Microbiological Analysis

The methods followed were that recommended by the *Bacteriological Analytical Manual* of the Food and Drug Administration (FDA, 2001). Each sample weighing 10 g was homogenized in a stomacher using 90 mL of peptone water 0.1% for 2 min. Decimal dilutions were carried out. Enumeration of total mesophilic microorganisms was assessed using plate count agar (PCA; Merck). The plates were incubated at 30 °C for 48 h. Yeasts and molds enumeration was assessed using yeast extract glucose chloramphenicol agar (YGC; Merck). Plates were incubated at 28 °C for 5 d. For *E. coli* enumeration, samples were plated in the appropriate culture media eosin methylene blue agar (EMB; Merck) and incubated at 37 °C for 24 h. Results were expressed as colony forming unit per gram (CFU g^{-1}).

Total Anthocyanin (TA), Total Phenolics (TP), Antioxidant Capacity (AC), Ascorbic Acid (AA), and VitC Content Analysis

Extract Preparation

For TA, TP, and AC determination, 5 g of homogenized strawberries were extracted with 75 mL of extraction solvent (80% acetone and 20% water). The mixture was homogenized for 1 min, sonicated for 15 min, and centrifuged at 12,000 *g* for 20 min at 4 °C. Supernatant was separated and used for analyses (triplicates).

TA Content

TA content was determined by the pH differential method according to Heo and Lee (2005). Results were converted as milligram pelargonidin-3-glucoside per kilogram of fresh weight.

TP Content

TP content was determined using the Folin–Ciocalteu reagent according to Singleton and Rossi (1965). Results were expressed as milligram gallic acid equivalents per kilogram of fresh weight.

AC

AC of the samples was estimated according to Sánchez-Moreno et al. (2003). The AC of strawberry extracts was expressed as milligram AA equivalent per kilogram of fresh weight.

AA and VitC Contents

AA and VitC contents were determined according to Van de Velde et al. (2012). Results were expressed as milligram AA per kilogram of fresh weight and milligram VitC per kilogram of fresh weight, respectively.

Color Measurement

Color was determined using a Minolta 508 d spectrophotometer (Minolta Co., Ltd., Tokyo, Japan) with D65 illuminant, 10° observer angle, and specular component excluded, evaluating the International Commission on Illumination (CIE) system parameters: L^* (luminosity), $C_{ab}{}^*$ (chroma value), h_{ab} (hue angle). A total of 10 measurements were performed per sample (Piagentini et al., 2012).

Soluble Solids (SS) and pH

Measurements of SS were made with a hand-held digital refractometer model PAL-ALPHA (Atago Co. Ltd., Tokyo, Japan), and pH value was determined with a pH meter model B-213 (Horiba, Ltd., Tokyo, Japan). In total, three measurements were performed per sample, and the results for SS were expressed in percentage.

Statistical Analysis

STATGRAPHICS Centurion XV 15.2.06 (Statpoint Technologies, Inc., Warrenton, VA, USA) was used to perform analysis of variance (ANOVA), to fit the experimental data to the second-order polynomial equations and obtain the coefficients of the equations. The *t* test was performed to state significant differences between storage time (0 and 7 d) and between predicted and experimental values in the validation experiment.

Results and Discussion

Initial Microbial Load, Bioactive Compounds, and General Quality Attributes of Control Strawberries

Total mesophilic microbial, mold, and yeast counts of control strawberries were 3.3, 3.7, and 3.6 log CFU g^{-1}, respectively.

Table 1. Physicochemical and bioactive attributes of control strawberries at day 0 and after 7 d (2 °C).

