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Effects of long-term controlled atmosphere storage, minimal processing, and packaging on quality attributes of *calçots* (*Allium cepa* L.)

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Abstract

Calcots are the immature floral stems of the second-year onion (Allium cepa L.) resprouts. Modified atmosphere packaging or vacuum packaging are suitable alternatives to preserve fresh-cut vegetables. The objective of this study was to evaluate the effect of postharvest storage time of raw vegetable stored under controlled atmosphere and used packaging system after minimal processing on the quality of fresh-cut calcots. Calcots used for minimal processing were stored under 1.0 kPa $O_2 + 2.0$ kPa CO₂ at 1 °C for 30 and 60 days. Fresh-cut *calcots* were packaged using passive modified atmosphere packaging or vacuum packaging and were stored at 4 °C for 15 days. Calcots stored under controlled atmosphere for 30 days presented better retention of quality and in turn, being more suitable for minimally processing. Vacuum packaging preserved the physicochemical quality of fresh-cut *calcots* better after 15 days. Mesophilic aerobic counts were also higher in fresh-cut calcots stored under modified atmosphere packaging, but all counts were below the recommended limits during and at the end of their shelf-life (15 days). The most suitable conservation strategy might be to store whole *calcots* under controlled atmosphere for 30 days and after minimally processing, packaged under vacuum in order to extend the shelf-life of fresh-cut calçots.

Keywords Fresh-cut, Allium, packaging, modified atmosphere, vacuum, quality

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). Although they are usually consumed grilled or roasted, there is currently an increasing trend toward consuming raw *calçots* in, for example, salads (<u>Simó et al., 2013; Zudaire et al., 2017</u>).

Controlled atmosphere (CA) is used to prolong the quality of fresh fruit and vegetables by modifying the atmospheric composition different from air while

supplementing proper temperature and relative humidity management during storage (Kader, 2016). United Fresh Produce Association defines fresh-cut produce as any fresh-cut fruit or vegetable or any combination of them that has been physically altered but remains in the fresh state. The production of fresh-cut produce is increasing due to the modern consumption trends which include health concerns and time and effort saving (Denoya et al., 2015). Moreover, fruit and vegetables have high vitamin and low calorie content with accurate sensory quality as suitable components of any healthy diet (Artés and Allende, 2014). Minimal processing includes operations such as peeling and cutting which cause wounding that significantly reduce the shelf-life of freshcut products. Those operations not only make the fresh-cut fruit and vegetables more susceptible to microbial attack compared to intact produce but also cause damage to tissues and cellular structure, leading to leakage of nutrients and cellular fluids (Heard, 2002). Physical preservative treatments such as modified atmosphere packaging (MAP) or vacuum packaging (VAC) have been developed in order to extend the shelf-life of whole and fresh-cut products (Zhang et al., 2014).

A large number of fresh-cut fruits and vegetables are stored and marketed in MAP in combination with chilled storage or other preservation procedures (Hodges and Toivonen, 2008). MAP refers to the technique of sealing actively respiring produce in polymeric film packages to modify the O₂ and CO₂ concentrations within the package atmosphere (Mir and Beaudry, 2016); those concentrations are reached by the natural interaction between the respiration rate of the product and the transfer of gases through the packaging material (Oliveira et al., 2015). One of the primary effects of MAP is a lower respiration rate, which reduces the rate of substrate depletion and oxidation reactions (Sothornvit and Kiatchanapaibul, 2009). MAP has been successfully used to maintain the quality of fresh-cut fruit and vegetables (Oliveira et al., 2015) such as onions (Hong and Kim, 2004; Martínez et al., 2005).

VAC, which removes the air from the package, is now commonly used to extend the shelf-life of whole and fresh-cut fruit and vegetables. The use of VAC inhibits the advance of oxidative reactions and inhibits the growth of aerobic microorganism, which generally triggers deterioration of fruit and vegetables during storage (<u>Rocha et al., 2003</u>). The disadvantage of this

method is the extremely low oxygen concentration that could increase the growth of anaerobic pathogens (<u>Lee et al., 2011</u>), fermentative metabolism, and accumulation of ethanol and acetaldehyde (<u>Denoya et al., 2015</u>). VAC systems have been investigated for example in onions (<u>Hong and Kim, 2004; Pérez-Gregorio et al., 2011</u>).

