

Effects of Several Sanitisers for Improving Quality Attributes of Minimally Processed *Fragaria vesca* Strawberry

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Abstract

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In order to decrease microbial contamination, fresh-cut industry commonly uses sodium hypochlorite as a disinfection agent, however, the by-products such as trihalometanes and chloramines are potentially harmful for human health making necessary the search for alternative disinfectant agents. A comparative study on the effectiveness of different disinfection methods on the quality of minimally processed *F. vesca* strawberry is presented. The fruit was processed in a clean room through the following steps: reception, cutting, washing, draining, and packaging. The processed strawberries were packaged in thermally sealed polypropylene trays using passive modified atmosphere. During a storage period of 8 days at 4°C, the quality parameters, sensory attributes, and microbial counts were determined. As conclusion, the use of lactic acid at a concentration of 2.5 g/l in the washing water was effective in reducing microbial counts, maintaining the sensory attributes and quality of the product during the storage. The present study demonstrates that the use of lactic acid in the washing water could be a good alternative of the use of sodium hypochlorite and suggests that strawberries could make an acceptable fresh-cut product.

Keywords: fresh cut; lactic acid; microbial counts; sodium hypochlorite

Ready to eat fresh fruits have become an important area of potential growth in the fast expanding produce industry (CORBO *et al.* 2000), presumably due, in part, to their characteristics of freshness, low caloric content, and commodity to be used. Nevertheless, it is well known that minimal processing alters the integrity of the fruit and induces surface damage increasing lightly the tissue respiration and leading to biochemical deterioration such as browning, off-flavour development, and texture breakdown decreasing the fresh-cut fruit quality (MARTÍN-BELLOSO *et al.* 2006).

The increasing public health concern related to the microbial safety of fruit has resulted in a considerable

amount of studies that analyse the efficiency of different methods for reducing the microbial load of fresh products (PARISH *et al.* 2003). Thus, the success of the washing depends on different factors such as the target microorganisms, characteristics of the product surfaces, type of washing, exposure time, dose, pH, temperature, etc. To examine the techniques, it is generally accepted that an ideal sanitising agent should have two important properties: a sufficient level of antimicrobial activity, and a negligible effect on the sensory quality of the product. Therefore, the sensory quality of the product should be also evaluated when selecting the optimal sanitising technique (MARTÍNEZ-SÁNCHEZ *et al.* 2006).

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Fresh-cut industry commonly uses sodium hypochlorite as the disinfection agent, however, the by-products such as trihalometanes and chloramines are potentially harmful for human healthy making necessary the search for alternative disinfectant agents. Organic acids, mainly citric, lactic, and acetic acids, which are in GRAS (Generally Recognised As Safe) status, have been investigated because of their bactericidal activity (ÖLMEZ & KRETZSCHMAR 2009).

Strawberries that are one of the most popular fruits worldwide are rich in nutrients but also highly perishable, being susceptible to mechanical injury, desiccation, decay, and physiological disorders during storage. The shelf life of fresh strawberries in the cold is usually less than 5 days; this time is reduced when the product is minimally processed. Improvements in the shelf-life can be achieved by using good quality raw products, giving special care during processing and along the trade chain, controlling the temperature, and using modified atmosphere packaging (NGUYEN-THE & CARLIN 1994).

The main purpose of this work was to determine how the use of different sanitisers in the washing water affects the microbial growth, sensory characteristics, and quality of minimally processed *F. vesca* strawberry during a storage period of 8 days at 4°C.

MATERIAL AND METHODS

Plant material. Strawberries were provided by Asociación para el Desarrollo del Sistema Productivo Vinculado a la Agricultura Onubense (ADESVA) (Huelva, Spain) in the season of 2010. The temperature in the clean room was 8°C. The processing steps were: (i) reception: strawberries were carefully selected for uniform size and colour as well as the absence of damage and defects; (ii) cutting: the calyx was cut off using special knives designed for fruit; (iii) washing: 5.0 kg of strawberries were washed during 90 s in 25 l of solution, applying the following treatments: FH1 (water), FH2 (5.0 g/l calcium lactate), FH3 (100 mg/l sodium hypochlorite (10% v/v) adjusted to pH 6.5), FH4 (5.0 g/l ascorbic acid), FH5 (2.5 g/l lactic acid), FH6 (5.0 g/l ascorbic acid + 5.0 g/l lactic acid). At the end of the treatment, the samples were immediately rinsed with tap water; (iv) draining: strawberries were drained with cold air using an industrial drying tunnel system by hot/cold

