Efficacy of chlorine, peroxyacetic acid and mild-heat treatment on the reduction of natural microflora and maintenance of quality of fresh-cut *calçots* (*Allium cepa* L.)

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Highlights:

- Minimally processing reduced microbial population of *calçots*
- Mild-heated *calçots* presented the highest microbiological counts after 15 d
- Similar microbiological quality in *calçots* treated with PAA or NaOCl
- High visual quality scores

Abstract

Calçots are the immature floral stems of the second-year onion (*Allium cepa* L.) resprouts with economic importance in Spain. The effect of sodium hypochlorite, peroxyacetic acid and mild heat treatment on the microbiological, physicochemical and visual quality of *calçots* after disinfection and for 15-day storage period at 4 °C was studied. Previous minimal processing operations for the disinfection helped to reduce the population of aerobic mesophilic bacteria and yeast and moulds compared to the harvest product. *Calçots* subjected to a mild-heat treatment (55 ºC for 60 s) presented the highest reduction of aerobic mesophilic bacteria. Despite being mildly thermally-treated they showed higher fresh weight loss (around 3 %) than the rest of the disinfected *calçots* during storage and they obtained the highest quality visual scores after 15 days of storage with a good acceptance of the product. Microbial reductions obtained after peroxyacetic acid treatment were in the same range as those obtained after the sodium hypochlorite treatment. In addition, the quality of both treated samples was maintained with no differences during refrigerated storage. The results indicate that mild-heat or peroxyacetic acid decontamination treatments resulted in a good strategy for obtaining a clean and high quality fresh-cut *calçot* product.

Keywords: minimally processed; disinfection; thermal; peroxyacetic acid; *Allium*

Abbreviations

PAA: peroxyacetic acid	BI: browning index
MHT: mild-heat treatment	TCD: Total Colour Difference
SSC: soluble solids content	TAPC: Total aerobic plate count
TA. Titratable acidity	
FWL: fresh weight loss	

1. Introduction

Calçots (*Allium cepa* L.) are the immature floral stems of second-year onion resprouts of the 'Ceba Blanca Tardana de Lleida' (BTL) onion landrace with an economic importance in Catalonia (northeast Spain). They usually consumed as grilled or roasted. The singularity of the production of this product has helped confer protected status from the European Union and 'Calçot de Valls' has been awarded Protected Geographical Indication (PGI) (EC No 905/2002) (Simó, Valero, Plans, Romero del Castillo, & Casañas, 2013). The demand and interest in *calçots* worldwide has motivated the food industry to explore postharvest techniques such as minimal processing, thus maintaining their nutritional and organoleptic characteristics and extending shelf-life.

Minimal processing is a critical step where the contamination probability and subsequent survival of microorganisms is very high (Vandekinderen, Van Camp, et al., 2009). Decontamination agents such as sodium hypochlorite and peroxyacetic acid could be added to the wash water to reduce the microbial load of fresh-cut *calçot* without affecting sensorial and nutritional quality. However, the minimally processed vegetables of *Allium* genus have an additional problem called 'telescoping' which is an extension growth of the white inner leaf bases and it can be avoided by the heat treatment, for example, in green onions (Cantwell, Hong, & Suslow, 2001; Hong, Peiser, & Cantwell, 2000) or leek (Tsouvaltzis, Siomos, & Gerasopoulos, 2013). In addition, it is generally accepted that an ideal sanitizing agent should have two important properties: a sufficient level of antimicrobial activity and a negligible effect on the sensory quality of the product (Allende, Selma, López-Gálvez, Villaescusa, & Gil, 2008).

Chlorine (Cl_2), or hypochlorite (OCl^-) in solution, are the most commonly employed aqueous sanitizers in the food industry and for drinking water disinfection due to their efficacy against pathogens. For commercial disinfection purposes, chlorine gas, sodium hypochlorite (NaOCl) solutions, or calcium hypochlorite ($Ca(ClO)_2$) are used to make aqueous hypochlorite solutions (Feliziani, Lichter, Smilanick, & Ippolito, 2016). However, the increasing public health concerns about the possible formation of chlorinated organic compounds and the emergence of new and more tolerant pathogens, have raised doubts in relation to the use of chlorine by the fresh-cut industry (Allende et al., 2008). Chlorate is formed as a by-product when using chlorine, chlorine dioxide or hypochlorite for disinfection purposes in food production. In the European Union, the washing of plant-derived food with chlorine disinfected water can be permitted under national regulations (EFSA, 2015). Taking into account these concerns, new chemical-based and physical-based disinfection methods are being investigated with the objective of ensuring the production of safe products.

Among commercial alternatives to chlorine, peroxyacetic or peracetic acid (PAA) is effective in killing pathogenic microorganisms in suspension at lower concentrations than would be required with chlorine (Ölmez & Kretzschmar, 2009). PAA is gaining an increase in interest with the claim that only harmless disinfection by-products have been identified thus far, such as acetic acid, water and oxygen that are formed from its spontaneous decomposition (Ölmez & Kretzschmar, 2009). However, PAA is a strong oxidant which could oxidized/affected the phytochemicals, nutritional compounds, as well as quality parameters of fruit and vegetables (Van de Velde, Grace, Pirovani, & Lila, 2016). For example, Van de Velde, Piagentini, Güemes, & Pirovani (2013) reported that disinfection with PAA at 80 mg L^{-1} for 120 s produced a reduction of approximately 30 % on the total content of anthocyanins, ascorbic acid and vitamin C of fresh-cut strawberries. Moreover, PAA could be able to interfere with activity of enzymes related to the colour changes, such as polyphenol oxidase (Silveira, Conesa, Aguayo, & Artes, 2008). However, in a study carried out with fresh-cut melon the disinfection with PAA had no effect on the physicochemical and sensorial quality (Botondi, Moscetti, & Massantini, 2016). On the other hand, mild heat treatment (MHT) is a thermal process that might reduce microbial load based on the use of water at temperatures between 45 and 60 °C, for durations ranging from a few seconds to 20 min. This technique is completely safe for humans and the environment (being residue-free and environmentally friendly) and of feasible use without registration rules. Hot water immersion treatment (HWT) is usually applied by a complete submersion or in the form of hot water rinsing and brushing (HWRB) in decay control (Usall, Ippolito, Sisquella, & Neri, 2016).

