Research Article

Received: 30 September 2016 Revised: 16 June 2017 Accepted article published: 3 July 2017 Published online in Wiley Online Library: 2 August 2017

(wileyonlinelibrary.com) DOI 10.1002/jsfa.8513

# Immature pea seeds: effect of storage under modified atmosphere packaging and sanitation with acidified sodium chlorite

**Elena Collado,[a] Tâmmila Venzke Klug,[a,b] Ascensión Martínez-Sánchez,[a,b] Francisco Artés-Hernandez,[a,b] Encarna Aguayo,[a,b] Francisco Artés,[a,b] Juan A Fernández[a,c] and Perla A Gómez[a]***

## Abstract

**BACKGROUND: Appropriate sanitation is a priority for extending the shelf life and promoting the consumption of immature pea seeds, as processing accelerates quality deterioration and microbial growth.**

**RESULTS: The combined effect of disinfection with acidified sodium chlorite (ASC) or sodium hypochlorite (SH) and packaging under a passive modified atmosphere (MAP) at 1 or 4 °C on quality was analysed. After 14 days, greenness and vitamin C had decreased, especially in the SH-disinfected samples. Total phenols and antioxidant capacity were not affected by disinfection. Proteins levels fell by around 27%, regardless of the sanitizer and storage temperature. Compared with the initial microbial load, samples stored at 1 °C showed an increase of 1 log CFU $g^{-1}$ in psychrophiles when treated with SH, whereas no increase of note occurred with ASC. In general, microbial counts were always below 3 log CFU $g^{-1}$ for all the treatments.**

**CONCLUSION: Immature pea seeds could be stored for 14 days at 1–4 °C under MAP with only minor quality changes. Disinfection with ASC resulted in better sensory quality, higher content of vitamin C and lower psychrophile counts. More research is needed to analyse the effect of these treatments on other quality parameters.**


**Keywords:** *Pisum sativum* L.; fresh cut; sanitation; microbial counts; antioxidant capacity; vitamin C

## INTRODUCTION

Legumes are an important source of proteins, carbohydrates, vitamins and minerals. In particular, green peas are a legume rich in proteins (~24%), complex carbohydrates, vitamins and minerals considered important for humans.[1] For that reason, peas should be consumed as part of a healthy diet to combat obesity and to help prevent diseases such as diabetes, heart disease and cancer.[2,3]

Our current lifestyles, with little time left to prepare balanced meals, combined with an increased interest in healthy food, has led consumers to demand natural, fresh and ready-to-eat vegetable products, as represented by fresh-cut or minimally fresh processed (MFP) fruits and vegetables.[4] The development of minimally processed immature pea seeds, therefore, would be a new format to promote the consumption of legumes. However, processing causes physiological stress, resulting in an increased respiration rate, membrane deterioration, water loss and higher susceptibility to microbial contamination. Few studies have dealt with the physiological response of peas to minimal processing,[5–7] and there are no recent references to immature fresh seeds. Nevertheless, in the few past years, there has been increased interest in this legume, especially concerning its behaviour during storage, susceptibility to oxidation, dehydration and loss of colour, for example.

Sanitation is one of the most critical steps in fresh-cut production, owing to the effects of microbial load on the quality, safety and shelf life of the final product. The industry has widely used sodium hypochlorite (SH) as a sanitizer because of its antimicrobial activity and low cost.[8] However, its use has been questioned because of the risk of trihalomethane synthesis resulting from contact with organic matter and its potentially harmful effect on health.[9]

As an alternative, it is possible to disinfect fresh-cut products with acidified sodium chlorite (ASC), which is obtained by lowering the pH of a sodium chlorite solution ($NaClO_2$), since it has none of the above-mentioned negative effects and is recognized as safe. ASC can be used[10] on raw agricultural commodities at chlorite concentrations of 500–1200 mg $L^{-1}$. Moreover, ASC has been seen to be effective at inactivating pathogens like *Escherichia coli* O157:H7 and *Salmonella*.[11]

* *Correspondence to: PA Gómez, Institute of Plant Biotechnology (IBV), Universidad Politécnica de Cartagena (UPCT), Campus Muralla del Mar s/n, 30202 Cartagena, Murcia, Spain. E-mail: perla.gomez@upct.es*

a *Institute of Plant Biotechnology (IBV), Universidad Politécnica de Cartagena (UPCT), Cartagena, Murcia, Spain*

b *Department of Food Engineering, Postharvest and Refrigeration Group, UPCT, Cartagena, Murcia, Spain*

c *Department of Horticulture, UPCT, Cartagena, Murcia, Spain*

The objective of this work was to study MFP pea seeds disinfected with ASC, packaged under passive modified atmosphere and stored at two temperatures (1 and 4 °C). Disinfection with SH was used as control. During storage, physicochemical, physiological, microbiological and sensory characteristics, as well as changes in bioactive components (total phenols and vitamin C), proteins and antioxidant capacity, were assessed.

## EXPERIMENTAL

### Plant material

Pea pods (var. Lincoln) were collected from an open field crop (Balsapintada, Cartagena, Spain) at immature physiological stage, when the pods were almost round (70% of seeds' full size[7]) and with a harvest temperature of 15 ± 2 °C. They were transported cold (5 ± 1 °C, 30 min) to the laboratory, where they were kept in darkness at 1 °C and 90–95% relative humidity (RH).