The objective of this study is to develop a novel ready-to-eat product as ingredient for salads or other dishes. In order to achieve this goal, the influence of CA storage's time and packaging type (MAP or VAC) on the microbial load and quality attributes of fresh-cut *calçots* was studied.

Materials and methods

Plant material

Calçots (*Allium cepa* L.) were provided by "Cooperativa Agricola Valls" (Tarragona, Spain) at commercial size. The *calçots* had the European quality label PGI "Calçot de Valls." They were cultivated in northeast Spain (41°13'47"N, 01°13'12"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 8000 plants per hectare. The resprouts arising in the autumn were covered with soil three times to increase the length of the edible white part and harvested in February.

Based on previous results (Zudaire et al., 2017), *calçots* used for minimal processing and storage were those stored under CA with 1.0 kPa O_2 + 2.0 kPa CO_2 at 1 °C with 85% of RH for 30 and 60 days which resulted effective to keep quality and nutritional parameters. Pre-conditioning was conducted according to the methodology previously described by <u>Aguiló-Aguayo et al. (2016)</u>.

Packaging of samples

Fresh-cut *calçots* were packaged using two different packaging methods: passive MAP without initial gas injection and VAC. MAP storage conditions were established following results obtained in a previous study carried out by <u>Aguiló-Aguayo et al. (2016)</u>. Samples were packaged in Cryovac® BN3050W bags (200 mm × 310 mm) with 48 µm of thickness (Sealed Air Global Headquarters, USA) and O₂ and CO₂ transfer rate of 5000 and 22,000 cm³ m⁻² d⁻¹ atm⁻¹ ¹ at 23 °C and 0% RH, respectively. In VAC conditions, vacuum bags (polypropylene, 150 × 300 mm) were sealed using JOLLY LCD vacuum-sealing machine (Lavezzini, Italy) with 93% of vacuum (one cycle of Step Vac and Soft Vac). In both MAP and VAC, 5 fresh-cut *calçots* were stored per bag (around 200 g in each pack) and all experiments were conducted in triplicate. Finally, bags were stored at 4 °C for 15 days and analyzed at days 0, 3, 6, 9 and 15 days for microbial and physicochemical quality and visual evaluation. The results of day 9 were not shown due a microbiological contamination in samples.

Headspace atmosphere

Headspace gas composition was measured according to the methodology previously described by <u>Iglesias et al. (2018)</u>. Briefly, before the microbiological analysis, headspace gas composition (CO₂ and O₂) was determined before opening the trays using a handheld gas analyzer (CheckPoint O_2/CO_2 ; PBI Dansensor, Denmark) at each sampling time. The gas composition in VAC packages was not measured because the film was completely fixed around the samples without free headspace. The results were expressed as kPa.

Soluble solids content, titratable acidity, and pH

Soluble solids content (SSC), titratable acidity (TA), and pH were measured as previously described by <u>Zudaire et al. (2018)</u>. pH, SSC, and TA were measured in the juice extracted by crushing *calçots* pieces in a blender. The results of SSC were expressed as °Brix and of TA as g of malic acid L⁻¹. Three determinations were performed per each packaging treatment at each sampling time.

Color

Color was measured as previously described by <u>Zudaire et al. (2018)</u>. The color of the white shaft was measured with a CR-200 Minolta Chroma Meter (Minolta, Inc., Tokyo, Japan). Color was measured using CIE L*, a*, b* coordinates with illuminant D65 and 10° observer angle. These values were used to calculate the browning index (BI) described by <u>Liu et al. (2016)</u>:

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

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

Fresh weight loss

Weight of individual fresh-cut *calçots* was recorded at each sampling date. Fresh weight loss (FWL) was calculated as described by <u>Son et al. (2015)</u>.