In this study, the effect of conventional and alternative decontamination techniques on the native microbial load of fresh-cut *calçot* were studied. In order to estimate the effect of decontamination on the quality of *calçots*, visual quality, weight loss, colour, firmness, pH, soluble solids and acidity were also studied.

2. Material and methods

2.1 Plant material

Calçots (*Allium cepa* L.) were provided by the 'Cooperativa Agricola Valls' (Tarragona, Spain) at commercial size. The *calçots* had the European quality label PGI 'Calçot de Valls' establishing that their diameter must be between 1.7 and 2.5 cm and the white shaft between 15-25 cm (D.A.R.P., 2009). They were cultivated in northeast Spain (41º13'47''N, 01º13'12''E), during the crop growing season of 2014 and 2015. In August 2014, the bulbs of 'Blanca Tardana de Lleida' onion were transplanted at a density of 8,000 plants per ha. The resprouts arising in the autumn were covered with soil three times to increase the length of the edible white part. The plants were manually harvested in February.

The minimal processing was carried out by a first manual step, including the cut of 4 cm over the last ligula of the *calçot* (white leaf which covered green leafs), the elimination of the first layer of sheath which contained the greatest amount of soil and the cut of the roots.

2.2 Decontamination: equipment, experimental set-up and procedures

The batch of 365 units of minimally processed *calçots* were divided into 4 batches for 4 different disinfection treatments: (1) control (no disinfection), (2) 100 mg L^{-1} sodium hypochlorite (10 % [w/v], Panreac, Barcelona, Spain), (3) 80 mg L^{-1} peroxyacetic acid (39 %, Sigma-Aldrich, Steinheim, Germany) and (4) mild heat treatment (MHT) at 55 °C. At each disinfection treatment, minimally processed *calçots* were immersed in 10 L disinfectant solution at room temperature under continuous agitation for 60 s. For all washing solutions, pH and oxidation reduction potential (ORP) or REDOX were determined before and after the *calçot* treatment (Table 1). Free chlorine was determined using a free and total chlorine photometer (HI 93734, Hanna Instruments, Eibar, Spain). ORP and pH were determined using a pH/ion/conductivity meter (Model GLP-22, Crison), with a pH electrode (Crison, 52-01) or an ORP electrode (Crison, platinum Ag/AgCl electrode 52-61), respectively. In addition, the microbial load of each washing solution after treatment was evaluated. To carry out such determinations, 1 mL of each washing solution was added to 9 mL of Dey-Engley neutralizing broth (Fluka, Sigma-Aldrich, St. Louis, US) and plated as described below. Afterwards, only samples treated with sodium hypochlorite were rinsed with water for 1 min. All samples were centrifuged at 210 rpm in centrifuge (Marrodán, Navarra, Spain) for 95 s, packaged in polystyrene trays with retractable film and stored at 4 °C for 15 d. The microbiological and quality determinations were carried out at the beginning of the experiment, after the treatments and after 3, 6, 9 and 15 d of shelf-life at 4 °C.

2.3 Enumeration of bacterial concentration on *calçots*

The enumeration of mesophilic microorganisms and yeast and moulds was performed in the manner described by Alegre, Viñas, Usall, Anguera, & Abadias (2011). Briefly, 10 g of the edible part of *calçots* were diluted in 90 g of buffered peptone water (BPW, Oxoid LTD, Basingstoke, Hampshire, England) in a sterile bag and homogenized in a masticator (IUL Masticator Basic 400 ml, IUL Instruments, Barcelona, Spain) for 90 s. Further ten-fold dilutions were made with saline peptone (SP; 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone) and plated in duplicate in Plate Count Agar (PCA, Biokar Diagnostics, Beauvais, France) and in Dichloran Rose Bengale Chloramphenicol Agar (DRBC, Biokar Diagnostics, Beauvais, France) and incubated at 30 ± 1 °C or 25 ± 1 °C for 3 and 5 days, respectively). Three determinations (in three trays) were performed at each sampling time. The results were represented in $\log_{10}$ cfu g^{-1}.

2.4 pH, soluble solids content and titratable acidity

pH, soluble solids content (SSC) and titratable acidity (TA) were measured in the juice extracted by crushing *calçots* pieces in a blender. pH was determined using a penetration electrode in a pH-meter model GLP22 (Crison Instruments SA, Barcelona, Spain). Soluble solids were measured at 20 °C with a hand-held refractometer (Atago Co. Ltd., Tokyo, Japan) and the results were expressed as °Brix. To measure titratable acidity, 10 mL of *calçots* juice was diluted with 10 mL distilled water and it was titrated with 0.1 mol L^{-1} NaOH (Panreac, Barcelona, Spain) up to pH 8.2. Results of titratable acidity were expressed as g of malic acid L^{-1}. Three determinations were performed per each treatment at each sampling time.

2.5 Fresh weight loss

The fresh weight of *calçots* was recorded in 15 samples that were randomly selected from each treatment at each period of storage. Data were expressed as percentage of fresh weight loss (FWL).

2.6 Firmness

To assess changes in texture, firmness (N) was measured at 5 cm from the roots set in transversal position using the TA.TX2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, England) attached with a Warner-Blatzler blade (HDP/BSK: Blade set with knife). The sample was placed into the press holder, and then the blade was moved downwards at different rates: pre-test rate: 5 mm s^{-1}; test rate: 1 mm s^{-1}; post-test rate: 10 mm s^{-1} to 60 mm below the bottom of the holder. Data acquisition rate was 200 pulses per sec. Eight individual and randomly chosen *calçots* per treatment at each sampling time were evaluated.

2.7 Colour

The colour of the white shaft was measured with a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan). Colour was measured using CIE L*, a*, b* coordinates with illuminant D65 and 10° observer angle. L* defines the lightness, and a* and b* define the red-greenness and blue-yellowness, respectively. Measurements were made in 17 randomly selected *calçots* per treatment and sampling time. CIE L* (lightness), a* (red-green) and b* (yellow-blue) parameters were measured through reflectance values. These values were used to calculate the browning index (BI) (Eq. (1)) and Total Colour Difference (TCD) (Eq. (2)). Changes in BI parameter have previously been shown to be effective in monitoring browning of fresh-cut apples (Liu, Ma, Hu, Tian, & Sun, 2016) :

$$BI = \frac{100(x-0.31)}{0.172} \quad (1)$$

where $x = (a^* + 1.75 \times L^*)/(5.645 \times L^*) + (a^* - (3.012 \times b^*))$.