### Processing, packaging and storage

The next day, the peas were shelled by hand in a cold room (5 ± 1 °C) and the obtained peas were immersed in cold water at 4 ± 1 °C. They were then sanitized by immersion in sodium hypochlorite (100 mg $L^{-1}$, pH 6.5, 2 min, 4 °C) or in ASC (300 mg $L^{-1}$, pH 1.8, 2 min, 4 °C) before rinsing with cold tap water (4 ± 1 °C, 1 min). Seeds (about 125 g) were packaged in 35 µm polypropylene bags (15 × 15 cm) with an $O_2$ permeability of 900 $cm^3\ m^{-2}\ d^{-1}$, $CO_2$ permeability of 1100 $cm^3\ m^{-2}\ d^{-1}$ at 23 °C and 0% RH. This film was selected based on earlier studies by our research group. Bags were previously sterilized with UV-C light (8 kJ $m^{-2}$) to prevent any kind of microbial contamination due to the packaging.

The bags were heat sealed to generate a passive modified atmosphere and stored at different temperatures (1 ± 0.5 and 4 ± 0.5 °C) and 90% RH for 14 days. At fixed times (0, 4, 8 and 14 days), samples were removed from storage and analysed. Five replicates per treatment and day of analysis were used.

### Atmosphere composition within packages

Throughout storage, the gas composition ($O_2$ and $CO_2$) inside the packages was monitored using the method developed by Rodríguez-Hidalgo *et al.*[12] For this, 1 mL of package headspace was extracted with a syringe, through a silicone septum placed over the film, and analysed in a gas chromatograph (7820A GC, Agilent Technologies, Waldbroon, Germany), using three replicates per treatment and per evaluation day. Samples were taken on days 1, 3, 5, 7 and 11 of storage.

### Physical quality

Pea colour was analysed using a Minolta colorimeter (CR-300 series, Ramsey, NJ, USA), obtaining $a^*$, $b^*$ and $L^*$ parameters. Hue angle ($H°$) and total colour difference ($\Delta E$) during storage were compared to their respective initial values.[13] The pH was analysed by pH-meter (GLP 21, Crison, Barcelona, Spain).

To evaluate the bioactive compounds (total phenolic and vitamin C), protein content and antioxidant capacity, samples were frozen in liquid $N_2$ and stored at −80 °C until analysis.

### Vitamin C

Vitamin C, in the form of ascorbic acid (AA) and dehydroascorbic acid (DHA), was measured according to the method of Zapata and Dufour,[14] with slight modifications. Derivatized samples (20 µL) were injected into a Gemini NX (250 mm × 4.6 mm, 5 µm) C18 column (Phenomenex, Torrance CA, USA), using a high-performance liquid chromatograph (Series 1100 Agilent Technologies) equipped with a G1322A degasser, G1311A quaternary pump, G1313A autosampler, G1316A column heater and G1315B photodiode array detector. The system was controlled by Chem Station Agilent v.08.03 software. AA and DHA were quantified using commercial standards (Sigma, St Louis, MO, USA). Total vitamin C, calculated as the sum of AA and DHA, was expressed as grams per kilogram, fresh weight basis (FW). All samples were tested in triplicate.

### Total phenolic content (TPC)

TPC was determined using the method developed by Singleton and Rossi,[15] with some modifications introduced by Martínez-Hernández *et al.*[16] Briefly, 2 g frozen samples was placed in glass bottles, and 3 mL methanol was added. The extraction was carried out in an orbital shaker (Stuart, Stone, UK) for 1 h at 200 × *g* in darkness inside a polystyrene box with an ice bed. The extracts were transferred to tubes and centrifuged at 15 000 × *g* for 10 min at 4 °C. Then, 19 µL TPC extract was placed in a well plate, and 29 µL of 1 mol $L^{-1}$ Folin–Ciocalteu reagent was added. The mixture was incubated for 3 min in darkness at room temperature. Then, 192 µL of a solution containing $Na_2CO_3$ (0.4%) and NaOH (2%) was added. After 1 h of incubation at room temperature in darkness, the absorbance was measured at 750 nm with a multiscan plate reader (Infinite M200, Tecan, Männedorf, Switzerland). TPC was expressed as grams of gallic acid equivalents (GAE) per kilogram FW. All samples were tested in triplicate.

### Total antioxidant capacity (TAC)

TAC was determined by the FRAP (fluorescence recovery after photobleaching) method.[17] The absorbance measurement was performed at 593 nm using a multiscan plate reader (Infinite M200). Briefly, a reaction solution containing sodium acetate buffer (pH 3.6), 10 mmol $L^{-1}$ TPTZ solution (in 40 mmol $L^{-1}$ HCl) and 20 mmol $L^{-1}$ $FeCl_3$ was prepared daily in a proportion of 10:1:1 (v/v/v) and incubated at 37 °C for 2 h in darkness. Then, 6 µL TAC extract was allowed to react with 198 µL of the FRAP solution for 40 min at room temperature in darkness. TAC was measured by the decrease in absorbance at 593 nm using the multiscan plate reader. The results were expressed as grams of Trolox equivalents per kilogram FW. All samples were tested in triplicate.

### Protein

To determine the protein content, 5 µL of the extract prepared for determining TAC was placed on a polystyrene plate (Greiner Bio-One, Frickenhausen, Germany), to which 250 mL Bradford reagent was added and allowed to react for 30 min in the dark and at room temperature. The absorbance at 595 nm was measured with the multiscan plate reader. The protein content was expressed as gram equivalents of albumin per kilogram FW. All samples were tested in triplicate.