Firmness

To assess changes on texture, firmness (N) was measured as previously described by <u>Zudaire et al. (2018)</u>. Briefly, firmness 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). A total number of eight samples were evaluated per replicate and sampling date.

Microbiological quality

The enumeration of mesophilic microorganisms and yeast and molds was performed as described by <u>Alegre et al. (2011)</u>. Briefly, 10 g of the edible part of *calçots* were diluted in 90 g of buffered peptone water (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 10-fold dilutions were made with saline peptone (8.5 g L⁻¹ NaCl and 1 g L⁻¹ peptone) and plated in duplicate in Plate Count Agar (Biokar Diagnostics, Beauvais, France) and in Dichloran Rose Bengale Chloramphenicol Agar (Biokar Diagnostics, Beauvais, France) and incubated at $30 \pm 1 \,^{\circ}$ C or $25 \pm 1 \,^{\circ}$ C for 3 and 5 days, respectively. The results were represented as log colony forming units (cfu) per gram basis on fresh weight. Three determinations (in three bags) were performed per each replicate at each sampling date.

Statistical analysis

Results were expressed as mean ± standard deviation. All data were firstly evaluated for normality (Shapiro-Wilk W Test) and homogeneity of variance (Levene's Test) of residues. Significant differences between results were calculated by using one-way analysis of variance (ANOVA). In case of nonnormality or unequal variances, the non-parametric equivalents (Wilcoxon/Kruskal-Wallis Tests) were used. Differences were significant at P < 0.05 (95% confidence level). In case of significant differences, multiple comparisons of means were established with the Post Hoc Tukey-Kramer HSD or Student's test. Moreover, two-way ANOVA (postharvest storage time and packaging type) was applied in order to know the incidence of these factors (Table 1). All statistical analyses were performed with JMP 8 software (SAS Institute Inc., Cary, NC, USA).

Factor	Browning index	Firmness	SSC	ТА	рН	Total aerobic count	Y&M
D0							
CA storage time	<.0001*	0.0017*	<.0001*	<.0001*	<.0001*	1.0000	1.0000
Packaging type	1.0000	1.0000	0.0222	0.0217*	<.0001*	0.4061	0.0421*
Storage time × packaging type	1.0000	1.0000	<.0001*	0.3264	0.0013*	1.0000	1.0000
D3							
CA storage time	0.3467	0.0002*	<.0001*	0.0233*	0.0004*	0.4622	_
Packaging type	0.2575	0.6264	0.0011	0.0191*	<.0001*	0.4592	-
Storage time × packaging type	0.3140	0.2622	0.0001*	0.6490	0.0016*	0.7142	
D6							
CA storage time	<.0001*	0.2707	0.0005*	0.0114*	<.0001*	<.0001*	0.0005*
Packaging type	0.0001*	0.5221	<.0001*	<.0001*	<.0001*	0.2417	0.0012*
Storage time × packaging type	0.0616	0.1508	<.0001	0.0081	<.0001*	0.0008*	0.0031*
D15							
CA storage time	0.4711	0.0983	<.0001*	0.0004*	<.0001*	0.0002*	0.2498
Packaging type	<.0001*	0.0429*	0.0023*	0.0009*	<.0001*	0.5962	0.0171*
Storage time × packaging type	0.1873	0.7300	0.4609	0.0011*	<.0001*	0.6758	0.0672

Effect of postharvest storage time under controlled atmosphere, packaging type after processing and postharvest storage time \times packaging type on physicochemical and microbiological quality of fresh-cut *calçots* at each sampling date. *P < 0.05 indicates a significant effect (single or interaction). SSC: soluble solids content; TA: titratable acidity; CA: controlled atmosphere; Y&M: yeast and mold.