air, Model Domino Junior Laboratorio (Turatti, Cavarzere, Italy); (v) packaging: 150.0 g of strawberries were placed in a polypropylene (PP) tray and thermally sealed with a PP film (50 mm thickness) in order to generate a passive modified atmosphere packaging (MAP). An industrial packaging, Model Verpackungs-Systeme (Western, Germany) was used; (vi) storage: the packaged samples were stored at 4°C under refrigeration for up to 8 days.

Soluble solids (TSS), titratable acidity (TA) and pH. TSS, TA and pH were measured in each independent homogenate, obtained from fresh fruit. TSS were measured by refractometry using an RE40 refractometer (Mettler Toledo, S.A.E., Coslada, Spain); the results were expressed as °Brix. The analyses of TA and pH were conducted using a DL50 Graphix automatic titrator (Mettler Toledo, S.A.E., Coslada, Spain). The results were expressed as g malic acid/100 g fresh weight.

Colour. Flesh colour was measured using a CR-200 tristimulus colorimeter (Minolta, Tokyo, Japan), with an 8 mm diameter viewing area and using illuminant D65. Chromatic analyses were conducted in accordance with the CIE (Commission Internationale de l'Éclairage) system of 1976. The values of L^* , a^* , and b^* were used to define a three-dimensional colour space (VOSS 1992; HUTCHINGS 1994). The hue angle (h^*) was calculated as $\arctg(b^*/a^*)$.

Firmness determination. Firmness was measured using a Texture Analyzer TA-XT2i (Stable Micro Systems, Aname, Spain) by means of a compression assay on the whole strawberry. The force was applied to produce a 2% deformation by a 100 mm aluminium plate. Force/deformation curves were recorded and the maximum force (N) was calculated. The mean values represent the average of 10 strawberries.

Head space gas composition. O₂ and CO₂ contents of all trays were measured using an O₂ and CO₂ meter PAK 12P (Control y Suministros S.A., Barcelona, Spain). Each tray was used for a single determination only.

Microbiological analyses. The following methods according to ISO 4833:1991, ISO 7954:1988, and ISO 7402:1993 were used and the results were expressed as log CFU/g:

Aerobic Colony Counts (mesophilic). 10 g of fruit was dissolved in 90 ml of 0.1% sterile peptone water (Universalpepton M66; Merck, Darmstadt, Germany); 1 ml of the solution was poured into each of two Petri plates and 10–15 ml of the plate count agar (Merck), prepared as directed by the manufacturer, was then added. After solidification, the

plates were incubated at 30°C for 72 h, and the bacterial colonies were then recorded.

Psychrotrophic. 1 ml of the solution used in the aerobic colony count determination was poured into each of two Petri plates and 10–15 ml of the plate count agar (Merck, Darmstadt, Germany), prepared as directed by the manufacturer, was then added. After solidification, the plates were incubated at 16°C for 5 days, and then bacterial colonies were recorded.

Yeast and moulds. A 1 ml aliquot of the solution used in the mesophilic determination was poured into each of two Petri plates and 10–15 ml of Glucose Chloramphenicol Agar (Merck, Darmstadt, Germany), prepared as directed by the manufacturer, was then added. After solidification, the plates were incubated aerobically at 25°C for 5 days. The colonies were recorded and identified.

Enterobacteriaceae. 1 ml of the solution used in the aerobic colony count determination was poured into each of two Petri plates, and 8–10 ml of violet red bile agar (Merck, Darmstadt, Germany), prepared as directed by the manufacturer, was then added. After solidification, a second layer of the culture media (8–10 ml) was added. After solidification, plates were incubated at 37°C for 48 h, and Enterobacteriaceae colonies were then recorded.