	Time (d)	
Parameter	0	7
SS (%)	(7.5 ± 0.4) a	(7.4 ± 0.2) a
pH	(3.6 ± 0.1) a	(3.6 ± 0.0) a
L^*	(28.9 ± 4.7) a	(28.1 ± 2.9) a
$C_{ab}{}^*$	(20.7 ± 2.2) a	(21.3 ± 2.3) a
h_{ab}	(18.0 ± 2.7) a	(18.1 ± 3.1) a
TP (mg GA kg^{-1})	(3000 ± 116) a	(2670 ± 105) b
TA (mg P3G kg^{-1})	(530 ± 17.2) a	(470 ± 20.1) b
AC (mg AA kg^{-1})	(4680 ± 352) a	(4430 ± 142) a
AA (mg AA kg^{-1})	(490 ± 10.0) a	(500 ± 14.4) a
VitC (mg VitC kg^{-1})	(550 ± 10.0) a	(550 ± 40.0) a

Mean ± standard deviations.
Different letters in the same row indicate significant differences ($P \leq 0.05$) by t test.
SS: soluble solids; TP: total phenolic; TA: total anthocyanin; AC: antioxidant capacity; AA: ascorbic acid; VitC: vitamin C.

After 7 d at 2 °C, the microorganism counts did not change, indicating that total load did not rise during the storage period at 2 °C. Moreover, there was absence of *E. coli* on strawberry control samples. No statistical significant differences were found in the content of AC, AA, and VitC, and general quality characteristics (SS, pH, and color parameters) of control strawberries were noted after 7 d of storage at 2 °C with respect to day 0 (Table 1). However, TP and TA content showed a significant reduction (around 10%) after storage. These results showed that the time of the storage at 2 °C affected the phenolic contents of whole strawberries (control samples) as it was reported by other authors (Ayala-Zavala et al., 2004; Van de Velde et al., 2016).

Effect of Spray Washing Disinfection with PAA on the Native Microbial and Inoculated *E. coli* Count Reductions

The experimental results obtained for TMCR, MCR, YCR, and EcoliCR under the design conditions of sanitizer concentrations and spraying times are presented in Table 2. Tests on residuals (normal probability, residuals vs. estimated values for the responses, and residuals vs. random order of runs) revealed that they satisfied the assumptions of normality, independence, and randomness (data not shown).

The ANOVAs for the responses $TMCR_0$, MCR_0, YCR_0, and $EcoliR_0$ at day 0 indicated that the developed models were adequate, exhibiting no significant lack of fit ($P > 0.05$). The coefficients of determination (R^2) were satisfactory (>70%). Based on all these tests, the models were accepted.

Based on Pareto chart (data not shown), the PAA concentration has more effect on $TMCR_0$, MCR_0, and YCR_0 than the spraying time, and the

Table 2. Experimental results of the microbiological and *E. coli* count reductions of sprayed strawberries with PAA on the processing day and after 7 d at 2 °C.

Run	Concentration (mg L^{-1})	Time (s)	*i* (d)	$TMCR_i$ (-log N_i/N_{ci})	MCR_i (-log N_i/N_{ci})	YCR_i (-log N_i/N_{ci})	$EcoliCR_i$ (-log N_i/N_{ci})
1	120.5	75	0	1.16	1.91	2.30	1.72
			7	1.65	2.41	2.48	2.41
2	240	75	0	1.15	2.48	3.60	2.82
			7	1.82	2.41	2.57	3.91
3	205	119	0	1.30	1.84	2.20	2.94
			7	1.12	3.59	2.70	2.45
4	120.5	11	0	0.75	0.59	0.73	1.47
			7	1.61	1.59	1.56	1.97
5	36	30	0	0.22	0.52	0.95	2.01
			7	1.48	1.41	1.50	1.56
6	120.5	75	0	1.07	2.26	2.30	1.60
			7	1.78	1.84	1.94	1.88
7	36	119	0	0.70	0.26	1.36	1.22
			7	1.33	1.25	0.49	1.58
8	205	30	0	0.94	1.91	2.43	1.12
			7	1.95	2.41	2.22	2.00
9	120.5	138	0	1.15	2.00	2.18	1.82
			7	1.58	2.05	2.00	3.90
10	120.5	75	0	1.20	1.51	1.95	2.18
			7	1.30	1.26	1.41	2.96
11	1	75	0	0.43	1.40	1.19	1.06
			7	0.86	2.19	1.50	1.71

$TMCR_i$: total mesophilic microbial count reduction at day *i*; MCR_i: mold count reduction at day *i*; YCR_i: yeast count reduction at day *i*; $EcoliCR_i$: *E. coli* count reduction at day *i*; N_i is the viable microorganism count of sprayed samples at day *i*; N_{ci} is the viable microorganism count of control samples at day *i*; *i*: analysis day, 0 or 7.

