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Optimisation of the peracetic acid washing disinfection of fresh-cut strawberries based on microbial load reduction and bioactive compounds retention

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Summary Total microbial count reduction (TMR), total anthocyanins (TAR), and ascorbic acid retention (AAR) after the operation at different PAA concentrations (0–100 mg L^{-1}), contact times (10–120 s), and temperatures (4–40°C) were used for multiple optimisation using Derringer's desirability function. Two optimization scenarios (OP 1 and OP 2) were studied. OP 1 was proposed with the goal to maximize TMR with 90% TAR and AAR; and OP 2 maximizing TAR and AAR with TMR of 2 log UFC g^{-1}. The optimized variable levels obtained were the following: 100 mg L^{-1} PAA, 24 °C and 50 s for OP 1 and 20 mg L^{-1} PAA, 18 °C and 52 s for OP 2. Additional validation experiments showed agreement between predicted and experimental results. OP 2 conditions are recommended to fresh-cut strawberries washing disinfection because of an acceptable TMR, higher TAR and AAR, better sensory attributes, and the economic convenience of lesser PAA consumption.

Keywords Derringer's optimization, fresh-cut fruits, peracetic acid.

Introduction

Strawberries (*Fragaria* × *Ananassa* Duch.) are one of the most consumed berries in Argentina (Williner *et al*., 2003). They are a relevant source of bioactive compounds due to their high levels of vitamin C and phenolic compounds. Total vitamin C (ascorbic acid plus dehydroascorbic acid) is found at concentrations between 40 and 60 mg 100 g^{-1} fresh weight (FW) in strawberries (Davey *et al*., 2000; Van de Velde *et al*., 2013a). The main phenolic compounds are anthocyanins, which are responsible for the appealing and bright red colour of the strawberries, with concentrations up to 65 mg 100 g^{-1} FW. Bioactive compounds present antioxidant activity and therefore a consequent beneficial effect on the maintenance of the consumer health and in the prevention of neuronal and cardiovascular illnesses, cancer, and diabetes (Cordenunsi *et al*., 2005; da Silva Pinto *et al*., 2008; Giampieri *et al*., 2012).

The minimal processing to obtain fresh-cut strawberries involves selection, prewash, calyx and peduncle elimination, cutting, washing disinfection, and packaging. The washing disinfection is an essential operation to eliminate foreign matter, micro-organisms on the surface of the vegetable product and in the wash water, and cellular fluids produced by cutting (Pirovani *et al*., 2004).

Peracetic acid (PAA), commercially available as a quaternary equilibrium of acetic acid, hydrogen peroxide, PAA and water, is a disinfectant with good antimicrobial properties that can be suitable in the fresh-cut industry in replace of the traditionally used sodium hypochlorite (Vandekinderen *et al*., 2009). The United States Code of Federal Regulations (CFR) recommends the use of peracetic acid in the washing water of fruits and vegetables up to 80 mg L^{-1} concentration (Code of Federal Regulations [CFR], 2007).

Peracetic acid is active at low temperatures (0–25 °C), in a pH range of 3–7.5, and it does not produce mutagenic by-products, as it happens with chlorine and its derivates (Kitis, 2004; Ölmez & Kretzschmar, 2009). The decomposition products of PAA are only oxygen and acetic acid (Silveira *et al*., 2008). Moreover, PAA is not susceptible to peroxidases and it retains in a better way its activity in the presence of organic loads or food residue when compared with chlorine (Malchesky, 1993). However, a disadvantage of using PAA in the fresh-cut industry is the higher cost in comparison with chlorine (Gorny, 2001).

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Peracetic acid's mechanism of action against micro-organisms is based in the denaturation of proteins and enzymes and increased cell wall permeability by oxidizing sulfhydril and disulfide bonds (Kitis, 2004). Moreover, PAA-based sanitizers contain a considerable amount of hydrogen peroxide that also possesses antimicrobial properties (Wagner *et al.*, 2002). However, when PAA is used a disinfection agent, its disinfection power predominates over the antimicrobial effect of the accompanying hydrogen peroxide (Small *et al.*, 2007). The effectiveness of PAA in reducing the initial microbiological load was demonstrated for several fresh-cut fruits and vegetables (Silveira *et al.*, 2008; Artés *et al.*, 2009; Vandekinderen *et al.*, 2009).

