Overall quality of minimally processed pea seeds stored in modified atmosphere packaging

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Abstract

Peas (*Pisum sativum* L. var. *saccharatum*) are an important source of protein, carbohydrates, vitamins and minerals. Pods are harvested before physiological maturity and stored at temperatures near 0°C. Because of their very high respiration rate, and even though they are classified as a non-climacteric product, loss of quality is fast. Most studies conducted on fresh peas have dealt with the fresh pods, but very little information is available on the optimum storage conditions for immature pea seeds, which are well adapted to preparation as a minimally processed product. The effects of sanitation with chlorine (100 ppm, pH 6.5) or, alternatively, with acidified sodium chlorite (300 ppm, pH 1.8) and passive modified atmosphere packaging (MAP) on overall quality of fresh pea seeds (cultivar 'Lincoln') were assessed during storage at 1 and 4°C. After 12 days, atmospheres within packages were 8 kPa CO_2/12 kPa O_2 and 11 kPa CO_2/10 kPa O_2 at 1 and 4°C, respectively. Spoilage microbial growth (mesophilics, enterobacteria, psychrotrophs, yeasts and mould) were around 2 log CFU g^{-1} and 3 log CFU g^{-1} at 1 and 4°C, respectively. However, enterobacterial growth was drastically reduced at 1°C regardless of the disinfectant used. Colour, firmness and sensory quality were acceptable, with best quality at 1°C. Low-temperature storage allowed a high-quality product to be obtained, even after 12 days of storage, with acidified sodium chlorite being a good alternative to chlorine. However, more research is needed to study its effects on other quality parameters.

Keywords: *Pisum sativum*, legumes, modified atmosphere, quality, microbial growth

INTRODUCTION

Legumes have been cultivated for centuries by a variety of cultures and, depending on the species, there have different origins. It seems certain that, together with cereals, they have been one of the staple foods of the population from the Neolithic era, when man began to cultivate the land and to practice agriculture as a complement to his primitive hunting activity (MAGRAMA, 2015).

Related to their composition, legumes are characterized by a high protein content, the presence of slow-assimilation carbohydrates, minerals (calcium, iron, zinc), fibre (soluble) and certain bioactive components. Thus, their inclusion in the diet helps in the prevention and control of chronic diseases such as diabetes, high cholesterol, heart disease and various types of cancers (Chuang et al., 2012).

Human consumption of vegetables is lower in Europe than in other regions of the world, even though it shows a wide variability. The possibility of using fresh, peeled and ready-to-eat vegetables can promote a healthier eating style, since they facilitate preparation.

Peas (*Pisum sativum* L. var. *saccharatum*) are an important source of protein, carbohydrates, vitamins and minerals. Pods are harvested before physiological maturity and

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stored at temperatures near 0°C. Because of their very high respiration rate, loss of quality is fast (Kader, 1992a). Most postharvest studies conducted on fresh peas have dealt with the fresh pod, and very little information is available about the optimum storage conditions of immature pea seeds, which are well adapted to preparation as a minimally processed vegetable. The effects of sanitation with chlorine (NaClO, 100 ppm, pH 6.5) or alternatively with acidified sodium chlorite ($NaClO_2$, 300 ppm, pH 1.8) and passive modified atmosphere packaging (MAP) (Kader, 1992b) on overall quality of fresh pea seeds were assessed during storage at 1 and 4°C. An advantage of the use of acidified sodium chlorite, compared with the more commonly used chlorine, is that trihalomethanes are not produced from organic contaminants, being safer for consumers.

MATERIALS AND METHODS

Plant material and processing conditions

Pods ('Lincoln') were collected at immature ripening stage (15 °Brix) from an open field crop in Cartagena (Murcia, Spain) and cold-transported 30 km to the Institute of Plant Biotechnology at the Technical University of Cartagena. Upon arrival, they were kept in darkness at 1°C. The next day, the plant material was peeled by hand in a disinfected cold room (8°C) and immature seeds were immersed in cold water (4°C, 1 min). After that, they were sanitized by immersion in sodium hypochlorite (SH) (100 ppm, pH 6.5, 4°C) or, alternatively, in acidified sodium chlorite (ASC) (300 ppm, pH 1.8, 4°C) for 1 min. They were then rinsed with cold tap water (4°C, 1 min) and drained in a wringer.