Sensory evaluation. Sensory quality 10.5 Statistical analysis. For statistical studies, SPSS 18.0 software was used (SPSS Inc., Chicago, USA). Correlations were estimated with the Pearson test at $P < 0.05$ significance level. The data were expressed as means \pm SD and were analysed using a one-way analysis of variance (ANOVA). When ANOVA detected significant differences between the mean values, the means were compared using Tukey's test.

RESULTS AND DISCUSSION

Total soluble solids, titratable acidity, and pH

The results for pH and TSS are presented in Figure 1. No significant differences occurred in TSS and titratable acidity during the storage days. pH of strawberries was very low (about 3.5) and during the storage did not change significantly ($P > 0.05$), within the limits 3 to 5 that promote the processes of copigmentation (BROUILLARD *et al.* 1991). The values for TSS and acidity found in this study were similar to other ones given for this fruit (CAMPANIELLO *et al.* 2008).

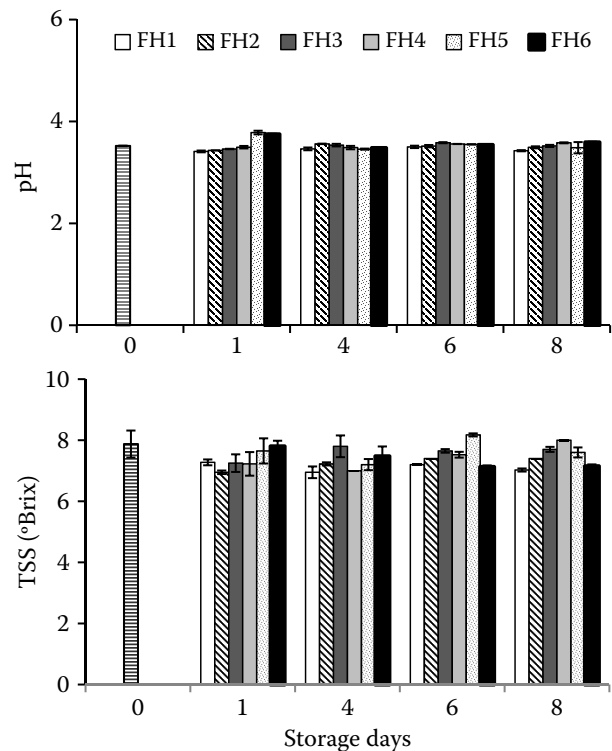


Figure 1. Evolution of pH and total soluble solids (TSS) for fresh cut strawberries throughout the storage at 4°C using different washing treatments: FH1 (water), FH2 (5.0 g/l calcium lactate), FH3 (100 mg/l sodium hypochlorite (10% v/v) adjusted to pH 6.5), FH4 (5.0 g/l ascorbic acid), FH5 (2.5 g/l lactic acid), FH6 (5.0 g/l ascorbic acid + 5.0 g/l lactic acid). Vertical bars represent standard deviation of the means ($n = 4$)

Colour

The values of L^* and h^* parameters offer the evidence of deterioration or browning of the cut zone of the product and determine the acceptance or rejection of the product. In our study, L^* and h^* showed no significant differences at the end of the storage days with all the treatments (data not shown), so we can conclude that the pigment conservation was good. A similar behaviour for minimally processed strawberries treated with chitosan as preservative was found by CAMPANIELLO *et al.* (2008).

Firmness determination

The values found (1.50 N approximately) were practically constant during storage (data not shown), however, a slight decrease occurred from 4th day due

to that the minimal processing alters the integrity of the fruit and induces surface damage leading to biochemical deterioration such as the texture breakdown (MARTÍN-BELLOSO *et al.* 2006). At the end of the storage, the firmness values obtained for all treatments were not significantly different.

Head space gas composition

A decrease in the headspace oxygen concentration along with an increase in the headspace carbon dioxide concentration was detected. The package headspace was monitored over 8 days of storage (data not shown). The O₂ and CO₂ changes were natural consequences of the progress of respiratory activity and the gas diffusion across the film. Starting from the atmospheric gas concentration, the levels of 18–19% O₂ and 3–4% CO₂ within the packages were established after 5 days and lasted until the end of the storage. The values found for O₂ and CO₂ levels imply that the PP film could be adequate for passive modified atmosphere packaging.