TCD was calculated in accordance with Martín-Diana et al. (2007):

$$TCD = [\left(L_f^* - L_i^*\right)^2 + \left(a_f^* - a_i^*\right)^2 + \left(b_f^* - b_i^*\right)^2]^{1/2} \quad (2)$$

where i = initial (without disinfection) and f = final.

2.8 Assessment of overall visual quality

The assessment was carried out in the manner described by Altisent, Plaza, Alegre, Viñas, & Abadias (2014). The visual quality was determined by an untrained panel based on the following hedonic scale: 9 = excellent; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible.

2.9 Statistical analysis

Results were expressed as mean ± standard deviation. Significant differences between results were calculated by using one-way analysis of variance (ANOVA). Differences were considered to be significant at $P<0.05$ (95 % confidence level). Significance of differences between means was determined by using Tukey's test and Student's *t*-test. All statistical analyses were performed with JMP 8 software (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1 Effect of decontamination treatments on microbial load

3.1.1 Effectiveness of sanitizers after decontamination

Reductions of microbial populations on fresh-cut *calçots* before and after washing are shown in Fig. 1. Mesophilic population of fresh harvested *calçots* (7.33 ± 0.19 $\log_{10}$ cfu g^{-1}) significantly decreased after the minimal processing operation (control) which included cutting and peeling operations. Hence, pre-processing steps helped to reduce natural microflora present in harvest *calçots* (Fig. 1A). Moreover, disinfection treatments also reduced mesophilic population and that reduction was more drastic with MHT (1.7-log units with regard to the control and 3.0-log units with regard to the harvested sample). Stringer, Plowman, & Peck (2007) report that hot water washing (52 °C, 90 s) reduced the initial population of aerobic mesophylls by < 1.2 log cfu g^{-1} on broccoli and green beans.

No significant differences ($P>0.05$) on the mesophilic counts obtained in *calçots* disinfected by 100 mg L^{-1} NaOCl or 80 mg L^{-1} PAA treatments were observed, reducing 2.3-log units with regard to harvested sample and 0.8-log units with regard to control. According to other studies, the effectiveness of PAA is dependent on the type of the vegetable product. Vandekinderen, Van Camp, et al. (2009) observed a reduction of 1-log units of natural microbiota (between 5.58 and 7.29 log cfu g^{-1}) after washing fresh-cut leeks (*Allium porrum* L.) with 80 mg L^{-1} PAA for 60 s. Dai et al. (2012) showed that the population of total aerobic bacteria of fresh-cut Chinese chives significantly decreased after disinfected with 150 mg L^{-1} PAA for 2 min or 100 mg L^{-1} PAA for 5 min (2.16-log units and 2.20-log units, respectively). Van de Velde, Güemes, & Pirovani (2014) reported that total microbial count of fresh-cut strawberries decreased 2.6-log units of initial microbiota after disinfecting with 100 mg L^{-1} PAA for 120 s (40 °C).

PAA is known to be an oxidising agent that is in general effective against a wide spectrum of microorganisms (Kyanko, Russo, Fernández, & Pose, 2010). PAA disinfects by oxidizing of the outer cell membrane of bacteria, yeast and moulds, and that oxidation consist on the transfer of electrons (Joshi, Mahendran, Alagusundaram, Norton, & Tiwari, 2013). It has been suggested that PAA acts primarily on lipoproteins in the cell membrane, and could be equally effective against outer-membrane lipoproteins, facilitating its action against Gram-negative cells (Botondi et al., 2016). The action on the membranes could be due different mechanisms: (1) Denaturation of proteins and enzymes and the increase of cell wall permeability by oxidizing sulfhydryl and disulphide bonds; (2) Disruption of cell membranes and the blockage of enzymatic and transport systems in microorganisms. PAA could also damage DNA and lipids through the production of reactive oxygen species (Vandekinderen, Devlieghere, De Meulenaer, Ragaert, & Van Camp, 2009).

The population of yeast and moulds (Fig. 1B) of fresh harvested *calçots* (3.94 ± 0.31 $\log_{10}$ cfu g^{-1}) decreased after minimal processing and after washing with all tested decontamination treatments. There were no significant differences ($P>0.05$) between disinfection treatments, achieving reductions of around 0.8-log units with respect to the control and about 2.5-log units with respect to the harvested sample. Stringer et al. (2007) observed that hot water washing (52 °C, 90 s) reduced the initial yeast population (2.0 log cfu g^{-1}) and mould population (1.1 log cfu g^{-1}) on broccoli. Similar results were obtained in the study carried out by López-Gálvez et al. (2010), where washing with NaOCl (100 mg L^{-1}, 60 s) produced a reduction of 1.2 ± 0.1-log units on fresh-cut iceberg lettuce (*Lactuca sativa* L.) in contrast to the unwashed lettuce (3.2 ± 1.4 log cfu g^{-1}).

3.1.2 Effect of sanitizers on microbial stability

Effect of disinfectant treatments on the microbial population of fresh-cut *calçot* stored for 15 d at 4 °C are shown in Table 2. TAPC was maintained in all samples except samples treated with MHT during the storage time and there were no significant differences between them at the end (15 days). The initial population of MHT-treated samples was lower than other samples. Those results were in agreement with those reported by Kim, Feng, Toshkov, & Fan (2005) where TPAC of fresh-cut green onions (*Allium fistulosum* L.) treated at 50 °C for 20 s increased during the 14-day storage period at 4 °C from 4.2 to 7.5 log cfu g^{-1}.

Furthermore, Silveira, Aguayo, Escalona, & Artés (2011) showed that the mesophilic population of fresh-cut 'Galia' melons (*Cucumis melo* var. *cantalupensis* Naud) treated by HWD at 60 °C for 60 s increased after 10 d at 5 °C, from 3.7 to 7.8 $\log_{10}$ cfu g^{-1}. Thermal treatment would disrupt physical barrier membranes liberating nutrients from the cells which facilitated access to nutrients leading to the greater proliferation of microorganisms (Goyeneche et al., 2015). MHT might alter the ability of the vegetable tissue to support growth of microorganism as it can inactivate plant defences and damage cell membranes which may release nutrients (Stringer et al., 2007).