Packaging and storage

Seeds (about 125 g) were packaged in polypropylene (PP) bags [15×15 cm, 35 μm thick, permeability 900 cm^3 O_2 m^{-2} day^{-1} atm^{-1} and 1100 cm^3 CO_2 m^{-2} day^{-1} atm^{-1} at 23°C and 0% relative humidity (RH)] in order to achieve a passive modified atmosphere. Bags were previously sterilized with UV-C light (8 kJ m^{-2}) to avoid any microbial contamination due to the packaging.

The bags were heat-sealed and stored at different temperatures (1 and 4°C) and 90-95% RH. Five replicates, each comprising a PP bag, per treatment and day of analysis, were prepared. Samples were analysed on days 0, 4, 8 and 14 during storage.

Analyses

1. Respiration rate, ethylene emission and gas composition within packages.

The respiration rate (RR) and ethylene emission were determined daily using a closed system. Three replicates of 100 g seeds were placed within sealed glass jars (2000 mL) and kept at two temperatures (1 and 4°C). Increases in CO_2 were monitored after closing the jars for 1 h. Headspace gas samples (1 mL) were withdrawn from the packages with a gas-tight syringe. Samples were analysed in a gas chromatograph (Agilent Technologies 7820A GC System) equipped with a thermal conductivity detector (200°C), oven (80°C), injector (150°C), and a Teknokroma NF 21152 F 80/100 400°C packed column and a Teknokroma NF 21150 C 80/100 275°C packed column. Helium (20 mL min^{-1}) was the carrier gas. Ethylene was analysed by a gas chromatograph (Agilent Technologies 7820A GC System) equipped with a flame ionization detector (200°C), oven (80°C), injector (150°C) and a Teknokroma NF 27849 FS 250°C packed column. In order to avoid accumulation of CO_2 and C_2H_4, a continuous flow of 30 mL humidified air min^{-1} was applied. RR was expressed as mL CO_2 kg^{-1} h^{-1} and ethylene as mL C_2H_4 kg^{-1} h^{-1}.

The O_2 and CO_2 partial pressures within packages were analysed by injecting 0.5 mL gas samples taken from the headspace into the same GC used for analysing RR.

2. Physicochemical parameters: pH, total soluble solids (TSS), titratable acidity (TA), colour (CIELab*) and firmness.

The pH was determined with a pH meter (GLP Crison 21, Barcelona, Spain). TSS were

determined by a hand refractometer (Atago, Zuzi, A43009) and expressed in °Brix at 20°C. TA (g citric acid 100 mL^{-1}) was analysed by titrating 5 mL juice with NaOH (0.1 M) to pH 8.1 (AOAC, 2009) with an electronic titrator (Metrohm 716 DMS Titrino). Colour was measured by using a compact tristimulus colorimeter (Minolta CR-300, Ramsey, NJ, USA) with a target of 8 mm in diameter, 2nd observer (Y=94.3; x=0.3142, y=0.3211, CIE standard illuminant). Values were expressed as CIELab* parameters: lightness (L*), colour saturation (Chroma) and tone (hue angle; H°). Firmness was measured by using a texture analyser (Brookfield, CT3-4500, USA) equipped with a 3.80 mm probe, 10.0 mm min^{-1} speed. Results were expressed in N.

3. Sensory quality.

Sensory quality was assessed by a 10-person trained panel (AENOR, 1997), taking into account the appearance, taste, aroma, texture, colour, browning and overall quality, on a 10-point scale (1 = extremely poor, 3 = poor, 5 = acceptable and limit of usability, 7 = good and 9 = excellent). Sensory quality was the determinant factor for assessing shelf-life of the product. Analyses were performed in a standardized tasting room with 10 panellists.

4. Microbiological analysis.

Each sampling day, 10 g seeds from each treatment were blended with 90 mL sterile peptone water (pH 7.0) (Scharlau Chemie S.A., Barcelona, Spain) for 1 min in a sterile stomacher bag (BA6/4/cpg, London, UK) by using a masticator (Seward Medical, London, UK). Serial dilutions were prepared in 9 mL sterile peptone water. From each dilution, 1 mL aliquots were aseptically pipetted for bacteria and 0.1 mL for yeasts and moulds. The following media and incubation conditions were used: plate count modified agar for mesophilic and for psychrophilic aerobic bacteria (incubated at 30°C for 48 h and at 7°C for 7 days, respectively); violet red bile dextrose agar (VRBD, pH 7.2) for enterobacteria (incubated at 37°C for 48 h); and rose Bengal agar for yeasts and moulds by spreading (incubated for 5 and 7 days at 22°C, respectively). All media were from Scharlau Chemie S.A., Barcelona, Spain. Microbial counts were reported as $\log_{10}$ CFU g^{-1}.

