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

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17

18 **Abstract**

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

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

35 **Abbreviations**

36 PAA: peroxyacetic acid BI: browning index

37 MHT: mild-heat treatment TCD: Total Colour Difference

38 SSC: soluble solids content TAPC: Total aerobic plate count

39 TA: Titratable acidity

40 FWL: fresh weight loss

41

42 1. Introduction

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

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

63 Chlorine (Cl_2), or hypochlorite (OCl^-) in solution, are the most commonly employed aqueous
64 sanitizers in the food industry and for drinking water disinfection due to their efficacy against
65 pathogens. For commercial disinfection purposes, chlorine gas, sodium hypochlorite (NaOCl)
66 solutions, or calcium hypochlorite ($\text{Ca}(\text{ClO})_2$) are used to make aqueous hypochlorite solutions
67 (Feliziani, Lichter, Smilanick, & Ippolito, 2016). However, the increasing public health

68 concerns about the possible formation of chlorinated organic compounds and the emergence of
69 new and more tolerant pathogens, have raised doubts in relation to the use of chlorine by the
70 fresh-cut industry (Allende et al., 2008). Chlorate is formed as a by-product when using
71 chlorine, chlorine dioxide or hypochlorite for disinfection purposes in food production. In the
72 European Union, the washing of plant-derived food with chlorine disinfected water can be
73 permitted under national regulations (EFSA, 2015). Taking into account these concerns, new
74 chemical-based and physical-based disinfection methods are being investigated with the
75 objective of ensuring the production of safe products.

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

95 complete submersion or in the form of hot water rinsing and brushing (HWRB) in decay control
96 (Usall, Ippolito, Sisquella, & Neri, 2016).

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

101 2. Material and methods

102 2.1 Plant material

103 *Calçots* (*Allium cepa* L.) were provided by the 'Cooperativa Agrícola Valls' (Tarragona, Spain)
104 at commercial size. The *calçots* had the European quality label PGI 'Calçot de Valls'
105 establishing that their diameter must be between 1.7 and 2.5 cm and the white shaft between 15-
106 25 cm (D.A.R.P., 2009). They were cultivated in northeast Spain (41°13'47''N, 01°13'12''E),
107 during the crop growing season of 2014 and 2015. In August 2014, the bulbs of 'Blanca
108 Tardana de Lleida' onion were transplanted at a density of 8,000 plants per ha. The resprouts
109 arising in the autumn were covered with soil three times to increase the length of the edible
110 white part. The plants were manually harvested in February.

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

114 2.2 Decontamination: equipment, experimental set-up and procedures

115 The batch of 365 units of minimally processed *calçots* were divided into 4 batches for 4
116 different disinfection treatments: (1) control (no disinfection), (2) 100 mg L⁻¹ sodium
117 hypochlorite (10 % [w/v], Panreac, Barcelona, Spain), (3) 80 mg L⁻¹ peroxyacetic acid (39 %,
118 Sigma-Aldrich, Steinheim, Germany) and (4) mild heat treatment (MHT) at 55 °C. At each
119 disinfection treatment, minimally processed *calçots* were immersed in 10 L disinfectant solution
120 at room temperature under continuous agitation for 60 s. For all washing solutions, pH and
121 oxidation reduction potential (ORP) or REDOX were determined before and after the *calçot*
122 treatment (Table 1). Free chlorine was determined using a free and total chlorine photometer
123 (HI 93734, Hanna Instruments, Eibar, Spain). ORP and pH were determined using a
124 pH/ion/conductivity meter (Model GLP-22, Crison), with a pH electrode (Crison, 52-01) or an
125 ORP electrode (Crison, platinum Ag/AgCl electrode 52-61), respectively. In addition, the
126 microbial load of each washing solution after treatment was evaluated. To carry out such

127 determinations, 1 mL of each washing solution was added to 9 mL of Dey-Engley neutralizing
128 broth (Fluka, Sigma-Aldrich, St. Louis, US) and plated as described below. Afterwards, only
129 samples treated with sodium hypochlorite were rinsed with water for 1 min. All samples were
130 centrifuged at 210 rpm in centrifuge (Marrodán, Navarra, Spain) for 95 s, packaged in
131 polystyrene trays with retractable film and stored at 4 °C for 15 d. The microbiological and
132 quality determinations were carried out at the beginning of the experiment, after the treatments
133 and after 3, 6, 9 and 15 d of shelf-life at 4 °C.