The populations of yeast and moulds were maintained during storage, regardless of disinfectant treatment applied (Table 2). Moreover, there were no significant differences between samples disinfected with different treatments after 15 days of storage at 4 °C. According to our results, in the study carried out by Silveira et al. (2008) there were no differences between fresh-cut melons treated with 80 mg L^{-1} PAA or 150 mg L^{-1} NaOCl for 60 s. However, Page, González-Buesa, Ryser, Harte, & Almenar (2016) reported that fresh-cut onions (*Allium cepa* L.) treated with 80 mg L^{-1} NaOCl had significantly lower mesophilic and yeast and mould counts than those treated with 80 mg L^{-1} PAA for 120 s throughout storage (14 d).

To the best of our knowledge, there is no previous microbiological study with this crop. *Calçots* are usually eaten roasted but nowadays are being introduced into some fresh salads. Then, the low microbial counts obtained from the experiment demonstrated that *calçots* are suitable to eat as a raw vegetables.

3.2 Effect of decontamination treatments on quality

3.2.1 Soluble solids content, titratable acidity, pH and fresh weight loss

Physicochemical characteristics of *calçots* at harvest are shown in Table 3. Changes in SSC, TA, pH and weight on non-disinfected samples (control) and disinfected samples during 15 d of storage at 4 ºC are shown in Table 4. In general, disinfection treatments had no remarkable effect on physicochemical characteristics of *calçots*. Despite the pH of the PAA, where disinfection treatment was around 3.4 before and after washing the *calçots* (Table 1), the acidic pH conditions did not affect their initial quality. These results contrasted with those observed by Vandekinderen, Devlieghere, Van Camp, et al. (2009), who reported a reduction by 0.6 pH-units after disinfecting fresh-cut carrots (*Daucus carota* L.) with 80 mg L^{-1} PAA for 300 s. They suggested that this pH-decrease was caused by the acidic pH of the PAA solutions used for disinfection. In addition, there were no significant differences (P>0.05) between disinfected samples and control after a 15-d storage period at 4 °C with regard to TA (ranging from 1.67 to 2.09 g malic acid L^{-1}). Only values of SSC and pH presented barely detectable statistically significant differences among treatments, being the highest values in the control sample (8.8 °Brix and pH 5.9) after 15 d of storage. In a study carried out with fresh-cut melon the disinfection with 100 mg L^{-1} Tsumani 100^{TM} solution (composed of 15.2 % peracetic acid and 11.2 % hydrogen peroxide) for 3 min (20 ± 1 °C) had no effect on the SSC (Botondi et al., 2016).

Regarding the MHT, fresh-cut *calçots* maintained similar quality levels as the rest of the disinfected samples. FWL values increased in control and disinfected *calçots* after 15 days of storage, ranging from 3.31 to 5.47 %. However, there were no significant differences between control and disinfected samples. Siddiq, Roidoung, Sogi, & Dolan (2013) observed the lowest weight loss in 50 °C-treated (60 s) fresh-cut yellow onions (*Allium cepa*) after 21-d of storage, which was significantly different from the other two heat treatments (60 °C and 70 °C) and they suggested that heat treatment beyond 50 °C affected the tissue structure, causing some shrinkage.

3.2.2 Firmness, colour and visual quality

A general increment in the firmness values was observed in all the samples during the 15-d storage period at 4 °C (Fig. 2A). Moreover, a direct possible relationship between FWL and firmness was observed and it was more pronounced from day 6 of storage. The firmness increase might be associated with a possible water loss during storage.

Despite the increment in TCD and BI values of samples during storage, disinfection conditions of MHT at 50 ºC for 60 s led to the lowest browning index in *calçots* throughout storage (Fig. 2B and 2C). This behaviour might be explained through the possible inactivation of PPO (Polyphenol oxidase) and POD (peroxidase) which play an important role in skin colour changes and a mild-heat level of temperature might have more effect than acidic medium in the inactivation of those enzymes.

The results obtained in visual overall quality (Fig. 2D) demonstrated that all disinfected samples maintained excellent and very good scores until day 6 of storage at 4 °C. Those results were in agreement with those reported by Alvaro et al. (2009), where no differences were found by a trained panel in whole tomatoes, peppers and cucumber washed with any applied treatment including PAA. Although all samples were above the limit of marketability, only *calçots* treated with MHT presented scores above 7 (very good) at the end of the 15-d storage period. Despite the weight loss, overall quality was not affected. Furthermore, visual quality decreased drastically for *calçots* treated with NaOCl and PAA, 4 and 3.3 points after the 15-d storage period, respectively. Hong et al. (2000) suggested that mild-heat level of treatment at 55 °C for 120 s was one of several heat treatments that was effective enough to control 'telescoping' of cut green onions (*Allium cepa* x *A. fistulosum*).

4. Conclusions

The physical and microbiological quality of fresh-cut *calçots* after disinfecting with NaOCl, PAA and MHT were studied. In general, although all samples presented physiochemical changes after disinfection and a 15-d storage period at 4 °C, *calçots* treated with MHT at 55 °C for 60 s retained those characteristics better than other samples. However, the population of aerobic mesophilic bacteria in mildly heated *calçots* increased during the 15-d storage period. It is remarkable that there were no significant differences with regard to the results of *calçots* treated with NaOCl and PAA. Therefore, our results have shown that sanitization treatment with PAA (80 mg L^{-1}) for 60 s could be an alternative to chemical methods based on chlorine, maintaining physicochemical and microbiological quality and consumer acceptance. Furthermore, this treatment could be a suitable disinfection treatment for other fresh-cut vegetables, such as leafy vegetables which usually have high initial microbial loads compared to other vegetables.