Table 1. Two-way ANOVA: Postharvest storage time and packaging type

Results and discussion

Changes of gas composition within the packages

The objective of passive MAP is to maintain O₂ concentrations between 1% and 5% in the headspace of the fresh-cut vegetables during storage. However,

finding the optimal O₂ concentrations for fresh-cut vegetables is guite difficult as it depends on the type of vegetable and the cultivar (Vermeulen et al., 2018). The headspace gas composition (CO₂ and O₂) of modified atmospherepackaged fresh-cut calcots during two postharvest storage times (30 and 60 days) was evaluated (Figure 1). The gas composition in VAC packages was not measured because the film was completely fixed around the samples without free headspace. No significant differences (P > 0.05) were observed between the O₂ and CO₂ concentrations of fresh-cut *calcots* with a previous storage period of 30 or 60 days indicating that postharvest storage time had no influence on the respiration rate of the fresh-cut product. As expected, the CO₂ concentrations in modified atmosphere-packaged samples increased to 4.9–5.8 kPa after 3 days of storage, while O₂ concentrations decreased drastically up to 1.4–1.8 kPa after the same period. Nevertheless, Lucera et al. (2011b) reported that O₂ concentrations of fresh-cut broccoli florets packaging under MAP decreased rapidly to zero during the first 40 h of storage. Moreover, they showed that observed decrease was different and it depended on the film applied. The respiration increase observed in the present study could be associated with a more active metabolism of samples producing a possible loss of sugars and other soluble substrates (Cantwell and Suslow, 2002). Moreover, the induction of wounding stresses in the cut tissues as a result of mechanical injury led to an increase in the respiration rate (Watada et al., 1996). However, the permeability used for fresh-cut calcots was adapted to the respiration of the product reaching a sustainable equilibrium of gas concentrations after 15 days of storage at 4 °C. Moreover, the low temperature (4 °C) led to a decrease of the respiratory rate of the freshcut calcots, which prolonged the shelf-life (Berno et al., 2014).

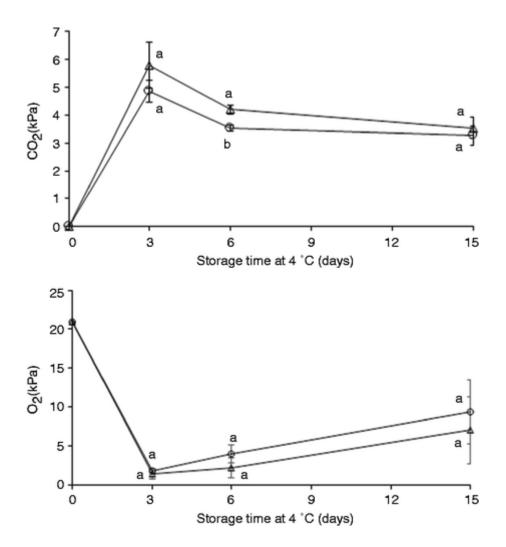


Figure 1. CO₂ and O₂ headspace concentration (kPa) inside fresh-cut *calçots* trays (passive MAP) throughout storage time at 4 °C and previously stored under CA for 30 days (\circ) or 60 days (Δ). Different letters indicate significant differences between CA storage times (P < 0.05) for each sampling date. The error bars represent the standard deviation with a 95% of confidence interval.

Similar results were observed in the study carried out by Page et al. (2016), where O_2/CO_2 concentrations changed during storage inside the bags contained sliced onions and they reached O_2 equilibrium after about 7 days of storage (1.5–3 kPa). In concordance, Ghidelli et al. (2014) reported that fresh-cut eggplant packaged under passive MAP reached an equilibrium on day 6 with the O_2 concentrations below 2 kPa and CO₂ concentrations of 15–18 kPa. Moreover, Fonseca et al. (2018) reported that O_2 consumption and CO₂ production rates evidence an increasing pattern with storage time after cutting red onions and the O_2 consumption and CO₂ production rate increased 2.0 times after 24 h at 4 °C.

SSC, TA, and pH

Two-way ANOVAs performed on the chemical parameters revealed significant variations (P < 0.05) depending on the postharvest storage time and packaging type studied (<u>Table 1</u>). Moreover, the analysis showed a significant interaction (postharvest storage time × packaging type) at each sampling date.