134 2.3 Enumeration of bacterial concentration on *calçots*

135 The enumeration of mesophilic microorganisms and yeast and moulds was performed in
136 the manner described by Alegre, Viñas, Usall, Anguera, & Abadías (2011). Briefly, 10 g of the
137 edible part of *calçots* were diluted in 90 g of buffered peptone water (BPW, Oxoid LTD,
138 Basingstoke, Hampshire, England) in a sterile bag and homogenized in a masticator (IUL
139 Masticator Basic 400 ml, IUL Instruments, Barcelona, Spain) for 90 s. Further ten-fold dilutions
140 were made with saline peptone (SP; 8.5 g L⁻¹ NaCl and 1 g L⁻¹ peptone) and plated in duplicate
141 in Plate Count Agar (PCA, Biokar Diagnostics, Beauvais, France) and in Dichloran Rose
142 Bengale Chloramphenicol Agar (DRBC, Biokar Diagnostics, Beauvais, France) and incubated
143 at 30 ± 1 °C or 25 ± 1 °C for 3 and 5 days, respectively). Three determinations (in three trays)
144 were performed at each sampling time. The results were represented in log₁₀ cfu g⁻¹.

145

146 2.4 pH, soluble solids content and titratable acidity

147 pH, soluble solids content (SSC) and titratable acidity (TA) were measured in the juice
148 extracted by crushing *calçots* pieces in a blender. pH was determined using a penetration
149 electrode in a pH-meter model GLP22 (Crison Instruments SA, Barcelona, Spain). Soluble
150 solids were measured at 20 °C with a hand-held refractometer (Atago Co. Ltd., Tokyo, Japan)
151 and the results were expressed as °Brix. To measure titratable acidity, 10 mL of *calçots* juice
152 was diluted with 10 mL distilled water and it was titrated with 0.1 mol L⁻¹ NaOH (Panreac,

153 Barcelona, Spain) up to pH 8.2. Results of titratable acidity were expressed as g of malic acid L⁻¹
154 ¹. Three determinations were performed per each treatment at each sampling time.

155 2.5 Fresh weight loss

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

159 2.6 Firmness

160 To assess changes in texture, firmness (N) was measured at 5 cm from the roots set in
161 transversal position using the TA.TX2 Texture Analyzer (Stable Micro Systems Ltd., Surrey,
162 England) attached with a Warner-Blatzler blade (HDP/BSK: Blade set with knife). The sample
163 was placed into the press holder, and then the blade was moved downwards at different rates:
164 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
165 of the holder. Data acquisition rate was 200 pulses per sec. Eight individual and randomly
166 chosen *calçots* per treatment at each sampling time were evaluated.

167 2.7 Colour

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

177
$$BI = \frac{100(x-0.31)}{0.172} \quad (1)$$

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

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

180
$$TCD = [(L_f^* - L_i^*)^2 + (a_f^* - a_i^*)^2 + (b_f^* - b_i^*)^2]^{1/2} \quad (2)$$