At the same time, the washing-disinfection operation can reduce nutrients and bioactive compounds of fresh-cut vegetables. To this respect, Vandekinderen *et al.* (2008) reported vitamin E losses about 37.5% and 75.1% after washing-grated carrots with PAA solutions at 80 and 250 mg L^{-1}, respectively, for 5 min. However, no changes in carotenes and phenolic contents were reported in these conditions.

In a previous work carried out in our laboratory, the changes in bioactive compounds (total anthocyanins and ascorbic acid) of fresh-cut strawberries (*Selva* and *Camarosa* varieties) as a consequence of washing disinfection with PAA solutions at different concentrations (0–100 mg L^{-1}), contact times (10–120 s), and temperatures (4–40 °C) were modeled using second-order polynomial equations (Van de Velde *et al.*, 2013b). According to our results, anthocyanins and ascorbic acid contents were principally affected by PAA concentration and processing time, and for these responses, no differences between strawberry cultivars were observed. The obtained models showed to be a useful predictive tool for processors to evaluate how to improve the bioactive compounds retention under different conditions of PAA, temperature, and contact time in the washing disinfection of fresh-cut strawberries. For instance, working at 80 mg L^{-1} PAA (the limit concentration established by CFR), the predicted total anthocyanins and ascorbic acid retention (AAR) are 70% and 64%, respectively, at the maximum time (120 s) and 22 °C (Van de Velde *et al.*, 2013b).

The objectives of the present study were the following: (i) to model the microbial load reduction in fresh-cut strawberries under different PAA concentrations, times, and temperatures, (ii) to optimize the washing-disinfection operation based on bioactive compounds retention and microbial load reduction, (iii) to validate, with additional confirmatory experiments, the optimization results, and (iv) to evaluate the characteristic sensorial properties of fresh-cut strawberries washed in the optimized operative conditions.

Materials and methods

Plant material

Cultivated strawberries (*Fragaria* × *ananassa* Duch.) of *Camarosa* variety were obtained from one planting at Arroyo Leyes (31°27′0″S, 60°40′0″W), Santa Fe, (Argentina) during December 2010 and November 2011. Fruits were harvested by skilled workers at full ripe stage (90% of the surface showing red colour). They were transported 20 km directly from the field to the laboratory in Santa Fe. Physicochemical characteristics of strawberries were: 8.5 ± 0.1 °Brix (soluble solids), pH 3.4 ± 0.1, and total acidity: 0.8 ± 0.1 mg anhydrous citric acid 100 g^{-1} FW.

Minimal processing

Processing methodology was the same as that presented in Van de Velde *et al.* (2013b). Strawberries were sorted, eliminating those fruits with signs of damage. Calyxes and peduncles were removed, and then, fruits were prewashed with tap water (20 °C, 2 min) and drained on absorbent paper. They were cut longitudinally into quarts, and 200 g of fresh-cut strawberries were subjected to washing disinfection according to experimental design with respect to initial PAA concentration, time, and temperature on each experimental run. The washing solution volume to fruit weight ratio was 3 L per kg. Finally, washed fresh-cut strawberries were drained in a basket and subsamples (approximately 10 g) of each run were transferred into sterile bags for microbiological analysis. Samples (10 g) of fresh-cut strawberries previous to washing disinfection were separated for microbiological analysis.

Peracetic acid solutions

A commercial sanitizer based on peracetic acid called Oxilac Plus (Indaquim S.A. Santa Fe, Argentina) was used in the washing-disinfection operation. Oxilac Plus is a stabilized mixture of peracetic acid (minimum 5%), hydrogen peroxide (minimum 20%), and water. Tap water was employed to prepare different PAA concentrations by appropriate dilutions of Oxilac Plus according to the experimental design. The concentration of PAA was verified by iodometric titration according to APHA-AWWA-WEF (1989). The pH values of the PAA solutions used were the following: 4.35 (20 mg L^{-1}); 4.15 (50 mg L^{-1}); and 3.80 (100 mg L^{-1}).