Values of SSC, TA, and pH of fresh-cut calcots packaged under MAP or VAC are shown in Figure 2. SSC values increased in samples with a postharvest storage of 30 days and decreased in those stored during 60 days after 15 days at 4 °C (Figure 2(a)). The increase observed in 30-day samples might be caused by the solubilization and synthesis of those sugars (Waghmare and Annapure, 2013). And the decrease observed in 60-day samples may be due to the increased metabolic activity as shown in the respiration value that accelerates the utilization of soluble solids (Han et al., 2016). Moreover, those samples packaged under MAP presented higher SSC values than those packaged under VAC at the same CA storage time (P < 0.05). Pinela et al. (2016) reported that a significant reduction in SSC was observed in fresh-cut watercress packaged under VAC and stored in the dark at 4 °C for 7 days. Han et al. (2016) reported that SSC decreased slowly in fresh-cut Welsh onions after 7 days storage at 4 °C, regardless of the cut type. Nevertheless, Gibe and <u>Kim (2013)</u> reported that values of SSC in fresh-cut winter squash increased vaguely from 17.2% to 18.6% and were maintained along 10 days of storage at 10 °C.

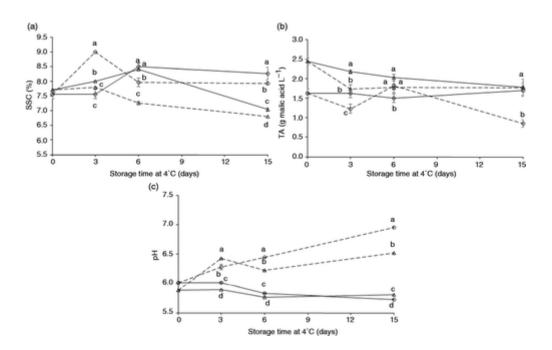


Figure 2. Soluble solids content (a), titratable acidity (b) and pH (c) of fresh-cut *calçots* packaged under passive MAP (—) or VAC (--) during 15-day storage time at 4 °C and previously stored under CA for 30 days (\circ) or 60 days (Δ). Different letters indicate significant differences (P < 0.05) between samples for each sampling date. The error bars represent the standard deviation with a 95% of confidence interval.

The permeability of the film used in the packaging could have an effect on the SSC of the fresh-cut fruits and vegetables. Indeed, <u>Becaro et al.</u> (2016) demonstrated that the SSC values of fresh-cut carrots packaged by LDPE and MS films were slightly higher than the values found for carrots packaged by MT films. They concluded that the lower SSC values for fresh-cut carrots packaged by MT film could be a result of lower permeability, which reduced the metabolic activity of the carrot tissue.

TA values of fresh-cut *calçots* decreased after 15 days at 4 °C, except for those stored previously under CA for 30 days and after packaged under MAP (Figure 2(b)). Overall, samples packaged under MAP presented higher TA values than those packaged under VAC at the same CA storage time (P < 0.05). Two-way ANOVA demonstrated the significant effect (P < 0.05) of postharvest storage time under CA and packaging type (separately) on the TA of fresh-cut *calçots* at each sampling date (Table 1). Indeed, TA values were higher in samples stored previously under CA for 60 days than those stored for 30 days due to the initial values (P < 0.05). This trend could be a response of the long postharvest storage time increasing the respiratory activity and the overall metabolic activities of whole *calçots* (Özden and Bayindirli, 2002).