### Sensory evaluation

A panel of seven people (aged 24–50), trained in sensory quality analysis, performed the evaluation. Before running the experiments, a consensus was reached among the panellists on those



  

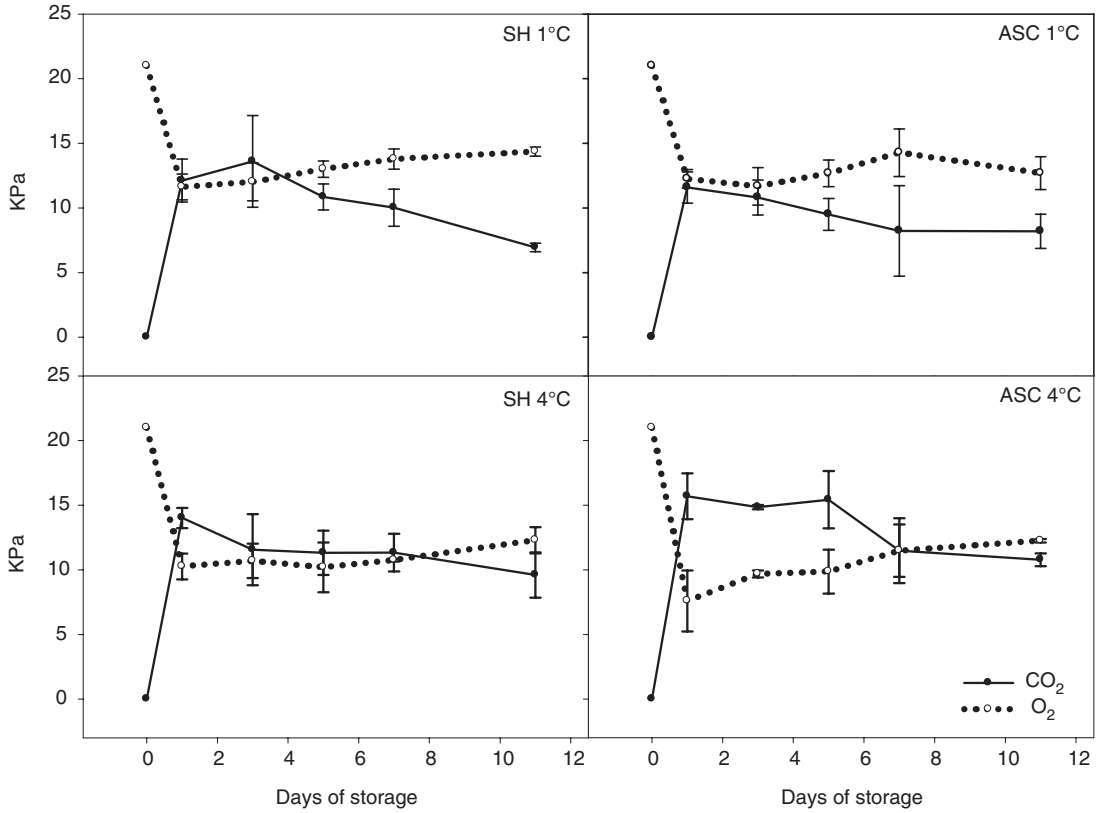


**Figure 1.** Changes in gas composition within packages of fresh pea seeds washed with different sanitizers (SH, sodium hypochlorite; ASC, acidified sodium chlorite) and stored in MAP for up to 14 days at 1 and 4 °C. Data are mean ± standard deviation ($n = 3$).

attributes that best described sensory changes. Samples (about 30 g) were served in randomly coded transparent glasses. Sensory analyses were performed according to international standards (ASTM STP 913 1986). Still mineral water was used as palate cleanser. Sensory quality was evaluated on the processing day and after 4, 8 and 14 days of storage at both 1 and 4 °C. A 9-point hedonic scale was scored for visual symptoms of dehydration (9 = none; 5 = limit of usability; 1 = extreme), and other parameters, such as visual appearance, flavour, aroma, texture, colour and overall quality, were scored as follows: 1 = extremely bad; 5 = limit of usability; 9 = excellent.

### Microbial growth

To determine the mesophilic and psychrophilic bacteria, enterobacteria, and yeast and mould growth, standard enumeration methods were used.[18] Samples of 10 g were homogenized in 90 mL sterile peptone saline solution (pH 7; Scharlau Chemie SA, Barcelona, Spain) for 30 s in a sterile stomacher bag (model 400, Bags 6141, London, UK) using a masticator (Colwort Stomacher 400 Lab, Seward Medical, London, UK). For the enumeration of each microbial group, tenfold dilution series were prepared in 9 mL sterile peptone saline solution. Mesophilic, enterobacteria and psychrotrophiles were pour plated, and yeast and mould were spread plated. The following media and incubation conditions were used: plate count modified agar (PCA) (Scharlau Chemie) for mesophilic and psychrotrophilic aerobic bacteria, incubated at 30 °C for 48 h and at 5 °C for 7 days, respectively; violet red bile dextrose agar (Scharlau Chemie) for enterobacteria, incubated at 37 °C for 48 h; and rose Bengal agar (Scharlau Chemie) for yeasts and moulds, incubated for 3–5 days at 22 °C. All microbial counts were reported as log colony-forming units per gram of product (log CFU $g^{-1}$). Each of the three replicates was analysed in duplicate.

### Statistical analysis

Analysis of variance was performed to compare the sanitizing treatments, storage times and temperatures at a significance level of $P \leq 0.05$, using PASW Statistics 23 for Windows (SPSS Inc., Chicago, IL, USA). In some cases, when significant differences were observed, the Tukey HSD (honestly significant difference) test was applied.