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References

Alegre, I., Viñas, I., Usall, J., Anguera, M., & Abadias, M. (2011). Microbiological and physicochemical quality of fresh-cut apple enriched with the probiotic strain *Lactobacillus rhamnosus* GG. *Food Microbiology*, *28*(1), 59–66. https://doi.org/10.1016/j.fm.2010.08.006

Allende, A., Selma, M. V., López-Gálvez, F., Villaescusa, R., & Gil, M. I. (2008). Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce. *Postharvest Biology and Technology*, *49*(1), 155–163. https://doi.org/10.1016/j.postharvbio.2007.12.010

Altisent, R., Plaza, L., Alegre, I., Viñas, I., & Abadias, M. (2014). Comparative study of improved vs. traditional apple cultivars and their aptitude to be minimally processed as 'ready to eat' apple wedges. *LWT - Food Science and Technology*, *58*(2), 541–549. https://doi.org/10.1016/j.lwt.2014.03.019

Alvaro, J. E., Moreno, S., Dianez, F., Santos, M., Carrasco, G., & Urrestarazu, M. (2009). Effects of peracetic acid disinfectant on the postharvest of some fresh vegetables. *Journal of Food Engineering*, *95*(1), 11–15. https://doi.org/10.1016/j.jfoodeng.2009.05.003

Botondi, R., Moscetti, R., & Massantini, R. (2016). A comparative study on the effectiveness of ozonated water and peracetic acid in the storability of packaged fresh-cut melon. *Journal of Food Science and Technology*, *53*(5), 2352–2360. https://doi.org/10.1007/s13197-016-2207-y

Cantwell, M. I., Hong, G., & Suslow, T. V. (2001). Heat treatments control extension growth and enhance microbial disinfection of minimally processed green onions. *HortScience*, *36*(4), 732–737.

D.A.R.P. (2009). ORDRE AAR/414/2009, de 21 de setembre, per la qual s'aprova el Reglament de la Indicació Geogràfica Protegida Calçot de Valls. Retrieved from http://portaljuridic.gencat.cat/ca/pjur_ocults/pjur_resultats_fitxa/?documentId=503886&language=ca_ES&action=fitxa

Dai, X., Luo, H., Jiang, L., Ling, L., Xue, Y., & Yu, Z. (2012). Efficacy of different sanitizing agents and their combination on microbe population and quality of fresh-cut chinese chives. *Journal of Food Science*, *77*(7), 348–353. https://doi.org/10.1111/j.1750-3841.2012.02770.x

EC No 905/2002. Commission Regulation (EC) No 905/2002 of 30 May 2002 supplementing the Annex to Regulation (EC) No 2400/96 on the entry of certain names in the 'Register of protected designations of origin and protected geographical indications' [2002] OJ L 142/27.

EFSA. (2015). Risks for public health related to the presence of chlorate in food. *EFSA Journal*, *13*(6), 1–103. https://doi.org/10.2903/j.efsa.2015.4135

Feliziani, E., Lichter, A., Smilanick, J. L., & Ippolito, A. (2016). Disinfecting agents for controlling fruit and vegetable diseases after harvest. *Postharvest Biology and Technology*, *122*, 53–59. https://doi.org/10.1016/j.postharvbio.2016.04.016

Goyeneche, R., Di Scala, K., Goñi, M. G., Vega-Gálvez, A., Quispe-Fuentes, I., & Roura, S. I. (2015). Application of ascorbic acid and mild heat shock to improve shelf life and ensure microbial safety of sliced radish (*Raphanus sativus* L.). *Journal of Food Processing and Preservation*, *39*(6), 3022–3031. https://doi.org/10.1111/jfpp.12567

Hong, G., Peiser, G., & Cantwell, M. I. (2000). Use of controlled atmospheres and heat treatment to maintain quality of intact and minimally processed green onions. *Postharvest Biology and Technology*, *20*(1), 53–61. https://doi.org/10.1016/S0925-5214(00)00112-5

Joshi, K., Mahendran, R., Alagusundaram, K., Norton, T., & Tiwari, B. K. (2013). Novel disinfectants for fresh produce. *Trends in Food Science and Technology*, *34*(1), 54–61. https://doi.org/10.1016/j.tifs.2013.08.008

Kim, H. J., Feng, H., Toshkov, S., & Fan, X. (2005). Effect of sequential treatment of warm water dip and low-dose gamma irradiation on the quality of fresh-cut green onions. *Food Microbiology and Safety*, *70*(3), 179–185.

Kyanko, M. V., Russo, M. L., Fernández, M., & Pose, G. (2010). Efectividad del acido peracético sobre la reducción de la carga de esporas de mohos causantes de pudrición poscosecha de frutas y hortalizas. *Informacion Tecnologica*, *21*(4), 125–130. https://doi.org/10.1612/inf.tecnol.4299it.09

Liu, C., Ma, T., Hu, W., Tian, M., & Sun, L. (2016). Effects of aqueous ozone treatments on microbial load reduction and shelf life extension of fresh-cut apple. *International Journal of Food Science and Technology*, *51*(5), 1099–1109. https://doi.org/10.1111/ijfs.13078

López-Gálvez, F., Allende, A., Truchado, P., Martínez-Sánchez, A., Tudela, J. A., Selma, M. V., & Gil, M. I. (2010). Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biology and Technology*, *55*(1), 53–60. https://doi.org/10.1016/j.postharvbio.2009.08.001

Martín-Diana, A. B., Rico, D., Barry-Ryan, C., Frías, J. M., Henehan, G. T. M., & Barat, J. M. (2007). Efficacy of steamer jet-injection as alternative to chlorine in fresh-cut lettuce. *Postharvest Biology and Technology*, *45*(1), 97–107. https://doi.org/10.1016/j.postharvbio.2007.01.013

Ölmez, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT - Food Science and Technology*. Swiss Society of Food Science and Technology. https://doi.org/10.1016/j.lwt.2008.08.001

Page, N., González-Buesa, J., Ryser, E. T., Harte, J., & Almenar, E. (2016). Interactions between sanitizers and packaging gas compositions and their effects on the safety and quality of fresh-cut onions (*Allium cepa* L.). *International Journal of Food Microbiology*, *218*, 105–

113. https://doi.org/10.1016/j.ijfoodmicro.2015.11.017

Siddiq, M., Roidoung, S., Sogi, D. S., & Dolan, K. D. (2013). Total phenolics, antioxidant properties and quality of fresh-cut onions (*Allium cepa* L.) treated with mild-heat. *Food Chemistry*, *136*(2), 803–806. https://doi.org/10.1016/j.foodchem.2012.09.023

Silveira, A. C., Aguayo, E., Escalona, V. H., & Artés, F. (2011). Hot water treatment and peracetic acid to maintain fresh-cut Galia melon quality. *Innovative Food Science and Emerging Technologies*, *12*(4), 569–576. https://doi.org/10.1016/j.ifset.2011.02.010