Table 3. Analysis of variance of total mesophilic microorganisms ($TMCR_0$), yeasts (YCR_0), molds (MCR_0), and *E. coli* ($EcoliCR_0$) count reductions at day 0.

		Sum of squares			
Source	df	$TMCR_0$	MCR_0	YCR_0	$EcoliCR_0$
A: concentration	1	0.6834***	2.5283*	4.1016***	1.3770**
B: time	1	0.2470**	0.3461	0.6220	0.2907
AA	1	0.2184**	0.0541	0.0192	0.0209
BB	1	0.0036**	0.0090	0.1024*	1.7030
AB	1	0.0769	0.9981	0.9570	0.0424**
Pure error	3	0.0336	1.4058	0.6576	0.3816
Lack of fit	2	0.0089	0.2817	0.0817	0.1875

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

increase in both variables (C and t) increases the microbial count reduction.

At the processing day, $TMCR_0$ model was affected by sanitizer concentration and time of spraying through the linear and the quadratic terms ($P \leq 0.05$; Table 3). The reduced model is shown in Eq. [7]:

$$TMCR_0 = -0.29 + 0.01 \times C + 0.012 \times t - 2.710^{-5} \times C^2 - 5.710^{-5} \times t^2 \quad (7)$$

MCR_0 and YCR_0 models were affected by sanitizer concentration through its linear term, and YCR_0 was also affected by spraying time through its quadratic terms ($P \leq 0.05$; Table 3). The reduced models obtained are shown in Eq. [8] and [9]:

$$MCR_0 = 0.71 + 6.610^{-3} \times C \quad (8)$$

$$YCR_0 = -0.43 + 8.510^{-3} \times C + 3.810^{-2} \times t - 2.1210^{-4} \times t^2 \quad (9)$$

The highest predicted reduction for $TMCR_0$, MCR_0, and YCR_0 using the reduced models were 1.3 (183 mg L^{-1} and 109 s), 2.3 (240 mg L^{-1}, regardless of time), and 3.3 (240 mg L^{-1} and 90 s) log, respectively.

$TMCR_0$ developed model is visualized in a surface plot diagram in Figure 1. It is shown that as concentration and time increases, the reduction of total mesophilic microorganism increases. Reductions greater than 1.2 log could be obtained at concentrations and spray washing times higher than 160 mg L^{-1} and 70 s, respectively.

In the case of YCR_0, reductions greater than 2 log could be achieved at concentrations and times higher than 120 mg L^{-1} and 50 s, respectively (Figure 2).

$TMCR_7$, MCR_7, and YCR_7 models represent the impact of the spray washing operation, previously applied at 0 d, on microbiological reductions of fresh-cut strawberries after 7 d of storage at 2 °C.

$TMCR_7$ and MCR_7 models were not affected by process variables (concentration of PAA and time of spraying; Table 4). The best estimate of the