Microbiological assessment

The specifications proposed by Regulation E.C. (No. 2073/2005) were used to determine the end of the shelf-life from the microbiological point of view, set at 6 log CFU/g for total aerobic count mesophiles.

The effects of the different sanitising treatments on microbial growth are presented in Figure 2. In all treatments, microbial growth increased from 4th day of storage, except for the Enterobacteriaceae growth. However, some treatments reduced the microbial count more than others. Initial psychrotrophic populations (5.1 ± 0.2 log CFU/g) significantly ($P < 0.05$) decreased after washing with all the tested aqueous solutions, although the efficacy varied depending on the washing solution type. The highest psychrotrophic reduction was observed after washing fresh-cut strawberry with calcium lactate (FH2), but on days 4 and 8 of storage, lactic acid (FH5) showed a reduction of 4.5 and 2.0 log CFU/g respectively, compared with the harvest samples (without treatment).

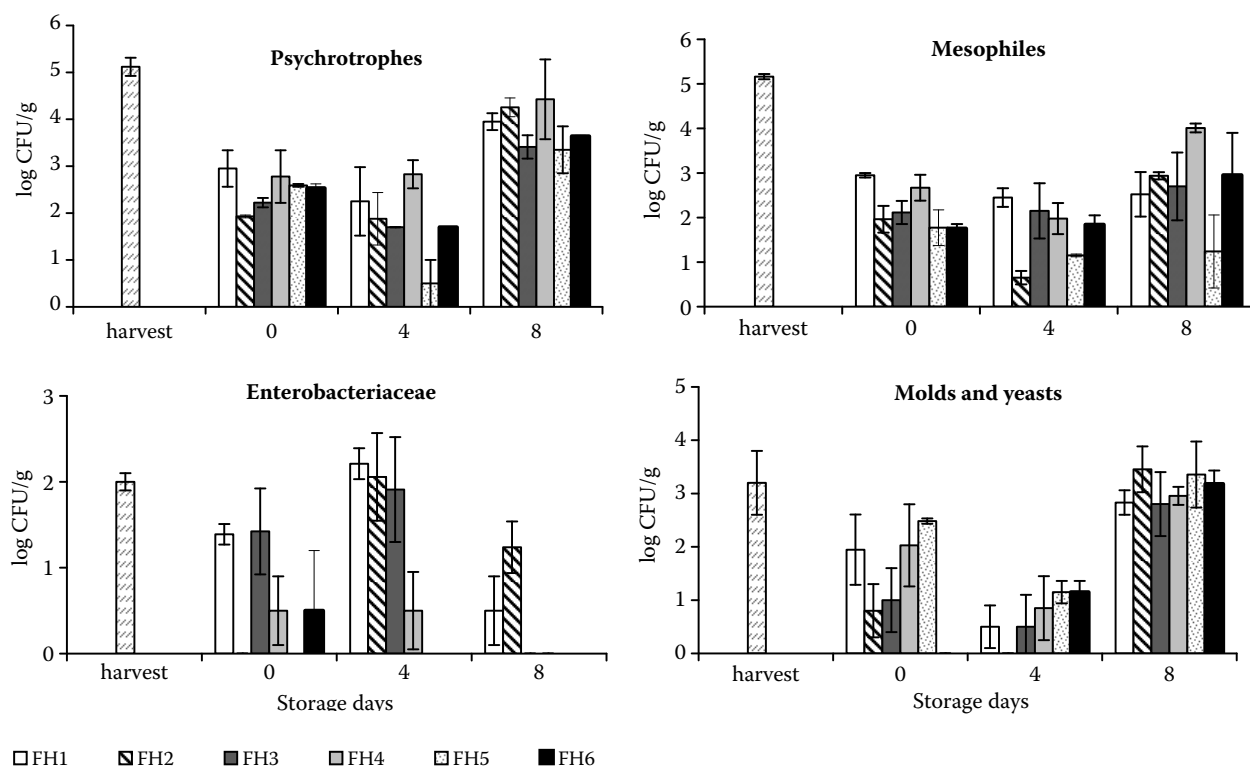


Figure 2. Effects of sanitising treatment [FH1 (water), FH2 (5.0 g/l calcium lactate), FH3 (100 mg/l sodium hypochlorite (10% v/v) adjusted to pH 6.5), FH4 (5.0 g/l ascorbic acid), FH5 (2.5 g/l lactic acid), FH6 (5.0 g/l ascorbic acid + 5.0 g/l lactic acid)] on microbial growth from fresh-cut strawberries stored in passive modified atmosphere packing at 4°C during 8 days. Vertical bars represent standard deviation of the means ($n = 3$)

Table 1. Sensory quality of minimally processed *Fragaria vesca* strawberries stored at 4°C after 8 days using different washing treatments: FH1 (water), FH2 (5.0 g/l calcium lactate), FH3 (100 mg/l sodium hypochlorite (10% v/v) adjusted to pH 6.5), FH4 (5.0 g/l ascorbic acid), FH5 (2.5 g/l lactic acid), FH6 (5.0 g/l ascorbic acid + 5.0 g/l lactic acid)

Washing treatments	Visual quality	Flavour	Browning	Firmness
FH1	3.74 ± 0.81	1.75 ± 0.98	3.18 ± 0.50	2.01 ± 0.58
FH2	5.55 ± 0.84	2.87 ± 0.81	2.07 ± 1.05	4.23 ± 0.95
FH3	5.94 ± 0.81	3.25 ± 0.98	1.94 ± 0.50	3.81 ± 0.58
FH4	5.74 ± 0.81	3.20 ± 0.98	1.84 ± 0.50	4.01 ± 0.58
FH5	6.74 ± 0.81	3.25 ± 0.98	2.04 ± 0.50	4.05 ± 0.58
FH6	6.04 ± 0.81	3.15 ± 0.98	1.99 ± 0.50	3.99 ± 0.58

Values are the mean of eight replicates ± standard deviation