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

182 2.8 Assessment of overall visual quality

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

187 2.9 Statistical analysis

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

193 3. Results and discussion

194 3.1 Effect of decontamination treatments on microbial load

195 3.1.1 Effectiveness of sanitizers after decontamination

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

205 No significant differences ($P > 0.05$) on the mesophilic counts obtained in *calçots* disinfected by
206 $100 \text{ mg L}^{-1} \text{ NaOCl}$ or $80 \text{ mg L}^{-1} \text{ PAA}$ treatments were observed, reducing 2.3-log units with
207 regard to harvested sample and 0.8-log units with regard to control. According to other studies,
208 the effectiveness of PAA is dependent on the type of the vegetable product. Vandekinderen,
209 Van Camp, et al. (2009) observed a reduction of 1-log units of natural microbiota (between 5.58
210 and $7.29 \log \text{ cfu g}^{-1}$) after washing fresh-cut leeks (*Allium porrum* L.) with $80 \text{ mg L}^{-1} \text{ PAA}$ for
211 60 s. Dai et al. (2012) showed that the population of total aerobic bacteria of fresh-cut Chinese
212 chives significantly decreased after disinfected with $150 \text{ mg L}^{-1} \text{ PAA}$ for 2 min or 100 mg L^{-1}
213 PAA for 5 min (2.16-log units and 2.20-log units, respectively). Van de Velde, Güemes, &
214 Pirovani (2014) reported that total microbial count of fresh-cut strawberries decreased 2.6-log
215 units of initial microbiota after disinfecting with $100 \text{ mg L}^{-1} \text{ PAA}$ for 120 s (40 °C).

216 PAA is known to be an oxidising agent that is in general effective against a wide spectrum of
217 microorganisms (Kyanko, Russo, Fernández, & Pose, 2010). PAA disinfects by oxidizing of the
218 outer cell membrane of bacteria, yeast and moulds, and that oxidation consist on the transfer of

219 electrons (Joshi, Mahendran, Alagusundaram, Norton, & Tiwari, 2013). It has been suggested
220 that PAA acts primarily on lipoproteins in the cell membrane, and could be equally effective
221 against outer-membrane lipoproteins, facilitating its action against Gram-negative cells
222 (Botondi et al., 2016). The action on the membranes could be due different mechanisms: (1)
223 Denaturation of proteins and enzymes and the increase of cell wall permeability by oxidizing
224 sulfhydryl and disulphide bonds; (2) Disruption of cell membranes and the blockage of
225 enzymatic and transport systems in microorganisms. PAA could also damage DNA and lipids
226 through the production of reactive oxygen species (Vandekinderen, Devlieghere, De Meulenaer,
227 Ragaert, & Van Camp, 2009).

228 The population of yeast and moulds (Fig. 1B) of fresh harvested *calçots* ($3.94 \pm 0.31 \log_{10} \text{cfu g}^{-1}$)
229 ¹⁾ decreased after minimal processing and after washing with all tested decontamination
230 treatments. There were no significant differences ($P > 0.05$) between disinfection treatments,
231 achieving reductions of around 0.8-log units with respect to the control and about 2.5-log units
232 with respect to the harvested sample. Stringer et al. (2007) observed that hot water washing (52
233 °C, 90 s) reduced the initial yeast population ($2.0 \log \text{cfu g}^{-1}$) and mould population ($1.1 \log \text{cfu}$
234 g^{-1}) on broccoli. Similar results were obtained in the study carried out by López-Gálvez et al.
235 (2010), where washing with NaOCl (100 mg L^{-1} , 60 s) produced a reduction of 1.2 ± 0.1 -log
236 units on fresh-cut iceberg lettuce (*Lactuca sativa* L.) in contrast to the unwashed lettuce ($3.2 \pm$
237 $1.4 \log \text{cfu g}^{-1}$).

238 3.1.2 Effect of sanitizers on microbial stability

239 Effect of disinfectant treatments on the microbial population of fresh-cut *calçot* stored for 15 d
240 at 4 °C are shown in Table 2. TAPC was maintained in all samples except samples treated with
241 MHT during the storage time and there were no significant differences between them at the end
242 (15 days). The initial population of MHT-treated samples was lower than other samples. Those
243 results were in agreement with those reported by Kim, Feng, Toshkov, & Fan (2005) where

244 TPAC of fresh-cut green onions (*Allium fistulosum* L.) treated at 50 °C for 20 s increased during
245 the 14-day storage period at 4 °C from 4.2 to 7.5 log cfu g⁻¹.

246 Furthermore, Silveira, Aguayo, Escalona, & Artés (2011) showed that the mesophilic
247 population of fresh-cut ‘Galia’ melons (*Cucumis melo* var. *cantalupensis* Naud) treated by
248 HWD at 60 °C for 60 s increased after 10 d at 5 °C, from 3.7 to 7.8 log₁₀ cfu g⁻¹. Thermal
249 treatment would disrupt physical barrier membranes liberating nutrients from the cells which
250 facilitated access to nutrients leading to the greater proliferation of microorganisms (Goyeneche
251 et al., 2015). MHT might alter the ability of the vegetable tissue to support growth of
252 microorganism as it can inactivate plant defences and damage cell membranes which may
253 release nutrients (Stringer et al., 2007).