Generally, pH values of fresh-cut *calcots* packaged under MAP decreased up to 5.8, whereas under VAC increased up to 7.0 after 15 days of storage at 4 °C (Figure 2(c)). However, Gibe and Kim (2013) showed that pH of fresh-cut winter squash packaged under MAP increased slowly during storage. Pinela et al. (2016) reported that passive MAP was the most appropriate treatment to maintain the initial pH value of fresh-cut watercress stored in the dark at 4 °C for 7 days. They suggested that those results could be related to the increase of organic acids, namely malic acid, and reduced glucose levels, probably due to fermentative processes. In both MAP and VAC, pH values were significantly higher (P < 0.05) in fresh-cut *calcots* with postharvest storage of 30 days than those stored for 60 days. The effect of postharvest storage time of whole calcots under CA could be confirmed by the two-way ANOVA (Table 1). In general, an inverse relationship was observed between total acidity and pH values of fresh-cut calcots. In the same way, Berno et al. (2014) reported that during storage, the acidity of fresh-cut purple onions increased gradually, while the pH decreased. Moreover, they remarked that dicing resulted in higher acidity and lower pH than slicing. However, Forney et al. (2012) reported that the pH of fresh-cut onions stored in sealed PLA, PE and vented PLA decreased until 5.4, 5.1 and 4.3 after 21 days at 4.5 °C, respectively. In concordance, Oliveira et al. (2012) reported that the pH of fresh sliced mushrooms was influenced by time (7 days) and temperature (0, 5, 10, or 15 °C), showing a slight decrease with both parameters.

Color, firmness, and weight loss

The effects of different packaging systems on the BI, firmness, and weight loss of fresh-cut *calçots* are shown in <u>Tables 2</u> and <u>3</u>. Overall, BI index values increased in fresh-cut *calçots* packaged under MAP and kept constant under VAC packaging conditions with the increase of storage time. BI values of modified atmosphere-packaged *calçots* were higher in those previously stored under CA for 60 days than for 30 days due to high initial values (P < 0.05). Two-way ANOVAs revealed that the effect of postharvest storage time or packaging type on BI depends on the sampling date. However, the analysis showed no significant postharvest storage time × packaging type interaction.

CA storage time	Sampling time (days)	Browning index	Firmness (N)
30 days	0	5.49 ± 1.11 Cb	$73.37 \pm 17.99 \text{ Bb}$
	3	$7.83\pm1.59~Bb$	114.95 ± 26.09 Aa
	6	$8.18\pm1.93~Bb$	92.23 ± 22.95 ABa
	15	9.47 ± 2.50 Aa	99.58 ± 35.92 ABa
60 days	0	7.74 ± 2.46 Ba	94.06±15.62 Aa
	3	8.97 ± 1.89 ABa	$72.33\pm17.04~\text{Ab}$
	6	9.17±2.18 Aa	95.73 ± 24.40 Aa
	15	9.27 ± 1.49 Aa	88.41 ± 8.72 Aa

Changes in browning index and firmness of fresh-cut *calçots* packaged under passive modified atmosphere packaging for 15-day storage period at 4 °C and previously stored in CA for 30 days or 60 days. Values are expressed as the mean \pm standard deviation. Different upper case letters in the same column indicate significant differences between sampling times (P < 0.05) for each CA storage time. Different lower case letters indicate significant differences between CA storage times (P < 0.05) for each sampling date. CA: controlled atmosphere.

Table 2. Modified atmosphere packaging

CA storage time	Sampling time (days)	Browning index	Firmness (N)
30 days	0	5.49±1.11 Ba	73.37 ± 17.99 Ab
	3	9.10 ± 5.78 Aa	102.32 ± 18.28 Aa
	6	$6.22\pm1.46~\text{Bb}$	100.44 ± 39.96 Aa
	15	6.16 ± 1.42 Ba	85.04±19.03 Aa
60 days	0	$7.74\pm2.46~\text{ABb}$	94.06±15.62 Aa
	3	$9.05\pm4.14~\text{Aa}$	$77.36\pm24.50~\text{Ab}$
	6	$8.49\pm2.41~ABa$	74.68 ± 20.52 Aa
	15	6.76±1.93 Ba	68.15±17.95 Aa

Changes in browning index and firmness of fresh-cut *calçots* packaged under vacuum packaging for 15-day storage period at 4 °C and previously stored in CA for 30 days or 60 days. Values are expressed as the mean ± standard deviation. Different upper case letters in the same column indicate significant differences between sampling times (P < 0.05) for each CA storage time. Different lower case letters indicate significant differences between CA storage times (P < 0.05) for each sampling date. CA: controlled atmosphere.