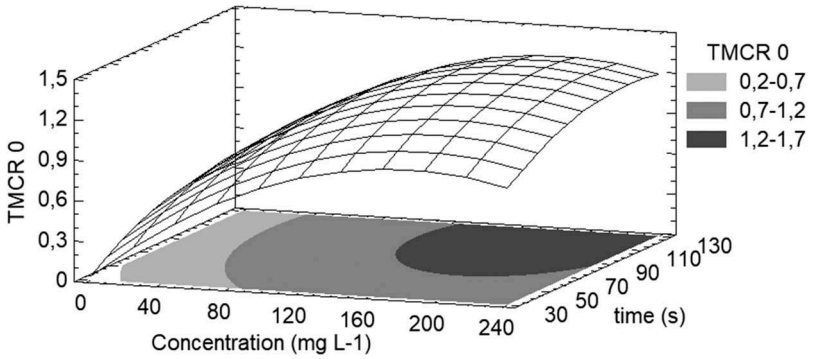

Figure 1. Surface and contour plots of the model describing the combined effect of the washing disinfection time and the peracetic acid concentration on the total mesophilic microbial count reduction at day 0 (TMCR0) obtained for fresh-cut strawberries.

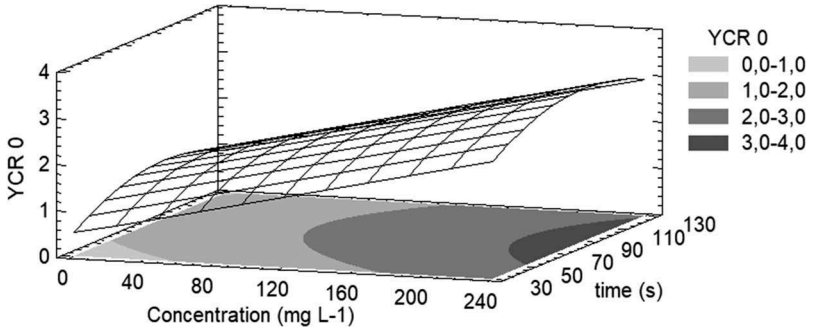

Figure 2. Surface and contour plots of the model describing the combined effect of the washing disinfection time and the peracetic acid concentration on the yeast count reduction at day 0 (YCR0) obtained for fresh-cut strawberries.

Table 4. Analysis of variance of total mesophilic microorganisms ($TMCR_7$), yeasts (YCR_7), molds (MCR_7), and *E. coli* ($EcoliCR_7$) count reductions at day 7.

		Sum of squares			
Source	df	$TMCR_7$	MCR_7	YCR_7	$EcoliCR_7$
A: concentration	1	0.3271	1.6663	2.4678*	2.4435
B: time	1	0.1494	0.3488	0.0011	1.2796
AA	1	0.0741	0.3756	0.0000	0.0125
BB	1	0.1156	0.4489	0.5550	0.0462
AB	1	0.0000	0.0018	0.0906	0.0013
Pure error	3	0.2450	1.1859	0.4817	2.9449
Lack of fit	2	0.1233	0.6613	0.5725	0.5833

*$P \leq 0.05$.

reduction of total mesophilic microbial load and molds is the average of all experimental results at 7 d (1.5 and 2.0 log reduction, respectively).

However, YCR_7 model was affected by sanitizer concentration through the linear term ($P \leq 0.05$; Table 4). The reduced model for YCR_7 is shown in Eq. [10]:

$$YCR_7 = 1.06 + 6.5710^{-3} \times C \quad (10)$$

Based on Eq. [10], the highest predicted value for YCR_7, obtained with this washing system, is 2.6 log reduction at the maximum sanitizer concentration (240 mg L^{-1}), regardless of spraying time.

To compare spray washing method, studied herein, with the washing disinfection by dipping using the same sanitizer, the work of Van de Velde et al. (2014) was analyzed. These authors studied the washing disinfection by immersion (or dipping) of quartered fresh-cut *Camarosa* strawberries with PAA and found that a dipping treatment at 100 mg L^{-1} PAA during 120 s allowed to reduce mesophilic aerobic microorganism population in 2.6 log. Spraying fresh-cut strawberries at the same PAA concentration during the same time results in lower reduction in mesophilic microorganisms (predicted value: 1.1 log reduction). These differences could be explained by the greater contact area between PAA solution and fresh-cut fruits in the immersion method. However, Rodgers et al. (2004) reported that whole strawberries washed with 80 mg L^{-1} of PAA by dipping during 5 min reduced molds and yeasts 1.1 and 1 log, respectively. In this study, at the same concentration (80 mg L^{-1}) but at lower time (2.3 min), slightly higher reductions were obtained (1.2 and 1.5 log for molds and yeasts, respectively).