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

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

266 3.2 Effect of decontamination treatments on quality

267 3.2.1 Soluble solids content, titratable acidity, pH and fresh weight loss

268 Physicochemical characteristics of *calçots* at harvest are shown in Table 3. Changes in SSC,
269 TA, pH and weight on non-disinfected samples (control) and disinfected samples during 15 d of
270 storage at 4 °C are shown in Table 4. In general, disinfection treatments had no remarkable
271 effect on physicochemical characteristics of *calçots*. Despite the pH of the PAA, where
272 disinfection treatment was around 3.4 before and after washing the *calçots* (Table 1), the acidic
273 pH conditions did not affect their initial quality. These results contrasted with those observed by
274 Vandekinderen, Devlieghere, Van Camp, et al. (2009), who reported a reduction by 0.6 pH-
275 units after disinfecting fresh-cut carrots (*Daucus carota* L.) with 80 mg L⁻¹ PAA for 300 s. They
276 suggested that this pH-decrease was caused by the acidic pH of the PAA solutions used for
277 disinfection. In addition, there were no significant differences (P>0.05) between disinfected
278 samples and control after a 15-d storage period at 4 °C with regard to TA (ranging from 1.67 to
279 2.09 g malic acid L⁻¹). Only values of SSC and pH presented barely detectable statistically
280 significant differences among treatments, being the highest values in the control sample (8.8
281 °Brix and pH 5.9) after 15 d of storage. In a study carried out with fresh-cut melon the
282 disinfection with 100 mg L⁻¹ Tsumani 100TM solution (composed of 15.2 % peracetic acid and
283 11.2 % hydrogen peroxide) for 3 min (20 ± 1 °C) had no effect on the SSC (Botondi et al.,
284 2016).

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

293 3.2.2 Firmness, colour and visual quality

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

298 Despite the increment in TCD and BI values of samples during storage, disinfection conditions
299 of MHT at 50 °C for 60 s led to the lowest browning index in *calçots* throughout storage (Fig.
300 2B and 2C). This behaviour might be explained through the possible inactivation of PPO
301 (Polyphenol oxidase) and POD (peroxidase) which play an important role in skin colour
302 changes and a mild-heat level of temperature might have more effect than acidic medium in the
303 inactivation of those enzymes.

304 The results obtained in visual overall quality (Fig. 2D) demonstrated that all disinfected samples
305 maintained excellent and very good scores until day 6 of storage at 4 °C. Those results were in
306 agreement with those reported by Alvaro et al. (2009), where no differences were found by a
307 trained panel in whole tomatoes, peppers and cucumber washed with any applied treatment
308 including PAA. Although all samples were above the limit of marketability, only *calçots* treated
309 with MHT presented scores above 7 (very good) at the end of the 15-d storage period. Despite
310 the weight loss, overall quality was not affected. Furthermore, visual quality decreased
311 drastically for *calçots* treated with NaOCl and PAA, 4 and 3.3 points after the 15-d storage
312 period, respectively. Hong et al. (2000) suggested that mild-heat level of treatment at 55 °C for
313 120 s was one of several heat treatments that was effective enough to control 'telescoping' of
314 cut green onions (*Allium cepa* x *A. fistulosum*).

315 **4. Conclusions**

316 The physical and microbiological quality of fresh-cut *calçots* after disinfecting with NaOCl,
317 PAA and MHT were studied. In general, although all samples presented physiochemical
318 changes after disinfection and a 15-d storage period at 4 °C, *calçots* treated with MHT at 55 °C
319 for 60 s retained those characteristics better than other samples. However, the population of

320 aerobic mesophilic bacteria in mildly heated *calçots* increased during the 15-d storage period. It
321 is remarkable that there were no significant differences with regard to the results of *calçots*
322 treated with NaOCl and PAA. Therefore, our results have shown that sanitization treatment with
323 PAA (80 mg L⁻¹) for 60 s could be an alternative to chemical methods based on chlorine,
324 maintaining physicochemical and microbiological quality and consumer acceptance.
325 Furthermore, this treatment could be a suitable disinfection treatment for other fresh-cut
326 vegetables, such as leafy vegetables which usually have high initial microbial loads compared to
327 other vegetables.