## RESULTS

### Atmosphere composition within packages

The generated passive modified atmosphere (MAP) was analysed to detect whether the sanitizers had any influence on the gas composition (Fig. 1). During the first day of storage there was an increase in the partial pressure of $CO_2$ and a decrease in $O_2$, both remaining constant from that time onwards (8 kPa $CO_2$/12 kPa $O_2$ and 11 kPa $CO_2$/10 kPa $O_2$ at 1 and 4 °C). No differences in the $O_2$ and $CO_2$ concentrations during storage were observed between sanitizing solutions. However, changes were influenced by the storage temperature. The concentration of $O_2$ was lower in the packs kept at 4 °C than at 1 °C, whereas the $CO_2$ concentration was always higher when stored at 4 °C than at 1 °C (Fig. 1).

### Physical quality analysis

No significant differences in pH were observed between sanitizing treatments, although a significant decrease was observed



  

**Table 1.** pH and colour of fresh pea seeds washed with different sanitizers (S) (SH, sodium hypochlorite; ASC, acidified sodium chlorite) and stored in MAP for up to 14 days at 1 and 4 °C. Data are mean ± standard deviation ($n = 3$)

| *t* | *T* | *S* | pH | *L** | *a* | *b* | Δ*E* |
|---|---|---|---|---|---|---|---|
| 0 | 1 °C | SH | 6.60 ± 0.00 | 44.53 ± 7.75 | −14.47 ± 1.31 | 29.86 ± 1.90 | 0.00 ± 0.00 |
| | | ASC | 6.63 ± 0.06 | 44.53 ± 7.75 | −14.47 ± 1.31 | 29.86 ± 1.90 | 0.00 ± 0.00 |
| | 4 °C | SH | 6.60 ± 0.00 | 44.53 ± 7.75 | −14.47 ± 1.31 | 29.86 ± 1.90 | 0.00 ± 0.00 |
| | | ASC | 6.63 ± 0.06 | 44.53 ± 7.75 | −14.47 ± 1.31 | 29.86 ± 1.90 | 0.00 ± 0.00 |
| 4 | 1 °C | SH | 6.40 ± 0.10 | 52.20 ± 4.13 | −13.43 ± 0.99 | 28.01 ± 2.04 | 6.54 ± 0.81 |
| | | ASC | 6.37 ± 0.06 | 47.55 ± 5.04 | −13.10 ± 0.97 | 26.65 ± 2.38 | 5.14 ± 0.69 |
| | 4 °C | SH | 6.37 ± 0.12 | 51.85 ± 4.61 | −13.68 ± 0.78 | 28.09 ± 1.60 | 5.23 ± 1.30 |
| | | ASC | 6.33 ± 0.06 | 51.00 ± 5.93 | −12.89 ± 1.05 | 26.30 ± 1.91 | 6.11 ± 0.31 |
| 8 | 1 °C | SH | 6.33 ± 0.15 | 50.62 ± 6.67 | −14.51 ± 1.31 | 29.67 ± 2.98 | 6.96 ± 0.97 |
| | | ASC | 6.37 ± 0.06 | 47.63 ± 4.28 | −14.08 ± 0.79 | 28.20 ± 1.26 | 6.16 ± 0.74 |
| | 4 °C | SH | 6.73 ± 0.40 | 49.24 ± 6.18 | −13.67 ± 1.22 | 27.97 ± 2.41 | 5.67 ± 1.30 |
| | | ASC | 6.43 ± 0.12 | 51.78 ± 5.57 | −14.08 ± 1.64 | 29.14 ± 3.50 | 6.51 ± 1.85 |
| 14 | 1 °C | SH | 6.17 ± 0.06 | 46.01 ± 3.34 | −13.28 ± 1.31 | 28.86 ± 3.07 | 6.51 ± 1.09 |
| | | ASC | 6.17 ± 0.06 | 50.01 ± 4.13 | −13.29 ± 0.66 | 27.92 ± 0.97 | 8.75 ± 1.20 |
| | 4 °C | SH | 6.13 ± 0.06 | 48.47 ± 5.28 | −12.40 ± 0.92 | 27.42 ± 2.33 | 7.20 ± 0.78 |
| | | ASC | 6.23 ± 0.06 | 42.18 ± 7.81 | −12.01 ± 1.43 | 27.81 ± 2.42 | 6.19 ± 0.67 |
| Sanitizer (*S*) | | | ns | ns | ns | ns | ns |
| Time (*t*) | | | *** | *** | *** | *** | *** |
| Temperature (*T*) | | | ns | ns | ns | ns | ns |
| *S* × *t* × *T* | | | ns | ns | ns | ns | ns |
| *S* × *t* | | | ns | ns | ns | ns | ns |
| *S* × *T* | | | ns | ns | ns | ns | ns |
| *t* × *T* | | | ns | ns | ns | ns | ns |

during storage (Table 1), with no notable difference between temperatures.

Colour is one of the most important visual attributes of peas. In this respect, storage time had a significant effect on lightness (*L**), colour difference (Δ*E*) and *a** and *b** parameters after 14 days of cold storage. As regards Δ*E*, which represents the colour change perceptible to consumers, its value increased with time, with no differences between sanitizing treatments or temperatures. Lightness also tended to increase, whereas *a** and *b** values tended to decrease, with no differences between treatments or temperatures. Colour changes were related to a loss in green colour and brightness, both corroborated by the sensory panel.

