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Physical, chemical, histological and microbiological changes in fresh green asparagus (*Asparagus officinalis*, L.) stored in modified atmosphere packaging

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Abstract

Modified atmosphere packaging (MAP) has been used to increase the shelf life of the green asparagus (*Asparagus officinalis*, L.), meeting the market demand for fresh high quality products available annually and without the use of additives whenever possible.

Green asparagus spears were stored under three different conditions until they were not fit for consumption: refrigeration at 2 °C, MAP at 2 °C, and MAP at 10 °C after 5 days at 2 °C. Gases (O_2 and CO_2), external appearance, weight loss, pH and acidity, vitamin C, texture and microbial quality, along with a microscopical analysis, were measured at regular intervals throughout the storage assays. Significant differences were found between packaged and non-packaged green asparagus in most of the parameters considered. Weight loss and hardening in the spears middle and basal sections increased markedly in refrigerated samples. Vitamin C contents decreased rapidly after storage in all treatments; however, this was more pronounced in refrigerated spears, while over the same time the ascorbic acid content was statistically higher in samples stored under MAP conditions. Also, MAP has a significant effect on the storage time, with the external appearance being the limiting factor for the shelf-life and reducing the microbial growth within the spears. Modified atmosphere, combined with refrigeration at 2 °C, showed the best results among the treatments in terms of retaining sensory and nutritional quality, increasing the safety and extending the shelf-life of green asparagus.

Keywords: Asparagus; Modified atmospheres packaging; Quality attributes

1. Introduction

Fresh vegetables have become an essential compound in the so-called "wealthy" diet; however, they are extremely perishable. In recent years, green asparagus destined for fresh consumption with minimal manipulation, that includes only the base cut and then tying in bundles, has increased its presence in the market. Mary Washington is the cultivar most commercialised and, derived from this cultivar, the University of California has developed many hybrids called UC.

Asparagus (*Asparagus officinalis*, L.) has a very short shelf-life due to its high respiration rate: 60 mg CO_2/kg/h at 5 °C (Kader, 1992), which continues after harvesting. Therefore, the internal and external commercialisation of the green asparagus has very interesting future prospects so long as it is possible to ensure a higher shelf-life by adequate post-harvest conservation. The high economic value of this crop and its very short shelf-life are factors that make asparagus a target product for considering methods to increase shelf-life, which would also be very profitable in terms of export.

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Due to this and to answer an increasing demand for fresh quality food by consumers (Day, 1995, 2000), modified atmospheres packaging (MAP) has been used to increase the shelf-life of asparagus. This method involves the alteration of the atmosphere surrounding the product by reducing the oxygen concentration and increasing the carbon dioxide content, without undertaking any active control of the concentrations of these gases. The atmosphere within the packaging changes over storage time due to factors such as product respiration and biochemical changes, as well as the slow diffusion of the gases through the packaging film. As a consequence, the gas composition inside the bag will depend on the balance reached between the metabolic reactions of the product and the diffusion characteristics of the film. There are many factors to take into account with this technique, such a film permeability (O_2, CO_2, water vapour) or temperature, that make it essential to fix the optimal conditions for each vegetable product (Fonseca, Oliveira, & Brecht, 2002; Gontard, Thibault, Cuq, & Guilbert, 1996; Hertog, Peppelembos, Tijskens, & Evelo, 1997; Kader, Zagory, & Kerbel, 1989).

MAP extends the shelf-life of vegetables by reducing the respiration rate, retarding the compositional changes associated with maturation and senescence, reducing microorganism growth and retaining all the attributes that consumers consider to be freshness markers. These changes are predominantly manifested in the form of wrinkled stems, loss of the characteristic bright green colour, hardness and losses of sugars, vitamins, water, flavour and aroma (King, Henderson, & Lill, 1986; Wills, McGlasson, Graham, & Joyce, 1999; Zagory & Kader, 1988;). Among these changes, texture is the main factor determining the sensory quality of asparagus and lignification is undesirable and can make the asparagus unacceptable as food. The humidity also, is an extremely important factor in determining its external appearance and losses of 3–6% make the product unacceptable for sale or consumption (Day, 1995).