Statistical analysis

The experiment was a 2×2×4 factorial design (disinfection treatment × temperatures × storage time), which was subjected to analysis of variance (ANOVA) using Statgraphics Plus (version 5.1) software. Figures represent mean values (n=5) ± SD.

RESULTS AND DISCUSSION

RR, ethylene emission and gas composition within packages

The RR of the fresh seeds was high; reaching values around 60 mg CO_2 kg^{-1} h^{-1} for 4°C and 40-50 mg CO_2 kg^{-1} h^{-1} for 1°C (Figure 1). These values remained, without showing large variations, but with a trend to decrease until the end of storage, with values around 30 and 40 mg CO_2 kg^{-1} h^{-1} for 1 and 4°C, respectively. The RR was significantly lower at the lowest temperature. In that way, our results are in agreement with Kader (1992a), who stated that peas are highly perishable when immature; therefore, they must be refrigerated and stored at temperatures near 0°C to prolong their shelf-life.

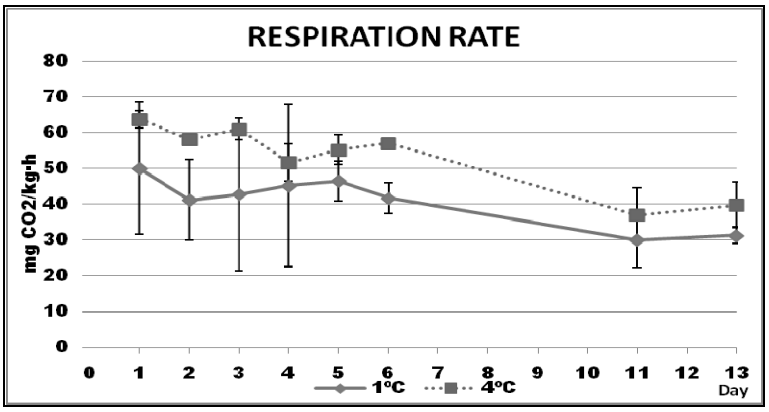

Figure 1. Evolution of respiration rate of immature pea seeds stored at 1 and 4°C (average of sanitizing treatments).

All MAPs showed a similar pattern for gas atmosphere changes during shelf-life (Figure 2). During the first day, there was an increase in the partial pressure of CO_2, as well as a decrease in O_2 inside the bags. Equilibrium was reached at day 2. From that day, modified atmospheres were reached with 8 kPa CO_2/12 kPa and 11 kPa CO_2/O_2 10 kPa for 1 and 4°C, respectively. That range of atmosphere compositions is in agreement with those that gave the best results for snow pea pods (Pariasca et al., 2001). In that study, higher CO_2 or lower O_2 concentrations had a detrimental effect on quality of stored pods, since they developed slight off-flavours, but this effect was reversible, since it was partially alleviated after ventilation. However, our report is about fresh seeds rather than pods.