Table 3. Vacuum packaging

<u>Hong and Kim (2004)</u> reported that color of fresh-cut bunched onions was not significantly influenced by MAP (active or passive) or VAC. However, <u>Waghmare and Annapure (2015)</u> reported that disinfection with sodium hypochlorite and packaging under MAP maintained the color of fresh-

cut cilantros at 25 days of storage. The maintenance of BI in freshcut *calçots* packaged under VAC (extremely low O₂ concentration) could be due to the suppression or the inhibition of enzymatic browning reactions because oxygen is a substrate of those enzymatic reactions (<u>Denoya et al.</u>, <u>2015</u>). In concordance, <u>Ghidelli et al. (2015</u>) reported that the passive MAP was more effective than the high O₂ atmosphere in order to control browning of fresh-cut artichoke.

The main factors influencing the conservation of fresh-cut vegetables are the storage temperature and the packaging system. Both should mainly aim to reduce the respiration rate, which will lead to a reduced degradation of texture and a better preservation of the quality (Vermeulen et al., 2018). The weight of packaged fresh-cut onions decreases during storage, depending on several factors such as respiration rate, temperature, microbial growth, and so on (Bahram-Parvar and Lim, 2018). However, in the present study, firmness (Table 2 and 3) and FWL (data not shown) of fresh-cut calcots under two packaging treatments and postharvest storage times were maintained after 15 days of storage at 4 °C. Those results agreed with the visual appearance (data not shown). Moreover, two-way ANOVA revealed that neither postharvest storage time nor packaging type had no effect on the firmness values at each sampling date (Table 1). Two of the major factors involved in the loss of texture are water loss and osmotic changes, and hence, FWL could be affected (Bico et al., 2009). However, Hong and Kim (2004) reported that FWL of fresh-cut bunched onions stored under MAP (active or passive) or VAC increased until 1.50–3.0% along storage at 10 °C for 14 days. Escalona et al. (2007) showed that the use of passive MAP helped maintaining firmness and induced good fresh quality of kohlrabi sticks during 14 days of storage at 0 °C, especially when those were placed in amide-PE bags. The maintenance of firmness during storage of VAC samples could be because moist did not dry out due to the absence of air which usually absorbs the moisture from the product (Rocha et al., 2003). Nevertheless, Pérez-Gregorio et al. (2011) reported that VAC produced produces water loss in fresh-cut onions after 16 days, generating an exudate.

Microbiological analysis

The overall shelf-life of a fresh-cut vegetable is decided by its physical and chemical quality as well as its microbial quality (Vandekinderen et al., 2008). Microbial growth is a key factor in the degradation of fresh-cut vegetables. Total bacterial counts represent the most relevant index to define quality of fresh-cut vegetables (Di Carli et al., 2016). The initial mesophilic aerobic bacteria count of fresh-cut *calcots* were 4.5 ± 0.4 and $4.3 \pm 0.2 \log c fu g^{-1}$ for 30 and 60 days, respectively (Figure 3(a) and (c)). In general, no significant differences (P > 0.05) were observed between the microbial population of fresh-cut samples with a previous postharvest storage of 30 days or 60 days, irrespective of the packaging method applied. However, two-way ANOVA revealed that postharvest storage time had effect (P < 0.05) only at day 6 and 15 of shelf-life study (Table 1). Counts were higher in those samples packaged under MAP than under VAC (P < 0.05). Ranjitha et al. (2015) reported that total aerobic plate count was suppressed more in fresh-cut green bell pepper packaged under MAP than in non-MAP. Moreover, the growth was gradual reaching values less than 6 cfu or CFU g^{-1} until day 8 of storage at 8 °C.