In the inoculation experiment, the initial count of *E. coli* in fresh-cut strawberries was 7.2 log CFU g^{-1} at day 0 and decreased around 1 log (6.4 log CFU g^{-1}) after 7 d of storage at 2 °C (inoculated control samples).

The reduced model obtained for $EcoliR_0$ through the stepwise regression procedure is shown in Eq. [11]

$$EcoliCR_0 = 2.37 - 7.3810^{-3} \times C - 0.01 \times t + 1.6210^{-4} \times C \times t \quad (11)$$

EcoliCR model at day 0 was affected by sanitizer concentration, through the linear term and the interaction term with time (Eq. [11]; Table 3). As it can be seen in Figure 3, at low PAA concentration, the *E. coli* reduction is about 1–2 log

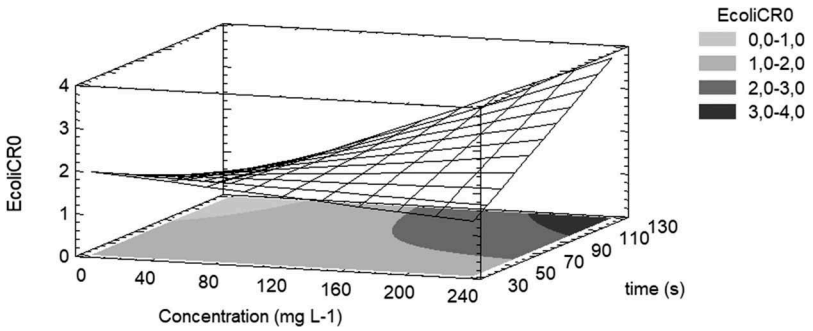

Figure 3. Surface and contour plots of E. *coli* count reduction at day 0 (EcoliCR0) obtained for fresh-cut strawberries.

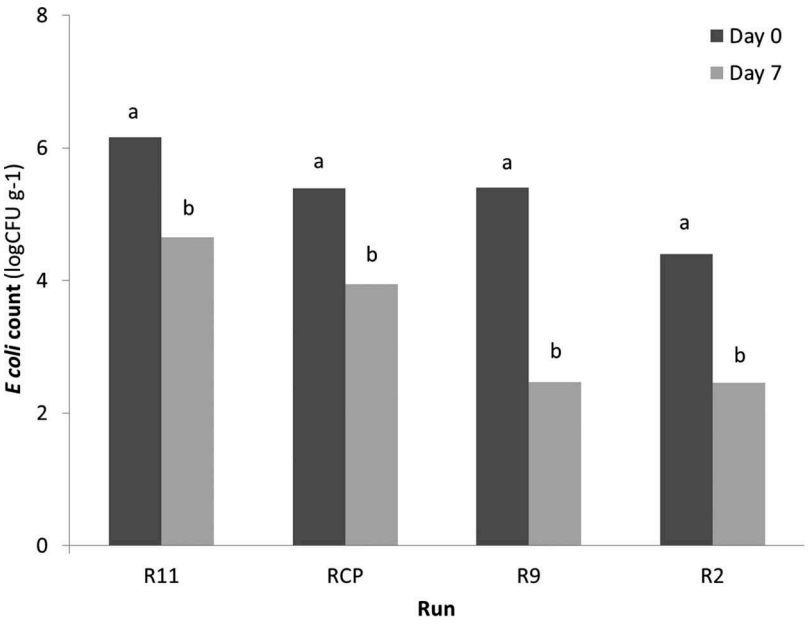

Figure 4. Effect of storage time at 2ºC on inoculated E. coli of fresh-cut strawberry sprayed with PAA. R11: sample treated with 1 mg L-1 and 74.5 s; RCP: mean of center points run of sample treated with 120.5 mg L-1 and 74.5 s; R9: sample treated with 120.5 mg L-1 and 138 s; R2: sample treated with 240 mg L-1 and 74.5 s. Different letters above the bars indicate significant differences (P≤0.05).