Experimental design

Response surface methodology using a Box–Behnken design (three factors at three levels in fifteen runs with two replicates in the central point) was used to study the operation of washing disinfection. It was assumed that there was a mathematical function for each response according to three independent factors related to processing:

$$Y = f\,(C, T, t)$$

where C = PAA initial concentration (mg L^{-1}), T = temperature of the washing-disinfection solution (°C) and t = time of treatment (s) and the three levels were as follows: C = 0, 50, and 100 mg L^{-1}, T = 4, 22 and 40 °C and t = 10, 65, and 120 s. The variables and levels were selected from previous research works as it was explained in Van de Velde *et al.* (2013b).

The studied response (Y) was the total microbial count reduction (TMR), and it was expressed as $-\log_{10} N_t/N_0$, where N_t represents the viable bacteria count after washing disinfection of fresh-cut strawberries and N_0 is the viable bacteria count of fresh-cut fruits before washing disinfection.

A second-order polynomial equation was proposed for TMR:

$$\begin{aligned}\mathrm{TMR} = {} & \beta_0 + \beta_C \times C + \beta_T \times T + \beta_t \times t + \beta_{CC} \times C^2 \\ & + \beta_{TT} \times T^2 + \beta_{tt} \times t^2 + \beta_{CT} \times C \times T \\ & + \beta_{Tt} \times T \times t + \beta_{tc} \times t \times C\end{aligned}$$

where: β_k are the coefficients of the model in non coded variables.

Microbiological analysis

Sample (10 g) was mixed with 90 mL 0.1 g 100 mL^{-1} peptone water and homogenized with a stomacher for 2 min. Further decimal dilutions, as required, were prepared with the same diluents. One mL was spread on a 3M™ Petrifilm™ aerobic plate count and incubated during 48 h at 30 °C. The followed procedure was that developed and outlined by 3M (St. Paul, MN, USA) and was performed in duplicate. Results were expressed as colony-forming units per gram (CFU g^{-1}).

Optimization procedure of the washing-disinfection operation

Derringer's desirability function was used for multiple response optimization of fresh-cut strawberries washing disinfection, according to Derringer & Suich (1980). The involved responses (Y_k) were the following: AAR, total anthocyanins retention (TAR), and TMR. AAR and TAR models of fresh-cut strawberries after washing disinfection were previously discussed and published by authors (Van de Velde *et al.*, 2013b). The method involves transformation of each predicted response, $\hat{Y}_k$ (AAR, TAR, and TMR), to a dimensionless partial desirability function, *di*. The *di*-values for each response (d_{AAR}, d_{TAR}, and d_{TMR}) are obtained by specifying the goals, that is, 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:

$$D = \left[d_{\mathrm{AAR}}^{p1} \times d_{\mathrm{TAR}}^{p2} \times d_{\mathrm{TMR}}^{p3}\right]^{1/3}$$

where p_1, p_2, *and* p_3 *are* the assumed relative importance of responses. They can range from 1 (least important) to 5 (most important). (Prakash Maran and Manikandan, 2012).

A weight factor, which defines the shape of the desirability function for each response, is then assigned. Weights must be between 0.1 and 10, with larger weights corresponding to more important responses. A weight factor of 1 was chosen for all individual desirabilities in this work. 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 optimum (Sivakumar *et al.*, 2007; Prakash Maran & Manikandan, 2012).

Confirmatory experiments at conditions obtained in optimization procedure

Methodology

Experiments were carried out to validate the models at PAA concentration, time, and temperature given by the optimization procedure. Samples of fresh-cut strawberries were prepared in the same way as it was previously described (Minimal processing). The AAR, TAR, and TMR experimental data were compared to values of these responses predicted by the models. Additionally, a sensory analysis of these samples was carried out by trained panel, and a preliminary study to evaluate the microbial load and bioactive compounds evolutions of fresh-cut strawberries washed both at OP1 and OP2 conditions when storing at 2 °C was achieved.

Microbiological analysis

The microbial count was determined as it was described under Materials and methods.

Total anthocyanins analysis

The total anthocyanins content was determined by the pH differential method according to Heo & Lee (2005). Results were converted to mg pelargonidin-3-glucoside 100 g^{-1} FW.