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- 459

460 **Figure captions**

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

465 **Fig. 2.** Effect of disinfectant treatments [control (◆), NaOCl (■), PAA (▲) and MHT (●)] after
466 60 s of treatment on firmness (A), TCD (B), BI (C) and overall visual quality (D) of fresh-cut
467 *calçots* stored for 15 d at 4 °C. TCD: Total Colour Difference; BI: Browning Index.
468 Replications: n = 8 (firmness) and n = 36 (colour). PAA: peroxyacetic acid; MHT: Mild heat
469 treatment.

470

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 \pm 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 \pm 0.10 Aa	4.98 \pm 0.04 Ab	5.00 \pm 0.32 Ab	4.14 \pm 0.30 BCc
	3	4.86 \pm 0.47 Aa	4.66 \pm 0.90 Aab	4.72 \pm 0.19 Aa	3.29 \pm 0.37 Cb
	6	5.12 \pm 0.07 Aa	4.58 \pm 0.89 Aa	4.26 \pm 0.35 Aa	4.36 \pm 0.36 Ba
	9	5.13 \pm 0.05 Aa	4.86 \pm 0.40 Aa	4.87 \pm 0.19 Aa	4.89 \pm 0.47 ABa
	15	4.89 \pm 0.50 Aa	5.28 \pm 0.90 Aa	4.88 \pm 0.49 Aa	5.35 \pm 0.18 Aa
Y&M (Log ₁₀ [cfu g ⁻¹])	0	2.29 \pm 0.50 Aa	1.41 \pm 0.00 Ab	1.51 \pm 0.17 Ab	1.41 \pm 0.00 Ab
	3	2.26 \pm 0.31 Aa	2.07 \pm 0.68 Aa	2.11 \pm 0.69 Aa	1.51 \pm 0.17 Aa
	6	2.40 \pm 0.00 Aa	1.61 \pm 0.17 Ab	1.51 \pm 0.17 Ab	1.41 \pm 0.00 Ab
	9	2.02 \pm 0.55 Aa	1.41 \pm 0.00 Aa	1.67 \pm 0.44 Aa	1.41 \pm 0.00 Aa
	15	1.61 \pm 0.17 Aa	1.41 \pm 0.00 Aa	1.61 \pm 0.34 Aa	1.71 \pm 0.29 Aa

Table 3.Physicochemical characteristics of *calçots* at harvest.