Silveira, A. C., Conesa, A., Aguayo, E., & Artes, F. (2008). Alternative sanitizers to chlorine for use on fresh-cut 'galia' (*cucumis melo* var. *catalupensis*) melon. *Journal of Food Science*, *73*(9), 405–411. https://doi.org/10.1111/j.1750-3841.2008.00939.x

Simó, J., Valero, J., Plans, M., Romero del Castillo, R., & Casañas, F. (2013). Breeding onions (*Allium cepa* L.) for consumption as 'calçots' (second-year resprouts). *Scientia Horticulturae*, *152*, 74–79. https://doi.org/10.1016/j.scienta.2013.01.011

Stringer, S. C., Plowman, J., & Peck, M. W. (2007). The microbiological quality of hot water-washed broccoli florets and cut green beans. *Journal of Applied Microbiology*, *102*(1), 41–50. https://doi.org/10.1111/j.1365-2672.2006.03065.x

Tsouvaltzis, P., Siomos, A. S., & Gerasopoulos, D. (2013). Effect of heat treatment on internal atmosphere and leaf extension of minimally processed leek stalks. *International Journal of Food Science and Technology*, *48*(10), 2105–2110. https://doi.org/10.1111/ijfs.12192

Usall, J., Ippolito, A., Sisquella, M., & Neri, F. (2016). Physical treatments to control postharvest diseases of fresh fruits and vegetables. *Postharvest Biology and Technology*, *122*, 30–40. https://doi.org/10.1016/j.postharvbio.2016.05.002

Van de Velde, F., Grace, M. H., Pirovani, M. É., & Lila, M. A. (2016). Impact of a new postharvest disinfection method based on peracetic acid fogging on the phenolic profile of strawberries. *Postharvest Biology and Technology*, *117*, 197–205.

https://doi.org/10.1016/j.postharvbio.2016.03.005

Van de Velde, F., Güemes, D. R., & Pirovani, M. E. (2014). Optimisation of the peracetic acid washing disinfection of fresh-cut strawberries based on microbial load reduction and bioactive compounds retention. *International Journal of Food Science and Technology*, *49*(2), 634–640. https://doi.org/10.1111/ijfs.12346

Van de Velde, F., Piagentini, A. M., Güemes, D. R., & Pirovani, M. E. (2013). 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*(5), 954–961. https://doi.org/10.1111/ijfs.12047

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*(8), 882–888. https://doi.org/10.1016/j.fm.2009.06.004

Vandekinderen, I., Devlieghere, F., Van Camp, J., Denon, Q., Alarcon, S. S., Ragaert, P., & De Meulenaer, B. (2009). Impact of a decontamination step with peroxyacetic acid on the shelf-life, sensory quality and nutrient content of grated carrots packed under equilibrium modified atmosphere and stored at 7 °C. *Postharvest Biology and Technology*, *54*(3), 141–152. https://doi.org/10.1016/j.postharvbio.2009.06.007

Vandekinderen, I., Van Camp, J., Devlieghere, F., Ragaert, P., Veramme, K., Bernaert, N., … De Meulenaer, B. (2009). Evaluation of the use of decontamination agents during fresh-cut leek processing and quantification of their effect on its total quality by means of a multidisciplinary approach. *Innovative Food Science and Emerging Technologies*, *10*(3), 363–373. https://doi.org/10.1016/j.ifset.2009.02.002

Figure captions

Fig. 1. Reductions of total mesophilic aerobic (A) and yeast and moulds (B) of fresh-cut *calçots* before and after disinfection (60 s). Values are expressed as the mean ± standard deviation (n = 3). Different capital letters indicate significant differences between treatments ($P<0.05$). Control: minimally processed *calçot*; PAA: peroxyacetic acid; MHT: Mild heat treatment

Fig. 2. Effect of disinfectant treatments [control (♦), NaOCl (■), PAA (▲) and MHT (●)] after 60 s of treatment on firmness (A), TCD (B), BI (C) and overall visual quality (D) of fresh-cut *calçots* stored for 15 d at 4 °C. TCD: Total Colour Difference; BI: Browning Index. Replications: n = 8 (firmness) and n = 36 (colour). PAA: peroxyacetic acid; MHT: Mild heat treatment.

Table 1.

Determination of pH. ORP (mV) and free Cl or PAA concentration of tested disinfectant water solutions before and after disinfection (60 s). ORP: oxidation-reduction potential. BT: Before treatment; AT: After treatment.

Treatment	pH		ORP (mV)		Free chlorine /PAA (mg L^{-1})	
	BT	AT	BT	AT	BT	AT
NaOCl						
100 mg L^{-1} 1 min	6.61	6.20	916	899	111.0	64.0
PAA						
80 mg L^{-1} 1 min	3.48	3.44	646	621	79.8	72.2

Table 2.

Effect of disinfectant treatments (60 s) on the microbial population (total mesophilic aerobic count – TAPC and yeast and moulds count –Y&M) of fresh-cut *calçots* stored for 15 d at 4 °C. Values are expressed as the mean ± standard deviation (n = 3). Different capital letters in the same column indicate significant differences between storage time ($P<0.05$) for each disinfectant treatment. Different lower case letters in the same row indicate significant differences between disinfectant treatments ($P<0.05$) for each storage time. Control: minimally processed *calçot*; PAA: peroxyacetic acid; MHT: Mild heat treatment.

Parameter	Day	Control	NaOCl (100 mg L^{-1} for 60 s)	PAA (80 mg L^{-1} for 60 s)	MHT (55 °C for 60 s)
TAPC (Log$_{10}$ [cfu g^{-1}])	0	5.79 ± 0.10 Aa	4.98 ± 0.04 Ab	5.00 ± 0.32 Ab	4.14 ± 0.30 BCc
	3	4.86 ± 0.47 Aa	4.66 ± 0.90 Aab	4.72 ± 0.19 Aa	3.29 ± 0.37 Cb
	6	5.12 ± 0.07 Aa	4.58 ± 0.89 Aa	4.26 ± 0.35 Aa	4.36 ± 0.36 Ba
	9	5.13 ± 0.05 Aa	4.86 ± 0.40 Aa	4.87 ± 0.19 Aa	4.89 ± 0.47 ABa
	15	4.89 ± 0.50 Aa	5.28 ± 0.90 Aa	4.88 ± 0.49 Aa	5.35 ± 0.18 Aa
Y&M (Log$_{10}$ [cfu g^{-1}])	0	2.29 ± 0.50 Aa	1.41 ± 0.00 Ab	1.51 ± 0.17 Ab	1.41 ± 0.00 Ab
	3	2.26 ± 0.31 Aa	2.07 ± 0.68 Aa	2.11 ± 0.69 Aa	1.51 ± 0.17 Aa
	6	2.40 ± 0.00 Aa	1.61 ± 0.17 Ab	1.51 ± 0.17 Ab	1.41 ± 0.00 Ab
	9	2.02 ± 0.55 Aa	1.41 ± 0.00 Aa	1.67 ± 0.44 Aa	1.41 ± 0.00 Aa
	15	1.61 ± 0.17 Aa	1.41 ± 0.00 Aa	1.61 ± 0.34 Aa	1.71 ± 0.29 Aa

Table 3.