### Vitamin C

Vitamin C (Fig. 2) decreased during storage with both sanitizing treatments and at both temperatures. The decrease was from 707 to 267 g kg$^{-1}$ FW at 1 °C and from 707 to 306 mg kg$^{-1}$ FW at 4 °C when SH was used, which was about 30% less drastic than in in the case of ASC (from 643 to 446 and 466 mg kg$^{-1}$ FW at 1 and 4 °C, respectively). In this way, at the end of the storage time, the vitamin C content had decreased to a greater extent after using SH, without any important effect of temperature being observed. For all cases, the concentration of DHA was always higher than that of AA. This fact is consistent with the results obtained by Martínez-Sánchez *et al.*[19]

### Total phenolic content

A tendency towards a slight increase (around 15%) was observed for all the treatments as storage time progressed (Fig. 3). On the processing day, the TPC was very similar for both SH and ASC washing solutions: 25.18 ± 4.73 and 26.95 ± 6.62 mg GAE 100 g$^{-1}$ FW, respectively, with no significant differences between them. At the end of storage, the TPC values were 30.83 ± 1.91 and 31.02 ± 6.08 mg GAE 100 g$^{-1}$ FW for seeds treated with SH at 1 and 4 °C, respectively, and 30.81 ± 6.37 and 30.46 ± 9.00 mg GAE 100 g$^{-1}$ FW for those treated with ASC at 1 and 4 °C.

In general, the TPC of fresh peas was not affected by the washing solutions. Moreover, the storage time did not have any detrimental effect either. However, although not significant, TPC increased between days 4 and 8 in the product treated with ASC at 4 °C before falling, to reach the same values as the other treatments.

### Total antioxidant capacity

The initial TAC of fresh-cut peas treated with SH and ASC was similar (27.41 ± 0.13 and 28.01 ± 1.04 mg Trolox equivalents 100 g$^{-1}$ FW, respectively). At the end of storage it had slightly decreased to 23.80 ± 3.98 and 27.40 ± 2.54 mg Trolox equivalents 100 g$^{-1}$ FW for SH-treated samples at 1 and 4 °C, respectively, and to 25.90 ± 5.20 and 24.04 ± 1.73 mg Trolox equivalents 100 g$^{-1}$ FW for ASC-treated samples at 1 and 4 °C, respectively.

Independently of this decreasing trend, and, as can be seen in Fig. 4, the TAC determined by FRAP method did not generally show any significant ($P > 0.05$) change during storage, with no differences between treatments or temperatures. However, although not significant, TAC increased during the first 4 days for the product treated with ASC at 4 °C and then fell to the same values as the other treatments.



  

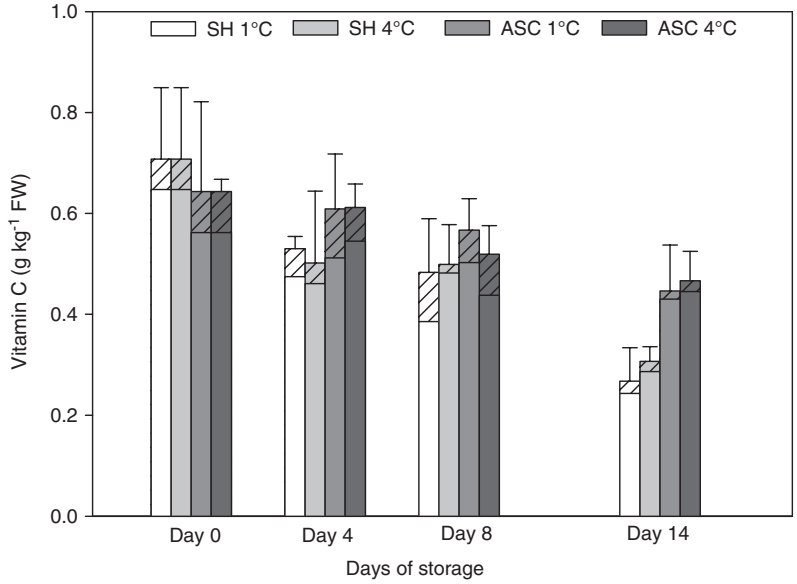


**Figure 2.** Evolution of vitamin C (AA + DHA) in MAP-stored immature pea seeds (1 and 4 °C) previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC) (DHA, dehydroascorbic acid; AA, ascorbic acid). Data are mean ± standard deviation ($n = 3$).

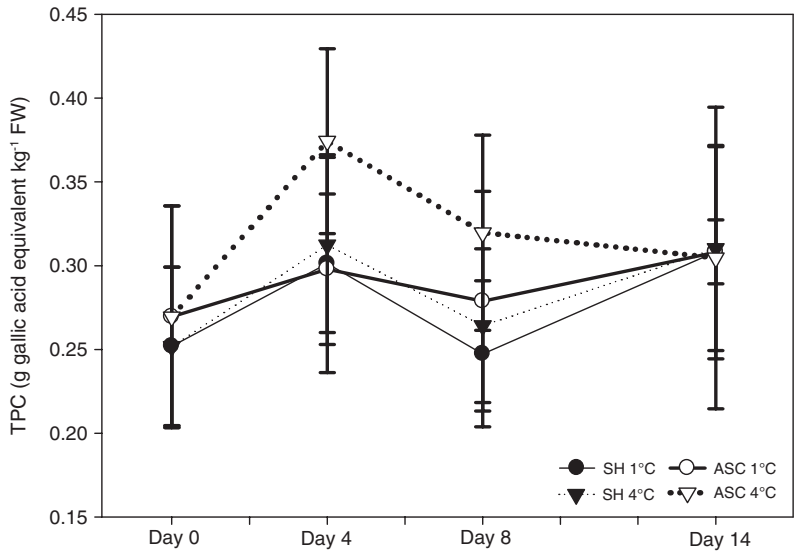


**Figure 3.** Evolution of total phenolic content (TPC) in MAP-stored immature peas seeds (1 and 4 °C), previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC). Data are mean ± standard deviation ($n = 3$).