Quality measurements	Mean \pm SD
SSC ($^{\circ}$ Brix)	10.03 \pm 0.21
TA (g malic acid L ⁻¹)	1.99 \pm 0.01
pH	5.92 \pm 0.03
Firmness (N)	61.84 \pm 10.45
Colour parameters	
L*	80.08 \pm 2.69
a*	-0.91 \pm 0.46
b*	5.18 \pm 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 \pm 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 \pm 0.1 Cc	8.2 \pm 0.0 Aa	7.9 \pm 0.0 Ab	7.1 \pm 0.1 Cd
	3	7.7 \pm 0.1 CDa	7.2 \pm 0.1 Db	7.2 \pm 0.2 Bb	7.7 \pm 0.2 ABa
	6	8.1 \pm 0.1 Ba	6.9 \pm 0.1 Ec	7.5 \pm 0.1 Bb	7.4 \pm 0.0 Bb
	9	7.5 \pm 0.1 Da	7.5 \pm 0.0 Ca	7.6 \pm 0.1 ABa	6.5 \pm 0.1 Db
	15	8.8 \pm 0.1 Aa	7.7 \pm 0.1 Bb	7.3 \pm 0.2 Bc	7.8 \pm 0.1 Ab
TA (g malic acid L ⁻¹)	0	1.90 \pm 0.04 BCa	1.84 \pm 0.13 BCa	1.70 \pm 0.10 Ca	2.01 \pm 0.20 BCa
	3	4.13 \pm 0.32 Aa	2.95 \pm 0.18 Ab	2.64 \pm 0.28 Bb	3.60 \pm 0.70 Aab
	6	1.77 \pm 0.06 BCb	1.98 \pm 0.17 BCab	2.19 \pm 0.09 Ba	2.31 \pm 0.17 BCa
	9	2.37 \pm 0.34 Ba	1.67 \pm 0.10 Cb	1.82 \pm 0.09 BCb	2.72 \pm 0.04 ABa
	15	1.75 \pm 0.18 Ca	2.09 \pm 0.07 Ba	1.67 \pm 0.16 Ca	1.78 \pm 0.23 Ca
pH	0	6.0 \pm 0.0 Aa	5.9 \pm 0.0 Ab	6.0 \pm 0.0 Aa	5.8 \pm 0.0 Ac
	3	5.7 \pm 0.0 Db	5.8 \pm 0.0 Ba	5.8 \pm 0.0 Ca	5.8 \pm 0.0 Aa
	6	5.9 \pm 0.0 Ba	5.9 \pm 0.0 Aa	5.9 \pm 0.0 Ba	5.8 \pm 0.0 Ab
	9	5.8 \pm 0.0 Ca	5.8 \pm 0.0 Ba	5.8 \pm 0.0 Ca	5.8 \pm 0.0 Aa
	15	5.9 \pm 0.0 Ba	5.9 \pm 0.0 Aa	5.9 \pm 0.0 Ba	5.8 \pm 0.0 Ab
FWL (%)	0	0.00 \pm 0.00 Ba	0.00 \pm 0.00 Ca	0.00 \pm 0.00 Ca	0.00 \pm 0.00 Aa
	3	0.56 \pm 0.40 Ba	0.51 \pm 0.75 Ca	0.85 \pm 0.21 BCa	3.02 \pm 4.85 Aa
	6	0.96 \pm 0.75 Ba	0.33 \pm 0.29 Ca	1.05 \pm 1.06 BCa	3.21 \pm 4.71 Aa
	9	1.18 \pm 1.15 Ba	1.47 \pm 0.31 Ba	2.04 \pm 0.69 Ba	3.75 \pm 5.15 Aa

15

 4.82 ± 1.19 Aa 3.42 ± 0.30 Aa 5.47 ± 0.69 Aa 3.31 ± 7.99 Aa

ACCEPTED MANUSCRIPT

Figure 1

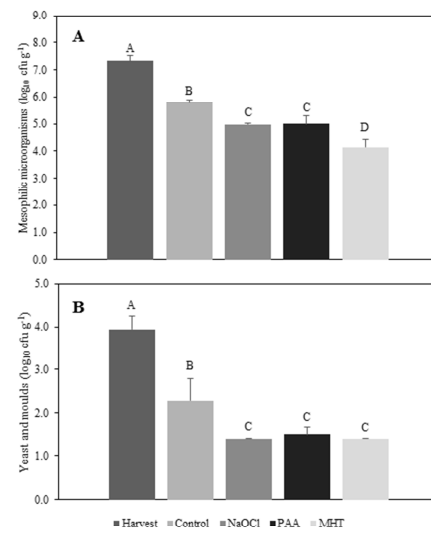
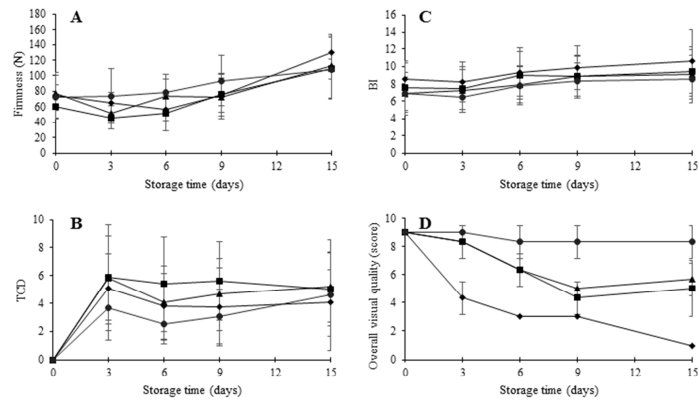


Figure 2



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