This study has been conducted to evaluate the effect of MAP storage and temperature conditions on shelf-life. Our objective was the study of two MAP conditions: the first one combined with refrigeration at 2 °C, is recommended for commercialisation and the second was carried out in order to compare these results with conditions simulating the storage conditions, i.e., transporting at 2 °C and finally displaying at 10 °C. At the same time, refrigeration storage at 2 °C and normal atmosphere conditions was carried out in order to establish a comparative assay. The evaluation was done by measuring the variation of physical, chemical, sensorial, microbiological and hystological characteristics over the storage periods.

2. Materials and methods

2.1. Plant material

Green asparagus of the hybrid *UC-157-F1*, specific for its production, was used in this research. The spears were harvested from the production site located in Guadalajara (Spain), during two consecutive seasons and similar dates (7 and 20 May, respectively). After harvesting, the samples were rapidly transported to the laboratory under refrigeration conditions.

2.2. Storage conditions and preparation of the samples

The asparagus samples were sliced to a length of 24 cm, washed and rinsed in tap water, drained and pre-refrigerated at 1 °C for 6 h. Three experiments were carried out with green asparagus under different storage conditions:

- Refrigeration at 2 °C and normal atmospheric conditions.
- MAP, combined with refrigeration at 2 °C.
- MAP, stored at 2 °C during 5 days, and then transferred to 10 °C storage to simulate transport and retailing conditions.

In the second season, only the first two above storage conditions were selected.

The modified atmosphere was established in a passive manner. The film used to package the asparagus was P-Plus (Danisco, Bristol, UK) (oriented polypropylene, 20×35 cm^2, 35 μm thickness, O_2 permeability of 14,000 cm^3/m^2/day/atm and water vapour transmission rate of 0.9 g/m^2/day). Each bag contained 500 g of spears and was heat-sealed (Rovebloc, mod. RU09, Barcelona, Spain) without gas packaging.

Each sample, containing three lots (500 g spears/lot), was included for each storage condition and sampling time. The analyses were conducted in triplicate for all the parameters with a frequency of 4–6 days, in order to provide regular and continuous data for the asparagus.

2.3. Gas analysis

The O_2 and CO_2 concentrations were measured with a CO_2/O_2 gas analyser (Abiss model TOM 12, Viry Chatillon, France), pumping 5 ml gas sample from the package; to avoid gas exchange with the surrounding atmosphere during the quantification, the needle perforated the bag through a small piece of adhesive foam (10×15 mm^2 and 5 mm thick).

2.4. External appearance and weight loss

The visual characteristics were determined over the storage time by a 6–8 member untrained panel, aware of sensory attributes and, despite this not being an objective parameter, it is a fundamental elemental criterion for determining the shelf-life of the product. The observed characteristics were turgidity (from fresh appearance, up to severe loss of turgidity), longitudinal striation (from no striation up to strong striation), desiccation of the bases (from no desiccation up to strong desiccation), colour changes (bright green, up to yellowing), the presence of off-odours (from no off-odours, up to appreciable off-odours) and lack of microorganism spoilage. A four-point scoring scale was employed (1, very good; 2, good; 3, acceptable; 4, unacceptable).

The weight of each lot was determined with a precision balance (Denver Instrument Company, AL-1800) during the entire storage time. This parameter gives very valuable information about the weight loss, which is due mainly to the loss in water that the product experiences over the storage time.

2.5. Texture

The texture was determined as the shear force measured by a texturometer (Aname, TA.XT2i/25, Stable Micro Systems, UK) provided with a Warner–Blatzler shear cell and specific software (Texture Expert for the windows Operating System).The amount of lignin in the asparagus increases from the tip to the base of the stalk, Therefore the shear force was measured at 20 (apical section), 10.5 (medium section) and 3 cm (basal section) from the base.