at any spraying time, but at high PAA concentration, it increases with increasing spraying time. The highest predicted reduction for EcoliCR$_0$ is 4 log, obtained at the maximum studied conditions of the spray washing (240 mg L^{-1} and 138 s).

After 7 d of storage at 2 °C, the concentration and time assayed on spray washing disinfection did not affect EcoliCR$_7$, and the mean reduction obtained for all treated samples was 2.4 log cycles (Table 4). As it can be seen in Figure 4, *E. coli* counts at day 7 were generally lower than the count of the same sample at day 0 ($P \leq 0.05$). Depending on the runs, the reduction in storage was between 1.2 (R11) and 2.9 log (R9). It is necessary to point out that the efficacy of decontamination methods is reflected in the microbiological reduction obtained but, even more important, in the maintenance of this reduction during storage (Abadias et al., 2011). The spray washing disinfection with PAA not only reduces the inoculated *E. coli* of fresh-cut strawberries but also prevents the increase in *E. coli* population during 7 d. This occurs probably due to the bacteria that are unable to repair the damage caused by PAA action (germicidal properties). This would explain the fact that despite the increased availability of readily biodegradable carbon (acetic acid), due to PAA decomposition, microbial counts decrease (Antonelli et al., 2006). Additionally, *E. coli* counts in inoculated control samples decreased about 1 log probably due to the low storage temperature (2 °C; Luo et al., 2010).

Table 5. Physicochemical and bioactive attributes retentions of fresh-cut strawberries after spray washing disinfection with PAA at day 0 and after 7 d of storage at 2 °C.

	Storage time (d)	
Parameter (%)	0	7
SSR_i	(99.2 ± 3.3) a	(98.4 ± 2.2) a
pHR_i	(98.4 ± 1.6) a	(100.0 ± 0.8) b
δLi*	(−1.2 ± 0.8) a	(−1.3 ± 0.6) a
δCabi*	(23.6 ± 5.1) a	(11.2 ± 3.3) b
δh_{abi}	(12.2 ± 3.9) a	(9.6 ± 3.7) a
TPR_i	(95.3 ± 3.4) a	(97.1 ± 2.2) a
TAR_i	(78.8 ± 4.5) a	(79.4 ± 6.1) a
ACR_i	(98.1 ± 6.8) a	(97.3 ± 9.8) a
AAR_i	(74.1 ± 7.5) a	(76.5 ± 6.6) a
$VitCR_i$	(83.0 ± 5.0) a	(85.5 ± 3.9) a

Mean ± standard deviation.

Different letters in the same row indicate significant differences ($P \leq 0.05$) by t test.

SSR_i: soluble solids retention at day *i*; pHR_i: pH retention at day *i*; δLi*: change in lightness at day *i*; δCabi*: change in chroma at day *i*; δh_{abi}: change in Hue at day *i*; TPR_i: total phenolic retention at day *i*; TAR_i: total anthocyanin retention at day *i*; ACR_i: antioxidant capacity retention at day *i*; AAR_i: ascorbic acid retention at day *i*; $VitCR_i$: vitamin C retention at day *i*; *i*: analysis day, 0 or 7.

Effect of Spray Washing on the Retention of General Quality Attributes and Bioactive Compounds

The ANOVAs of predictive models of the retention of general quality attributes (SS; pH; and L^*, Cab*, and h_{ab}), bioactive compounds (TP, TA, AA, and VitC), and the AC of treated fruits compared to control samples showed that the process variables (concentration PAA and spraying time) did not affect significantly these responses; therefore, no model could be obtained. For this reason, the best estimation is the average of their retentions within the experimental range (Table 5).