Physicochemical characteristics of *calçots* at harvest.

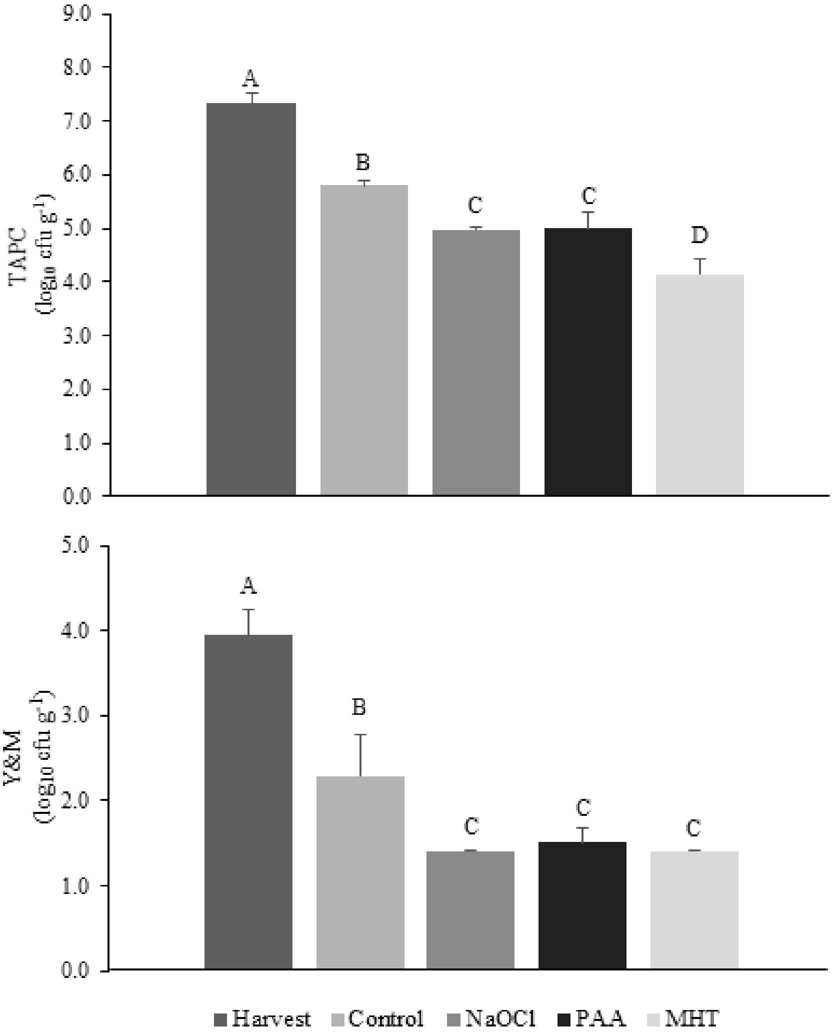
Quality measurements	Mean ± SD
SSC (°Brix)	10.03 ± 0.21
TA (g malic acid L^{-1})	1.99 ± 0.01
pH	5.92 ± 0.03
Firmness (N)	61.84 ± 10.45
Colour parameters	
L*	80.08 ± 2.69
a*	-0.91 ± 0.46
b*	5.18 ± 1.24

Table 4.

Changes in SSC, TA, pH and weight on non-disinfected samples (control) and disinfected samples during 15 d of storage at 4 °C. Values are expressed as the mean ± standard deviation (n = 3). Different capital letters in the same column indicate significant differences between storage time (P<0.05) for each disinfectant treatment. Different lower case letters in the same row indicate significant differences between disinfectant treatments (P<0.05) for each storage time. Control: minimally processed *calçot*; SSC: Soluble Solids Content; TA: Titratable Acidity; FWL: Fresh Weight Loss; PAA: peroxyacetic acid; MHT: Mild heat treatment