Ascorbic acid analysis

The ascorbic acid content was determined by HPLC according to Van de Velde *et al.* (2012) Results were expressed as mg ascorbic acid 100 g^{-1} FW.

Sensory analysis

The evaluation of the characteristic sensorial properties of fresh-cut strawberries washed at optimized conditions was performed by a descriptive quantitative test. An additional fresh-cut strawberry sample (control), only washed with water for 2 min, was also evaluated. A trained sensory panel of eight judges (three men and five women), which had previously participated in evaluating fresh fruits and vegetables, was used. During the specific training (5 × 30 min sessions), the panelists discussed and agreed on sensory attributes (general appearance, genuine odor, genuine aroma, genuine flavor, firmness, sour taste, and sweet taste), defects (off-flavor, off-odor, and browning), and anchored terms. The anchored terms were indicated from left to right as: very poor and excellent for general appearance; slight and strong for genuine odor, aroma and flavor; weak and strong for sour and sweet taste; very soft and very firm for firmness; and slight and severe for off-flavor, off-odor, and browning. The judges indicated their perception of each quality attribute intensity on a 10 cm unstructured line, with anchored terms located 1 cm from each end. They scored the perceived intensity of each attribute by placing a vertical line across the unstructured scale line. Quantification was accomplished by measuring the distance from the left end (0.00) to the vertical line. Each panelist performed the sensory test individually in separate booths with white incandescent lightning (sufficient to provide 700 lx.). Samples were coded with three-digit random numbers.

Statistical analysis

All data were analyzed using STATGRAPHICS Centurion XV 15.2.06 (Statpoint Technologies, Inc., Warrenton, Virginia, USA). First, tests to verify that the residuals satisfied the assumptions of normality, independence, and randomness were done for TMR. Then, TMR experimental data were subjected to ANOVA analysis, and second-order polynomial equation was fitted. For verification of the model adequacy, the lack of fit, the coefficient of determination (R^2), and the absolute average deviation (AAD), between predicted and observed data, were calculated. R^2 should be as close as possible to 1.0, and AAD value should be as small as possible (Baş & Boyaci, 2007). The significance of each term of the TMR model was evaluated referred to the pure error. The elimination of nonsignificant terms of the model was done by means of the linear stepwise regression procedure.

The numerical optimization procedure was performed through Derringer's desirability function.

Additionally, experimental data responses (AAR, TAR, and TMR) obtained in each confirmatory experiment were compared to values predicted from the developed models by a *t*-test analysis.

Moreover, sensory data, obtained in the confirmatory experiments, were analyzed by a one-way ANOVA. Significant differences were determined by LSD test.

Results and discussion

Effects of washing disinfection on the microbial reduction

The initial total microbial count of fresh-cut *Camarosa* strawberries before washing disinfection varied from 3.7 to 3.9 log CFU g^{-1}. The TMR experimental results at design conditions of PAA concentration (0–100 mg L^{-1}), time (0–120 s) and temperature (4–40 °C) of fresh-cut *Camarosa* strawberries and ANOVA analysis for TMR model are presented in Tables S1 and S2, respectively. The obtained model for TMR described the experimental data adequately. The lack of fit was not significant ($P > 0.05$), and the coefficients of determination (R^2) and the AAD were acceptable (Table S2). The coefficients of the reduced model by eliminating nonsignificant terms are shown in Table S3. The recalculated R^2 and AAD for the model were acceptable.

Total microbial count reduction model was affected by initial PAA concentration and washing temperature through the lineal terms, and by time through its quadratic term ($P \leq 0.05$). Figure S1 shows that higher TMR was obtained as PAA concentration and time increased at 22 °C. The predicted highest TMR using the reduced model was 2.6 log UFC g^{-1} at maximum washing-disinfection conditions: 100 mg L^{-1} PAA, 40 °C and 120 s.

According to other researchers, the effectiveness of PAA treatment was dependent on the type of the vegetable product. Kim *et al.* (2006) studied the effectiveness of 40 and 80 mg L^{-1} PAA solutions for 1 and 5 min in killing *Enterobacter sakazakii* inoculated onto the surface of apples, tomatoes, and lettuce. Authors reported that these treatments caused ≥4.00 log CFU/apple reduction in *E. sakazakii*, regardless of treatment time. The treatment of tomatoes with 40 mg L^{-1} PAA solution for 1 min decreased the number of *E. sakazakii* to <1.70 log UFC/tomato and in the case of lettuce, the reduction in that condition was 2.45 log UFC/lettuce.