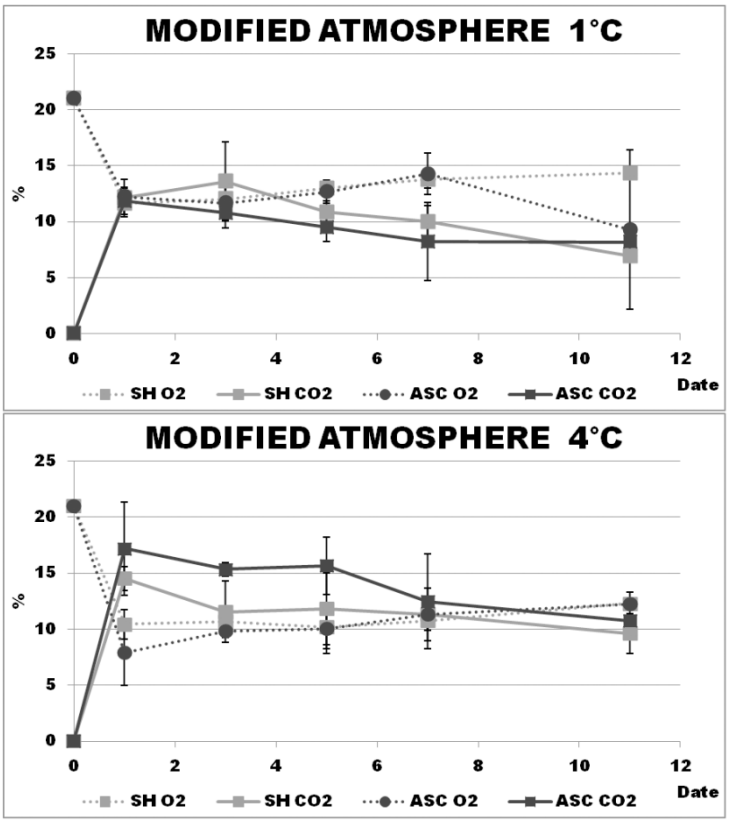

Figure 2. Evolution of modified atmospheres for immature pea seeds stored at 1 and 4°C (average of sanitizing treatments).

The ethylene emission rate was low (around 1.5 µL C_2H_4 kg^{-1} h^{-1}) and declined from the beginning of the experiment until day 5, from when it remained stable until the last day of the study. The higher initial values may be related to the stress induced by processing (peeling and washing).

Physicochemical parameters

Analysis of physical and chemical parameters indicated that pH decreased progressively. Consequently, TA showed a significant increase (from 0.04 to 0.22 g acid 100 mL^{-1} and from 0.04 to 0.18 g acid 100 mL^{-1} for 1 and 4°C, respectively, without differences between sanitizing treatments). TSS decreased from 17.67 to 14.67 °Brix and from 17.27 to 15.07 °Brix for 1 and 4°C, respectively, independent of the sanitizer, probably because they were used as substrate for keeping the high respiration rate. Lightness (L*) and saturation (Chroma) were stable, whereas tone (H°) showed a slight downward trend, indicating a decrease in greenness, while firmness remained stable (around 4 N). All these changes were more pronounced at 4°C than at 1°C.

Sensory quality

Related to sensory evaluation, peas presented the best quality on days 0 and 4. At day 8, they remained acceptable for consumption. On day 14, they showed lower organoleptic quality, but remained a good-quality product, especially at 1°C (Figure 3). As the storage time increased, all the treatments presented a slight to moderate visual dehydration and some loss of aroma, texture and flavour.

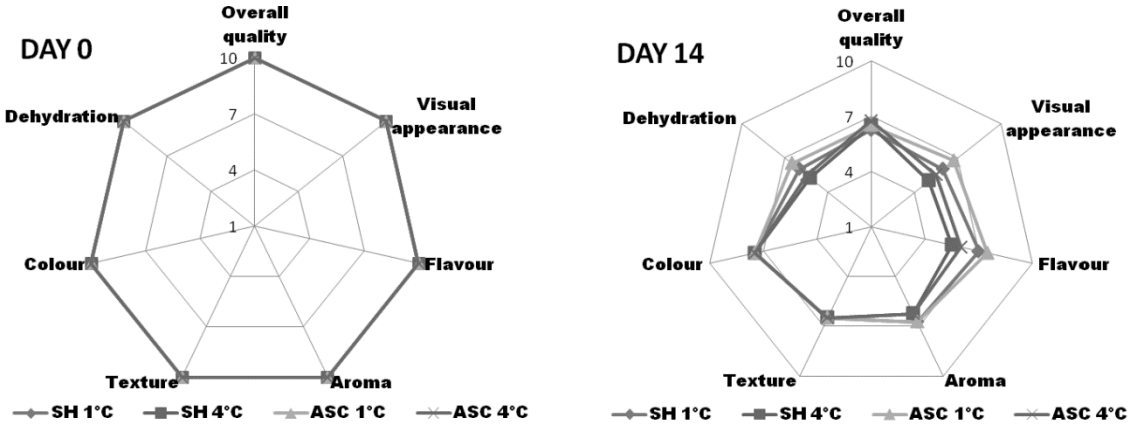

Figure 3. Sensory quality of MAP-stored immature pea seeds (1 and 4°C) on days 0 and 14, previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC).

Microbiological analysis

Peas presented an initial mesophilic load of 1.5 log, while it was 1.0 log for enterobacteria, 1.0 log for psychrophilic bacteria and 2.0 log for moulds and yeasts (Figure 4). Mesophilic and psychrophilic microorganisms showed continuous growth, but with differences between treatments, the lowest being for product disinfected with ASC. Regarding temperatures, growth was higher at 1°C than at 4°C. At the end of storage, spoilage microbial growth was around 2 and 3 log CFU g^{-1} at 1 and 4°C, respectively. Counts of moulds and yeasts remained uniform, and there was no detectable growth of enterobacteria.