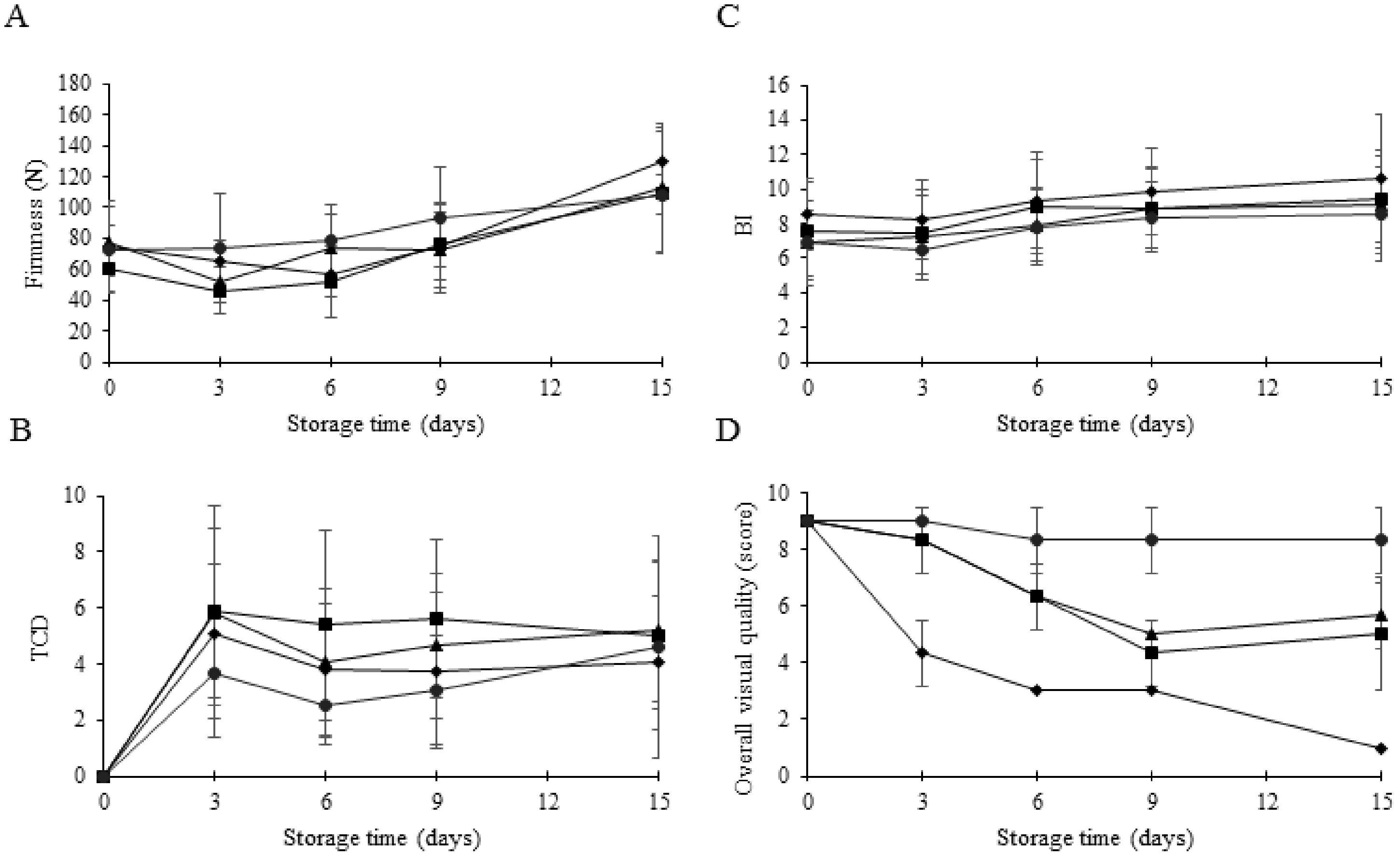
Parameter	Day	Control	NaOCl 100 mg L^{-1}	PAA 80 mg L^{-1}	MHT 55 °C
SSC (°Brix)	0	7.8 ± 0.1 Cc	8.2 ± 0.0 Aa	7.9 ± 0.0 Ab	7.1 ± 0.1 Cd
	3	7.7 ± 0.1 CDa	7.2 ± 0.1 Db	7.2 ± 0.2 Bb	7.7 ± 0.2 ABa
	6	8.1 ± 0.1 Ba	6.9 ± 0.1 Ec	7.5 ± 0.1 Bb	7.4 ± 0.0 Bb
	9	7.5 ± 0.1 Da	7.5 ± 0.0 Ca	7.6 ± 0.1 ABa	6.5 ± 0.1 Db
	15	8.8 ± 0.1 Aa	7.7 ± 0.1 Bb	7.3 ± 0.2 Bc	7.8 ± 0.1 Ab
TA (g malic acid L^{-1})	0	1.90 ± 0.04 BCa	1.84 ± 0.13 BCa	1.70 ± 0.10 Ca	2.01 ± 0.20 BCa
	3	4.13 ± 0.32 Aa	2.95 ± 0.18 Ab	2.64 ± 0.28 Bb	3.60 ± 0.70 Aab
	6	1.77 ± 0.06 BCb	1.98 ± 0.17 BCab	2.19 ± 0.09 Ba	2.31 ± 0.17 BCa
	9	2.37 ± 0.34 Ba	1.67 ± 0.10 Cb	1.82 ± 0.09 BCb	2.72 ± 0.04 ABa
	15	1.75 ± 0.18 Ca	2.09 ± 0.07 Ba	1.67 ± 0.16 Ca	1.78 ± 0.23 Ca
pH	0	6.0 ± 0.0 Aa	5.9 ± 0.0 Ab	6.0 ± 0.0 Aa	5.8 ± 0.0 Ac
	3	5.7 ± 0.0 Db	5.8 ± 0.0 Ba	5.8 ± 0.0 Ca	5.8 ± 0.0 Aa
	6	5.9 ± 0.0 Ba	5.9 ± 0.0 Aa	5.9 ± 0.0 Ba	5.8 ± 0.0 Ab
	9	5.8 ± 0.0 Ca	5.8 ± 0.0 Ba	5.8 ± 0.0 Ca	5.8 ± 0.0 Aa
	15	5.9 ± 0.0 Ba	5.9 ± 0.0 Aa	5.9 ± 0.0 Ba	5.8 ± 0.0 Ab
FWL (%)	0	0.00 ± 0.00 Ba	0.00 ± 0.00 Ca	0.00 ± 0.00 Ca	0.00 ± 0.00 Aa
	3	0.56 ± 0.40 Ba	0.51 ± 0.75 Ca	0.85 ± 0.21 BCa	3.02 ± 4.85 Aa
	6	0.96 ± 0.75 Ba	0.33 ± 0.29 Ca	1.05 ± 1.06 BCa	3.21 ± 4.71 Aa
	9	1.18 ± 1.15 Ba	1.47 ± 0.31 Ba	2.04 ± 0.69 Ba	3.75 ± 5.15 Aa
	15	4.82 ± 1.19 Aa	3.42 ± 0.30 Aa	5.47 ± 0.69 Aa	3.31 ± 7.99 Aa

Figure 1



9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
TAPC
(log10 cfu g-1)
A
B
C
C
D
5.0
4.0
3.0
2.0
1.0
0.0
Y&M
(log10 cfu g-1)
A
B
C
C
C
■ Harvest
■ Control
■ NaOCl
■ PAA
■ MHT

Figure 2


A
180
160
140
120
100
80
60
40
20
0
Firmness (N)
0
3
6
9
12
15
Storage time (days)
C
16
14
12
10
8
6
4
2
0
BI
0
3
6
9
12
15
Storage time (days)
B
10
8
6
4
2
0
TCD
0
3
6
9
12
15
Storage time (days)
D
10
8
6
4
2
0
Overall visual quality (score)
0
3
6
9
12
15
Storage time (days)