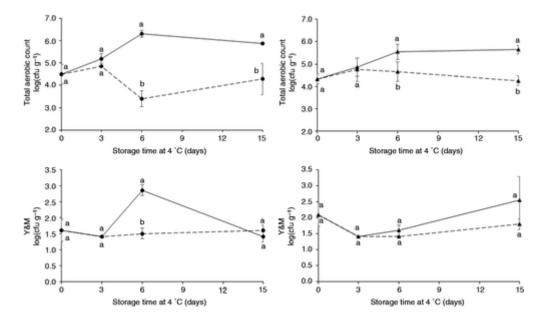


Figure 3. Development of total aerobic counts (a and c) and yeast and mold (b and d) in freshcut *calçots* packaged under passive MAP (—) or VAC (---) during 15-day storage time at 4 °C and previously stored under CA for 30 days (•) and 60 days (•). Different letters indicate significant differences (P < 0.05) between modified atmosphere- and vacuum-packaged samples for each sampling date. The error bars represent the standard deviation with a 95% of confidence interval.

Hong and Kim (2004) also observed slightly lower microbial populations (around 6 log cfu g⁻¹) in cut bunched onions stored under VAC than in MAP up to 28 days at 10 °C. This trend was directly related with the limitation of O_2 inside the package because low O_2 concentrations could inhibit the growth of the aerobic microbiota (Farber, 1991). Nevertheless, the counts at the end of storage in both modified atmosphere- and vacuum-packaged samples were kept below 6 log cfu g⁻¹ indicating that the concentrations of O_2 in the packages were effective in controlling the growth of mesophilic aerobic bacteria. Similar results were obtained in a studies carried out with fresh-cut green beans and fresh-cut broccoli florets packaged under MAP (Lucera et al., 2011a, 2011b). Mesophilic aerobic bacteria counts of all studied fresh-cut *calçots* not exceeded the recommended limit of 8 log cfu g⁻¹ proposed by <u>CNERNA-CNRS (1996)</u>.

Regarding the yeast and mold load, the initial counts were 1.6 ± 0.3 and $2.1 \pm 0.4 \log$ cfu g⁻¹ for 30 and 60 days, respectively (Figure 3(b) and (d)). Overall, there were no significant differences (P > 0.05) between counts of modified atmosphere- and vacuum-packaged *calçot* except at day 6 in those with a postharvest storage of 30 days. The population of freshcut *calçots* packaged under VAC was maintained along storage time and there were no significant differences (P > 0.05) between samples. Moreover, twoway ANOVA revealed that postharvest storage time had no effect on the yeast and molds count at each sampling date. Waghmare and Annapure (2015) reported that the population of yeast and molds increased along the storage time (25 days) in fresh-cut cilantros disinfected and packaged under MAP, but that increase was lower regarding to the rest of treatments. Similar results were obtained in a study carried out with fresh-cut carrots stored during 12 days at 5 °C (Fai et al., 2016).

The highest values $(2.55 \pm 0.73 \log \text{ cfu g}^{-1})$ at the end of the storage were observed in *calçots* packaged under MAP with a postharvest storage of 60 days. It is noteworthy that any of the studied fresh-cut *calçots* presented yeast and mold counts more than 5 log cfu g⁻¹ at the end of the refrigerated storage time, which is the limit proposed by <u>CNERNA-CNRS (1996)</u>.

Conclusions

Packaged fresh-cut *calcots* is a new product that could satisfy the consumer's ready-to-eat demands. The physicochemical and microbiological qualities of fresh-cut calcots after packaging under passive modified atmosphere or vacuum were evaluated. Overall, TA and BI values were higher, and SSC and pH values were lower in fresh-cut *calcots* previously stored under CA for 60 days than 30 days. The mesophilic population of fresh-cut calcots packaged under both passive MAP and VAC was lower than the maximum acceptable contamination value after 15 days of storage at 4 °C. Freshcut calcots packaged under vacuum presented better retention of quality attributes than those packaged under passive MAP. Hence, the most suitable conservation strategy could be to store whole calcots under CA (1.0 kPa $O_2 + 2.0$ kPa CO_2) for 30 days and after minimally processing, packaged under vacuum to extend the shelf-life maintaining physicochemical and microbiological quality. Future studies should focus on the microbiological monitoring for a better evaluation, with the objective of determining the period that the product can be used without compromising consumer health.

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