Meanwhile, Vandekinderen *et al.* (2009) modeled the washing disinfection with PAA in four types of fresh-cut vegetables (grated carrots, white cabbage, iceberg lettuce, and leek), varying the PAA concentration (0, 25, 80, 150, and 250 mg L^{-1}) and contact times (1, 5, and 10 min). The predicted microbial reductions were the following: 0.5–3.5 log CFU g^{-1} for carrots and white cabbage, 0.4–2.4 log CFU g^{-1} for iceberg lettuce, and 0.4–1.4 log CFU g^{-1} for leek. Authors reported that the models describing the microbial reductions in carrots and white cabbage as a function of contact time and PAA concentration showed a similar evolution: at PAA concentrations <75 mg L^{-1}, the increase in the treatment time (>4 min) had a slight effect on the microbial reduction. At higher PAA concentrations (>100 mg L^{-1}), the increase in contact time had a pronounced effect on the obtained microbial reduction. Probably, PAA instantly reacts with organic materials released from the vegetable tissues after minimal processing at the beginning of the washing treatment. Because of the reduction on the available disinfectant concentration at low PAA concentration, the treatment time may have smaller effect on the microbial inactivation (Vandekinderen *et al.*, 2009). Our predictions were in agreement with these latter results. Predicted TMR at 75 mg L^{-1} PAA concentration and 22 °C varied between 1.4 and 2.1 log UFC g^{-1} when the washing time ranged between 10 and 65 s. Meanwhile, these predicted reductions varied between 2.1 and 2.2 log UFC g^{-1} when the washing time ranged between 65 and 120 s. Therefore, the influence of the washing time on the microbial reduction would be less pronounced after 65 s at 75 mg L^{-1} PAA concentration, which is in agreement with the studies of Vandekinderen *et al.* (2009).

However, other issues should be probably taken into account as Sapers (2001) has pointed out. His investigation stated that other factors could limit the efficacy of conventional washing and sanitizing treatments in fresh-cut fruits and vegetables such as bacterial adherence to produce surfaces, bacterial attachment in inaccessible sites, formation of resistant biofilms, and internalization of micro-organisms within commodities.

Washing-disinfection optimization

A multiple response optimization of the fresh-cut strawberries washing-disinfection operation with PAA was conducted using the Derringer's desirability function. For the optimization procedure, the chosen responses were TMR, TAR, and AAR. The selection was made on the basis that anthocyanins are the main phenolic compounds in strawberries and they are related to the appealing red colour of the berries. Moreover, ascorbic acid was selected because it is a powerful natural antioxidant, with nutritional relevance, found at high levels in strawberries (Tarola *et al.* 2013).

The coefficients of the developed models for TAR and AAR and their associated parameters of goodness of fit are summarized in Table S3.

The development of two optimization scenarios (OP 1 and OP 2) allowed finding conditions of the variables which satisfied the different assumptions. The criteria for the optimization of responses at OP 1 and OP 2 are shown in Table S4. OP 1 criteria have been proposed for selecting the conditions when the microbial reduction was maximized, whereas the bioactive compounds retention was fixed at 90%. OP 2 criteria were applied for selecting conditions where bioactive compounds retention was maximized with an acceptable microbiological reduction target (2 log UFC g^{-1}).

The global desirability function value in OP 1 was equal to 0.72, and the obtained operative conditions were the following: 100 mg L^{-1} PAA concentration, 50 s washing time, and 24 °C washing temperature. In the same way, the global desirability function value in OP 2 was equal to 0.74, and the obtained operative conditions were the following: 20 mg L^{-1} PAA concentration, 52 s washing time, and 18 °C washing temperature.

Figure S2a,b shows the graphical representation of the maximum global desirability function reached at OP 1 and OP 2. The best compromise was obtained at the top of the graphics.

As it can be seen, the global desirability function values for both optimization scenarios were almost similar and the higher PAA concentration and washing temperature value obtained in OP 1 were in agreement with the proposed optimization criterion of selecting the conditions when the microbial reduction was maximized.