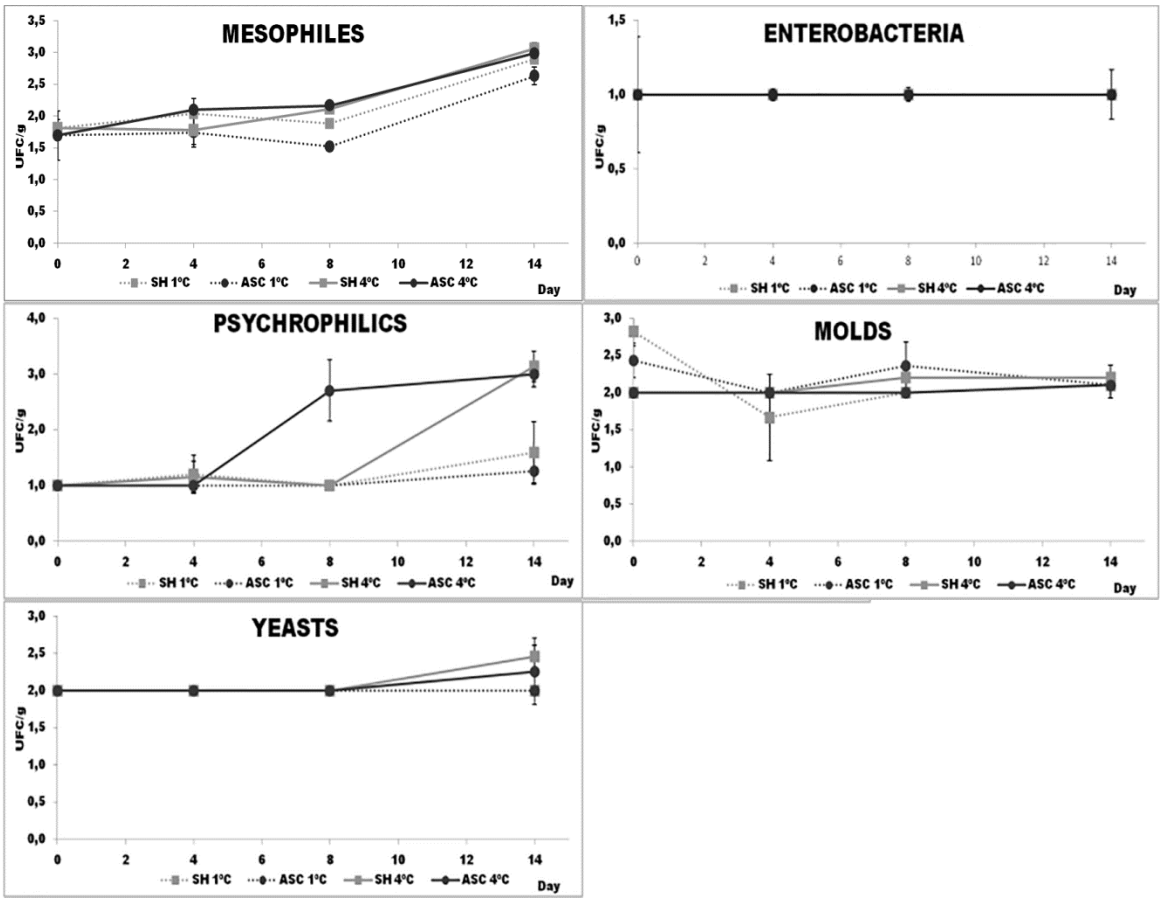

Figure 4. Evolution of microorganisms in MAP-stored immature pea seeds (1 and 4°C) on days 0 and 14, and previously sanitized with sodium hypochlorite (SH) or acidified sodium chlorite (ASC).

CONCLUSIONS

The results of this research suggest that quality parameters of MAP-stored immature peas seeds were acceptable, being better at 1°C. This lower temperature, when compared with 4°C, allowed a high-quality product to be obtained even after 12 days of storage, independent of the sanitizer. The atmosphere reached within packages was appropriate for avoiding important quality deterioration. Additionally, sodium chlorite might be seen as a good alternative to sodium hypochlorite. However, more research is needed to study its effects on other quality parameters, especially those related to healthy properties of peas.

ACKNOWLEDGEMENTS

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