Quality of fresh-cut fruits is a combination of parameters including appearance, texture, flavor, and nutritional value (Kader, 2002). Color has been considered to have a key role in food choice, food preference, and acceptability and may even influence taste thresholds, sweetness perception, and pleasantness (Rico et al., 2007). In our study, the mean percentage of L^* change was negative (around 1%), indicating that fresh-cut strawberries spray washed with PAA were slightly darker than control samples and did not change during storage at 2 °C. A positive value in the average of percentage of the Cab* change indicated that the color of PAA spray–washed fresh-cut strawberries was more vivid (around 20%) than control samples, and this change was significantly lower after storage. Positive values in h_{ab} changes indicated that PAA-washed fresh-cut strawberries were less red (around 12%) than control samples and did not change during storage. Other important quality parameters are SS and pH. Our results showed that the processing and storage conditions did not affect the retention of SS of fresh-cut strawberries with respect to control, but the retention of pH was slightly modified (Table 5).

Table 6. Predicted and experimental results of spraying washing disinfection with peracetic acid fresh-cut strawberries under optimal conditions (240 mg L^{-1} and 97 s).

	Storage time (d)			
	0		7	
Response	Predicted	Experimental	Predicted	Experimental
$TMCR_i$ (-log N_i/N_{ci})	1.2a	1.0a	1.5*a	2.0a
MCR_i (-log N_i/N_{ci})	2.3a	2.4a	2.0*a	2.0a
YCR_i (-log N_i/N_{ci})	3.3a	2.6a	2.6a	2.7a

*Mean value of all experimental runs.

$TMCR_i$: total mesophilic microbial count reduction at day *i*; MCR_i: mold count reduction at day *i*; YCR_i: yeast count reduction at day *i*; *i*: analysis day, 0 or 7.

Different letters in the same row indicate significant differences between predicted and experimental values in the same optimization conditions ($P \leq 0.05$) by *t* test.

Van de Velde et al. (2013) found that washing disinfection of fresh-cut strawberry by immersion with 20 mg L^{-1} of PAA and 52 s maximized the retention of AA and TA with moderate microbial load reduction (2 log). At these conditions, fresh-cut strawberries showed retentions of 87.2% for TA and 93.3% for AA. The retentions of AA and TA in fresh-cut strawberries washed by the spray system were lower (74% and 79%, respectively). However, the retention of total VitC (L-AA + L-dehydroascorbic acid) of spray-washed fresh-cut strawberries was around 84%, indicating its partial oxidation to L-dehydroascorbic acid due to PAA but keeping its biological function (Hernández et al., 2006). Simultaneously, there was a great retention of AC (around 98%), probably due to the high retention of the phenolic compounds (around 96%; Table 5).

Optimization and Model Validation

Maximizing the responses of the $TMCR_0$, YCR_0, MCR_0, and YCR_7 models, the optimum processing conditions achieved were 240 mg L^{-1} and 97 s. In Table 6, the results of validation experiments at this optimal condition are shown. As it can be seen, no differences were found between predicted and experimental values, validating the models obtained. The predicted reduction of *E. coli* counts under the optimal conditions was 3.4 log.

Conclusion

The bioactive compounds and the quality attributes were slightly modified after the spray washing disinfection and storage (7 d and 2 °C). The reduction of mesophilic aerobic microorganism, molds, yeast, and *E. coli* after the spray washing process has been suitably modeled. The models developed allowed to find the optimal conditions of the process (240 mg L^{-1} and 97 s), where a significant microbiological reduction could be obtained.

The spray washing disinfection with PAA could be considered a good alternative or complementary method for disinfection of fresh-cut strawberries due to the high reductions of microbial load with minimal changes in quality and nutritional attributes.

The results obtained herein could be a useful tool for processors as an approach to quantify the effect of concentration and time on their spray systems.

Conflict of Interest

The authors declare no conflict of interest.

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