Confirmatory experiments at conditions obtained in optimization

Fresh-cut strawberries washing-disinfection experiments were carried out at OP 1 and OP 2 conditions. The studied responses (TMR, TAR, and AAR) were determined on each washed sample. Table S5 shows predicted and experimental mean values at both optimized conditions. Experimental confirmatory results showed adequate agreement ($P > 0.05$) with predicted ones. As it can be seen, there was only approximately one log reduction on TMR results between OP 1 and OP 2 conditions. However, there was significant higher bioactive compounds retention at OP 2 scenario. As PAA is more expensive than chorine, the OP 2 scenario, with lower PAA concentration (20 mg L^{-1} PAA), could be more economically convenient for processors.

A preliminary study to evaluate the microbial load and bioactive compounds evolutions of fresh-cut strawberries washed both at OP1 and OP2 conditions when storing at 2 °C was carried out. According to results, fresh-cut strawberries washed at OP1 conditions (100 mg L^{-1} PAA, 24 °C and 50 s) and stored at 2 °C showed an increase in the microbial load from 2.1 log CFU g^{-1} on day 0 to 4 log CFU g^{-1} on day 15. In the other hand, fresh-cut strawberries washed at OP2 conditions (20 mg L^{-1} PAA, 18 °C and 52 s) showed an increase in the microbial load from 3.1 log CFU g^{-1} on day 0 to 5.8 log CFU g^{-1} on day 15 at 2 °C. The microbiological limit at the end of the shelf life, which separates good quality from marginally acceptable quality of fresh-cut product, has been set at 6 log CFU g^{-1} for total aerobic count mesophiles by legislation of Spain (IFPA, 2003). As it can be seen, results obtained at both optimization scenarios (OP1 and OP2) satisfy this criterion until day 15. The ascorbic acid content of fresh-cut strawberries sanitized at OP1 and OP2 conditions showed no changes with storage time at 2 °C. However, anthocyanin content after 15 days at 2 °C showed a similar reduction for both scenarios (approximately 25%). Further investigation is necessary to evaluate the microbial load, bioactive compounds, and other quality attributes evolution with storage time at different temperatures.

A sensorial analysis using a trained panel was performed with OP 1, OP 2, and control samples of fresh-cut strawberries. The mean score of sensory quality attributes (general appearance, genuine odor, genuine aroma, genuine flavor, firmness, sour taste, and sweet taste) and defects (off-flavor, off-odor and browning) for the three samples (OP 1, OP 2, and control) are presented in Table S6. These samples did not differ ($P > 0.05$) in general appearance, firmness, genuine aroma, genuine flavor, and sour and sweet taste.

The genuine odor was better in the control strawberries, not being detected differences in this sensorial attribute between strawberries washed in OP 1 and OP 2 conditions.

Panelists reported slight off-flavor in strawberries washed in OP 1 conditions. Moreover, this sensorial defect in strawberries washed in OP 2 (20 mg L^{-1} PAA, 18 °C, 52 s) condition was not significantly different from control strawberries (Table S6). Furthermore, slight higher off-odor defect was detected in OP 1 strawberries compared to OP 2 and control strawberries.

Therefore, the higher PAA concentration in OP 1 (100 mg L^{-1}) caused the off-odor and off-flavor occurrences of the washed strawberries in this condition.

Conclusions

The TMR model, developed herein, could be used to evaluate the microbial count reduction under specific PAA concentration, temperature, and time after washing disinfection of fresh-cut strawberries. The multiple (TMR, AAR, and TAR) response optimization through the Derringer's desirability function allowed finding conditions of washing disinfection in two scenarios (OP 1 and OP 2). When the objectives were to maximize the total microbial reduction with a target of 90% TAR and AAR retention, the predicted operative variable levels were the following: 100 mg L^{-1} PAA, 24 °C and 50 s (OP 1). On the other hand, when the objectives were to maximize TAR and AAR retention with 2 log UFC g^{-1} microbial reduction, the obtained operative variable levels were the following: 20 mg L^{-1} PAA, 18 °C and 52 s (OP 2). Additional experiments, done in both scenarios, showed good agreement within experimental and predicted responses. Washed fresh-cut strawberries at OP 2 conditions showed 1,4 log UFC g^{-1} microbial count reduction, 88,4% TAR and 95,2% AAR, and neither off-odor nor off-flavor occurrence. Based on latter results and the economic convenience of lesser PAA consumption, OP 2 conditions are recommended.