For the mesophilic growth, after 8 days of storage treatment FH5 (lactic acid) reduced the mesophilic growth by 1.5 log CFU/g compared with FH1 (water) and 4.0 log CFU/g compared with the harvest samples. The rest of treatments showed increases as compared with FH1.

On day 0, Enterobacteriaceae counts of fresh-cut strawberry washed with FH5 were smaller than 0.5 log CFU/g. This initial reduction provided a lower enterobacteriaceae load on days 4 and 8. The rest of treatments showed similar reductions. Consistent molds and yeasts levels between 2.5 and 3.5 log CFU/g were found in all treatments at the end of storage.

Higher values of psychrotrophic bacteria were found by CAMPANIELLO *et al.* (2008) in control samples of minimally processed strawberries. Similar reductions in the microbial counts were found by SILVEIRA *et al.* (2010) studying emerging sanitisers on fresh-cut melon cv. Galia.

The treatment FH5 (2.5 g/l lactic acid) was selected as the best sanitiser in washing water according to the results obtained.

Effect of washing treatment on sensory quality

The results obtained are shown in Table 1. The visual quality of minimally processed strawberries was excellent after washing and decreased slightly during storage. The same behaviour was found for the promotion of browning in the cutting area. These samples maintained the typical flavour during storage. No off-odour was detected in the washed samples at any storage time, without evidence for anaerobic fermentation after 8 days

of storage at 4°C. The firmness of strawberries slightly decreased during storage but on day 8, all samples maintained a moderate crispy texture without significant differences among them. Consequently, lactic acid in washing water did not affect the sensory quality of fresh-cut strawberries during storage. Similar results were found with strawberries treated with 1-MCP and CaCl (AGUAYO *et al.* 2006), where the panelists rejected the fruit visually from day 9 of storage.

CONCLUSIONS

The present study has demonstrated that lactic acid at a concentration of 2.5 g/l in the washing water was the most effective in reducing the microbial counts, maintaining the sensory characteristics and quality of minimally processed *F. vesca* strawberry during the storage, and thus it could be a good alternative to sodium hypochlorite. The results suggest that strawberries can make an acceptable fresh-cut product.

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