### Proteins

The initial protein concentration was 23.35 mg albumin equivalents 100 $g^{-1}$ FW for the seeds treated with SH, and 21.49 albumin equivalents 100 $g^{-1}$ FW for the group treated with ASC, while the final concentrations were 14.77 and 13.78 albumin equivalents 100 $g^{-1}$ FW in the group treated with SH at 1 and 4 °C, respectively, and 13.43 and 11.98 mg albumin equivalents 100 $g^{-1}$ FW for those treated with ASC at 1 and 4 °C, respectively. As shown in Fig. 5, the protein concentration fell during storage, regardless of the type of sanitizer used and storage temperature. This downward trend was more pronounced from day 4 onwards.

### Sensory evaluation

The effects of the different washing treatments on the sensory quality of immature seeds are shown in Fig. 6. The mean scores for all sensory attributes at day 0 indicated an optimal quality, with no differences between the washing solutions as regards overall quality, taste and colour. During storage, overall quality, taste and colour declined, although all the scores indicated good sensory quality with no significant differences between washing treatments. From day 8, the peas showed symptoms of dehydration. At day 14, especially in the case of samples stored at 4 °C, those sanitized with ASC presented a better sensory quality than those treated with SH. Dehydration and taste were worse for SH than ASC. However, all the samples were scored as being above the acceptable limit for fresh consumption.

### Microbial analysis

The total mesophilic counts of fresh peas stored at 1 and 4 °C are shown in Fig. 7. Initial values were relatively low: 1.81 and 1.70 log CFU $g^{-1}$ for SH and ASC, respectively.



  

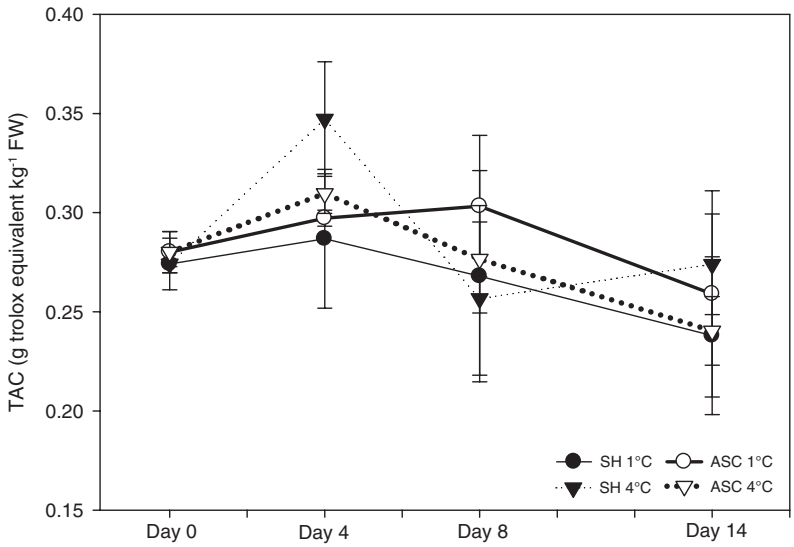


**Figure 4.** Evolution of total antioxidant capacity (TAC) measured by FRAP method in MAP-stored immature peas seeds (1 and 4 °C) previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC). Data are mean ± standard deviation ($n = 3$).

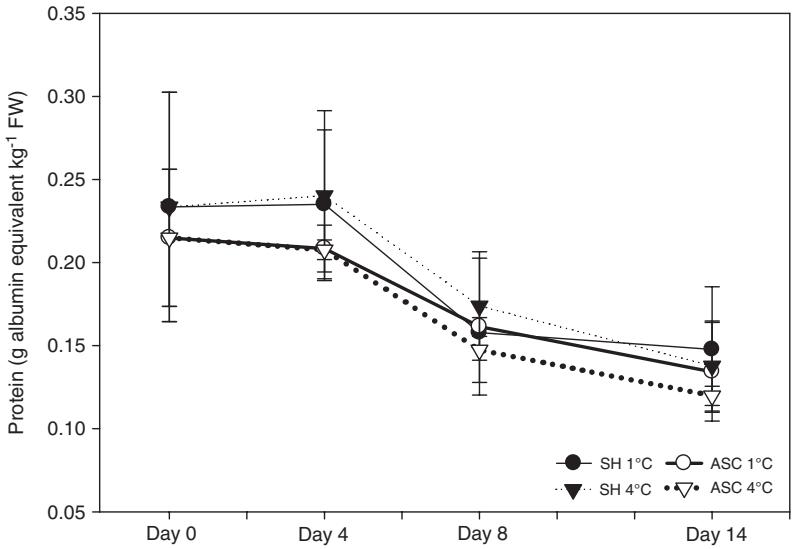


**Figure 5.** Evolution of proteins in MAP-stored immature peas seeds (1 and 4 °C) previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC). Data are mean ± standard deviation ($n = 3$).

During the first week of storage, mesophilic bacteria counts remained almost unchanged, but after 8 days they increased for all the samples. After 14 days at 1 °C the mesophilic counts reached 2.90 and 2.63 log CFU $g^{-1}$ for SH and ASC, respectively, and 3.00 and 2.99 log CFU $g^{-1}$ for SH and ASC at 4 °C, respectively. A comparison of the antimicrobial effect of ASC and SH after washing provided similar results, and no significant differences were observed during storage.

Psychrophile counts for samples stored at 4 °C remained unchanged until day 4 for samples treated with ASC, and until day 8 for those sanitized with SH, both then increasing by 2 log units. By contrast, samples stored at 1 °C showed an increase of just 1 log unit at the end of storage for SH, and no noticeable increase for the ASC treatment.

Mould and yeast counts remained constant (2.00 log CFU $g^{-1}$) throughout the storage period, whereas no enterobacteria were detected in any treatment (values below the detection limit).