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References

APHA-AWWA-WEF (1989). *Standard Methods for the Examination of Water and Wastewater*, 20th edn. Washington D.C.: American Public Health Association.

Artés, F., Gómez, P., Aguayo, E., Escalona, V. & Artés Hernández, F. (2009). Review: sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology*, **51**, 287–296.

Baş, D. & Boyaci, I.H. (2007). Modelling and optimization I: usability of response surface methodology. *Journal of Food Engineering*, **78**, 836–845.

Code of Federal Regulations [CFR] (2007). Online reference included in Secondary Direct Food Additives Permitted in Food for Human Consumption: Chemicals used in washing or to assist in the peeling of fruits and vegetables. Chlorine dioxide. Available in http://frwebgate3.access.gpo.gov/cgi-bin. Acceded on November 20, 2012.

Cordenunsi, B.R., Genovese, M.I., Oliveira do Nascimento, J.R., Hassimotto, N.M.A., dos Santos, R.J. & Lajolo, F.M. (2005). Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. *Food Chemistry*, **91**, 113–121.

da Silva Pinto, M., Lajolo, F. & Genovese, M. (2008). Bioactive compounds and quantification of ellagic acid in strawberries (*Fragaria × ananassa* Duch.). *Food Chemistry*., **107**, 1629–1635.

Davey, M.W., Van Montagu, M., Inzé, D. *et al.* (2000). Review: plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture*, **80**, 825–860.

Derringer, G. & Suich, J. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology*, **12**, 214–219.

Giampieri, F., Tulipani, S., Alvarez-Suarez, J.M., Quiles, J.L., Mezzetti, B. & Battino, M. (2012). The strawberry: composition, nutritional quality, and impact on human health. *Nutrition*, **28**, 9–19.

Gorny, J.R. (2001). *Food Safety for the Fresh-cut Produce Industry*, 4th edn. Pp. 107–120. Arlington, VA: International Fresh-cut Produce Association.

Heo, J.H. & Lee, C.J. (2005). Strawberry and its anthocyanins reduce oxidative stress-induced apoptosis in PC12 cells. *Journal of Agricultural and Food Chemistry*, **53**, 1984–1989.

IFPA International Fresh-cut Produce Association, (2003). *Food Safety Guidelines for the Fresh-cut Produce Industry* (edited by M.I. Gil & J.R. Gorny). Pp. 192–197. Alexandria, VA: International Fresh-cut Produce Association.

Kim, H., Tyu, J.H. & Beuchat, L.R. (2006). Survival of *Enterobacter sakazakii* on fresh produce as affected by temperature, and effectiveness of sanitizers for its elimination. *International Journal of Food Microbiology*, **111**, 134–143.

Kitis, M. (2004). Disinfection of wastewater with peracetic acid: a review. *Environmental International*, **30**, 47–55.

Malchesky, P.S. (1993). Peracetic acid and its application to medical instrument sterilization. *The International Journal of Artificial Organs*, **17**, 147–152.

Ölmez, H. & Kretzschmar, U. (2009). Review: potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT-Food and Science Technology*, **42**, 686–693.

Pirovani, M., Piagentini, A.M., Güemes, D.R. & Arkwright, S. (2004). Reduction of chlorine concentration and microbial load during washing disinfection of shredded lettuce. *International Journal of Food Science and Technology*, **39**, 341–347.

Prakash Maran, J. & Manikandan, S. (2012). Response surface modelling and optimization of process parameters for aqueous extraction of pigments from prickly pear (*Opuntia ficus-indica*) fruit. *Dyes Pigments*., **95**, 465–472.

Sapers, G.M. (2001). Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products. *Food Technology and Biotechnology*, **39**, 305–311.

Silveira, A.C., Conesa, A., Aguayo, E. & Artés, F. (2008). Alternative sanitizers to chlorine for use on fresh-cut "Galia" (*Cucumis melo* var. *Catalupensis*) melon. *Journal of Food Science*, **73**, 405–411.