## DISCUSSION

Peas are a highly perishable product and their shelf life and visual quality greatly depend on the storage conditions, including temperature and atmosphere composition.[5,20,21] A high storage temperature increases the respiration rate, leading to greater decay.[22] Green beans have intense respiration and heat emission, which limits their postharvest shelf life to 3–4 weeks maximum. This can be partly attributed to the intense metabolic activity of immature seeds inside the pods.[22] The results presented here show that a cold temperature and modified atmosphere can extend the shelf life of peas. Changes in gas composition inside the packages were influenced by the temperature. These data are consistent with those of Martínez-Sánchez *et al.*[23] The atmospheric composition within the packages obtained in our study can be considered as appropriate for this product, in accordance with previous studies.[6] However, those studies were based on pea pods, whereas the results presented here refer to immature seeds.



  

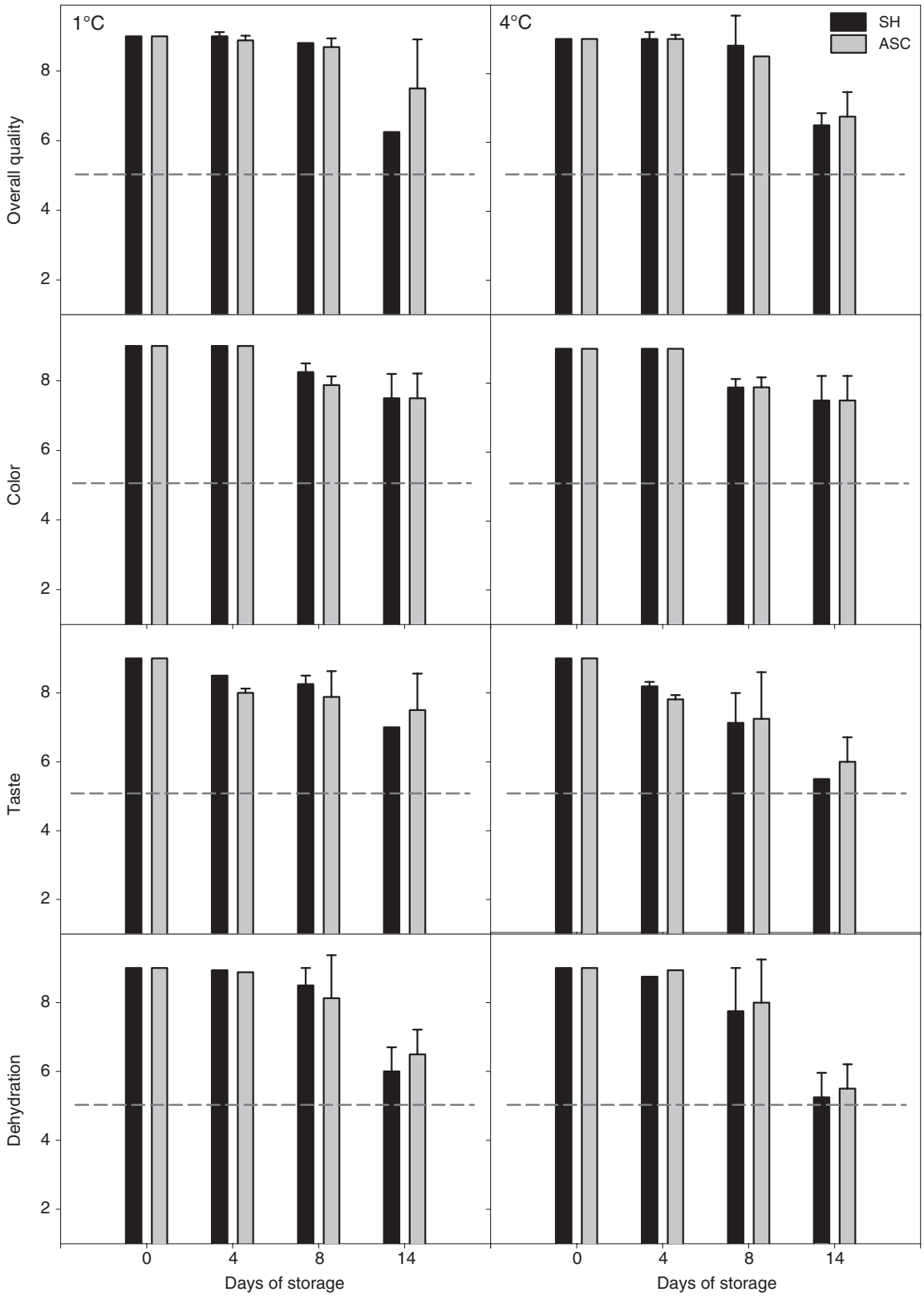


**Figure 6.** Evolution of sensory quality of MAP-stored immature pea seeds (1 and 4 °C) previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC) at 1 and 4 °C. Dashed horizontal line represents the limit of marketability. Data are mean ± standard deviation ($n = 3$).



  

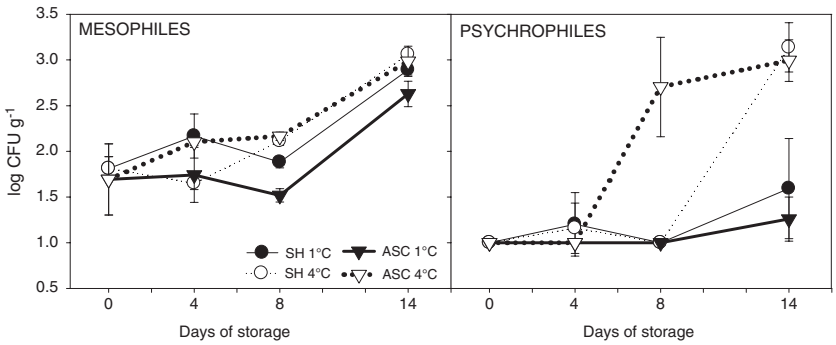


**Figure 7.** Evolution of mesophiles and psychrophiles in MAP-stored immature peas seeds (1 and 4 °C) previously sanitized with chlorine (SH) or acidified sodium chlorite (ASC). Data are mean ± standard deviation ($n = 3$).

Colour is one of the most important quality parameters determining consumer acceptance. $\Delta E$ levels gradually increased during storage. However, storing fresh immature pea seeds at the lower temperature (1 °C) and with a suitable adequately modified atmosphere induced lower colour changes. The maintenance of colour during storage was also confirmed by sensory analyses, since the scores for colour and overall quality were above the acceptance limit. In addition, no significant differences in colour between ASC and SH treated samples were observed.

The seeds presented a low initial degree of microbial contamination, which was probably due to the fact that they were within the pods and, consequently, protected from external bacteria. Moreover, good management practices during processing preserved the safety of the product. The antimicrobial effect of sanitizers after application is very important, but the maintenance of their antimicrobiological effect during storage is also essential. In this study, aerobic microflora, psychrophiles and moulds and yeasts were below 3 log CFU $g^{-1}$ at the end of cold storage, independently of the sanitizer used. In shredded carrots, Ruiz-Cruz *et al.*[24] observed that aerobic bacteria grew rapidly during cold storage for all the concentrations of ASC used, while Escalona *et al.*[25] detected a fast increase in mesophilic bacteria on minimally processed summer-harvested watercress washed with ASC (250 mg $L^{-1}$). Our results agree with those reported by Tomás-Callejas *et al.*,[26] who observed that after using ASC (300 mg $L^{-1}$) on fresh-cut tatsoi baby leaves the total aerobic mesophilic bacteria remained stable throughout storage for 11 days at 5 °C. The antibacterial capacity of ASC is attributed to chlorous acid, which is formed by the acidification of chlorite.[27] Chlorous acid gradually decomposes to form chlorate ions, chlorine dioxide and chloride ions. These reactive intermediates are highly oxidative, with broad-spectrum germicidal activity.[28] Moreover, the low pH of ASC solutions (~1.8) probably affected the ability of cells to maintain pH homeostasis, disrupting substrate transport and inhibiting metabolic pathways.[29]

Phenolic compounds in plants are largely responsible for metabolism and defensive mechanisms. The induction of phenolic compounds is a physiological response to infections or injuries. Any changes in the environment surrounding the product during storage stimulate the cells to induce more phenols in an attempt to initiate the response of the defence mechanism.[7] In general, the total phenolic content of pea seeds was not affected by the washing solutions or temperature (Fig. 3), but did show an upward trend during storage, which could be attributed to the stressful condition during processing. Similar trends were reported by Anurag *et al.*[7] and Selcuk and Erkan,[30] in this last case for pomegranate arils.

The retention in antioxidant capacity observed in our study could be attributed to the retention of total phenols. Similar FRAP values were observed for different sanitizers and temperatures, with no significant changes. In agreement with this, a very slight decrease in antioxidant activity was recorded in broccoli samples, irrespective of treatments, with the advancement of storage time.[31]

The present results related to the significant retention of phenolic compounds and the antioxidant capacity are important, since phenolic compounds have therapeutic properties for various diseases such as diabetes, bacterial and viral infections, hypercholesterolaemia, gastrointestinal ulcers, cancer and cardiovascular diseases due to their antiradical properties.[32]

Ascorbic acid (AA) is considered to be highly sensitive to processing and shelf life conditions and it is often used as a marker for product quality deterioration.[7] In our study, vitamin C levels fell during the storage period. This decrease may be associated with the accelerated rate of biochemical changes caused by processing, which can sometimes increase even at low temperatures,[33] and is consistent with previous studies.[7,31,34,35] Most of the vitamin C content was due to DHA (Fig. 2), perhaps because during processing pea seeds suffer oxidative stress that transforms AA to DHA. The vitamin C content of the fresh peas treated with ASC was higher at the end of storage, when a considerably lower decrease (30%) was evident compared to the value attained with SH. The low pH of the ASC washing solution could favour that trend.

Statistical analysis of the obtained data revealed, as reported by Sánchez-Mata *et al.*,[22] significant variations in the total protein content during storage. In most of the analysed samples, a general and significant decrease in the protein content was observed in the final stages of storage (Fig. 5), especially in peas stored at 4 °C, probably due to the more intense protein and amino acid degradation that occurs in this period. Other authors have reported a general decrease in total solids during shelf life, as indicated by Kinyuru *et al.*[35] for snap bean.

## CONCLUSION

Immature green pea seeds can be stored for 14 days under MAP at temperatures between 1 and 4 °C without any noticeable quality loss. The use of ASC as a sanitizer during processing presents itself as a good alternative to SH since it led to seeds with a better sensory quality and a higher content of vitamin C. More research is needed in the future to analyse the effect of these treatments on other important aspects of pea quality, such as the possible presence of antinutritional factors.



  

## ACKNOWLEDGEMENTS

The authors are grateful to the EUROLEGUME Project, funded by the European Union under the 7th Framework Programme for Research, Technological Development and Dissemination, No. 613,781. They also express their gratitude to CNPq (Council for Scientific and Technological Development, Brazil) for a doctoral grant (232758/2014-0) to T Venzke Klug. A Martínez-Sánchez has a postdoctotal grant ('Juan de la Cierva') from the Spanish Government (MINECO).

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