Sivakumar, T., Manavalan, R. & Valliappan, K. (2007). Global optimization using derringer's desirability function: enantioselective determination of ketoprofen in formulations and in biological matrices. *Acta Chromatographica*, **19**, 29–47.

Small, D.A., Chang, W., Toghrol, F. & Bently, W.E. (2007). Comparative global transcription analysis of sodium hypochlorite, peracetic acid, and hydrogen peroxide on *Pseudomonas aeruginosa*. *Applied Microbiology and Biotechnology*., **76**, 1093–1105.

Tarola, A.M., Van de Velde, F., Salvagni, L. & Preti, R. (2013). Determination of Phenolic Compounds in Strawberries (*Fragaria ananassa* Duch) by High Performance Liquid Chromatography with Diode Array Detection. *Food Analytical Methods*, **6**, 227–237.

Van de Velde, F., Pirovani, M.E., Cámara, M.S., Güemes, D. & del Bernardi, C.M.H. (2012). Optimization and validation of a UV–HPLC method for vitamin C determination in strawberries (*Fragaria ananassa* Duch.), using experimental designs. *Food Analytical Methods*, **5**, 1097–1104.

Van de Velde, F., Tarola, A., Güemes, D. & Pirovani, M. (2013a). Bioactive compounds and antioxidant capacity of *Camarosa* and *Selva* strawberries (*Fragaria* × *ananassa* Duch.). *Foods*, **2**, 120–131.

Van de Velde, F., Piagentini, A., Güemes, D.R. & Pirovani, M.E. (2013b). Modelling changes in anthocyanins, total vitamin C, and colour as a consequence of peracetic acid washing disinfection of two cultivars of strawberries for fresh-cut processing. *International Journal of Food Science and Technology*, **48**, 954–961.

Vandekinderen, I., Van Camp, J., Devlieghere, F. *et al*. (2008). Effect of decontamination agents on the microbial population, sensorial quality, and nutrient content of grated carrots (*Daucus carota* L.). *Journal of Agricultural and Food Chemistry*, **56**, 5723–5731.

Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Ragaert, P. & Van Camp, J. (2009). Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. *Food Microbiology*, **26**, 882–888.

Wagner, M., Brumelis, D. & Gehr, R. (2002). Disinfection of waste water by hydrogen peroxide or peracetic acid: development of procedures for measurements of residual disinfectants and application to a physicochemically treated municipal effluent. *Water Environment Research*, **74**, 33–50.

Williner, M.R., Pirovani, M.E. & Güemes, D.R. (2003). Ellagic acid content in strawberries of different cultivars and ripening stages. *Journal of the Science of Food and Agriculture*, **83**, 842–845.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Effect of time and peracetic acid (PAA) concentration on total microbial count reduction (TMR) of *Camarosa* freshcut strawberries at 22 °C.

Figure S2. Derringer's overall desirability obtained for OP 1 (a) and OP 2 (b) optimization of the responses (ascorbic acid retention, total anthocyanins retention, and total microbial count reduction).

Table S1. Experimental data for total microbial count reduction (TMR) on washed *Camarosa* fresh-cut strawberries at the Box–Behnken design conditions.

Table S2. Analyses of variance of the total microbial count reduction quadratic model (TMR model) of washed *Camarosa* fresh-cut strawberries.

Table S3. Coefficients of the model terms, coefficients of determination (R^2), and absolute average deviations (AAD) on the microbial count reduction (TMR), total anthocyanins (TAR), and ascorbic acid retention (AAR) models of washed fresh-cut *Camarosa* strawberries.

Table S4. Optimization criteria based on total microbial count reduction (TMR, $-\log N_t/N_0$), total anthocyanins retention (TAR,%), and ascorbic acid retention (AAR,%) after the washing disinfection with peracetic acid of *Camarosa* fresh-cut strawberries

Table S5. Comparison between predicted responses and experimental results of washing disinfection with peracetic acid of *Camarosa* fresh-cut strawberries under conditions that satisfied OP 1 and OP 2 optimization criteria.

Table S6. Sensory analysis of fresh-cut strawberries washed in OP 1 and OP 2 conditions and washed with tap water for 2 min (control).