2.6. pH, acidity and vitamin C

For analysing the pH and acidity, 15 g of fresh product were homogenised in an Omnimixer (Omni, model 17106, USA) and made up to a final volume of 100 ml using distilled water. After centrifugation (Hettich, mod. Universal 16R, Germany), 50 ml of the supernatant were taken and the pH was measured directly with a pH-meter (Crison, micropH-2000). The acidity was determined by titration of 50 ml of the supernatant with 0.1 N NaOH, and the reaction concludes when the pH reaches a value of 8.2 (Simon & Cerrolaza, 1993).

Vitamin C was measured by the method of Brubacher, Müller-Mulot, and Southgate (1985), which includes an extraction with metaphosphoric acid solution. Ascorbic acid was oxidised to dehydroascorbic acid by activated carbon. A fluorescent complex of quinoxaline was then formed by the addition of 1,2-phenylenediamine solution and measured in a fluorimeter (Perkin–Elmer, mod. LS-3, UK) at 350 (excitation) and 430 nm (emission).

2.7. Microscopy analysis

Samples obtained from diverse portions of the stalk (base, middle and apex) were used in the anatomical study. The spears were prepared and fixed, following the protocol of D'Ambrogio de Argüeso (1986), which consists of fixation (samples were placed in 2.5% glutaraldehyde in sodium cacodylate tampon), postfixation (1% osmium tetroxide), washing with distilled water and dehydration through a series of acetone solutions, infiltration and embedding with Spurr's resin and ultramicrotome sectioning (semi-thin and ultra-thin sections).

After this preparation, the semi-thin sections were stained with Richardson blue for light microscopy observations, while the ultra-thin sections were treated with uranyl acetate and lead citrate for transmission electron microscopy observations (Zeiss mod. EM-902, Germany).

2.8. Microbial analysis

Asparagus was removed aseptically from each conservation treatment. Due to the solid nature of the sample prior to the analysis, the sample was homogenised with peptone water in a stomacher (masticator IUL Instrument) for 1 min. After serial dilution in peptone water (10^{-1}, 10^{-2} and 10^{-3}), the samples were plated on specific media as follows: total mesophiles were determined by mass sowing on plate count agar (PCA, Oxoid CM463) and incubated at 30 °C for 72 h. Total anaerobic were determined by sowing on the surface of a Petri dish of Schaedler agar (pH 7.6) (Oxoid CM437). The dishes were incubated in an anaerobic atmosphere at 37 °C for 48 h. Yeast and moulds were determined in Sabouraud media (pH 5.6) (Oxoid CM41). The Petri dishes were incubated at 25 °C for 5 days.

2.9. Statistical analysis

Results are presented in tables as means ±SD. Data were statistically examined by ANOVA with mean separation by Duncan multiple range test ($\alpha = 0.05$). The statistical software was SAS. version 8.1.

3. Results and discussion

3.1. Atmospheric composition inside the bags

Table 1 illustrates the level changes of respiratory gases inside the MAP over the storage time ($p \leqslant 0.001$). The O_2 and CO_2 levels reached an

Table 1
Respiratory gases evolution in green asparagus stored under MAP conditions[1]

Storage condition	Days	O_2	CO_2
First season			
	0	21.00 ± 0.00^{a}	0.30 ± 0.00^{c}
	5	16.30 ± 1.83^{b}	6.00 ± 1.14^{b}
MAP 2 °C	9	14.77 ± 0.95^{c}	7.14 ± 1.07^{a}
	14	14.87 ± 0.46^{bc}	8.13 ± 0.21^{a}
	20	14.70 ± 0.85^{c}	7.61 ± 0.54^{a}
	26	14.60 ± 1.22^{c}	8.07 ± 0.36^{a}
	33	14.22 ± 0.44^{c}	8.08 ± 0.19^{a}
MAP 10 °C	0	21.00 ± 0.00^{a}	0.30 ± 0.00^{d}
	5	16.30 ± 1.83^{b}	6.00 ± 1.14^{c}
	9	12.84 ± 1.07^{c}	9.06 ± 0.61^{b}
	14	8.93 ± 0.46^{d}	12.97 ± 1.33^{a}
	20	5.13 ± 0.42^{e}	14.03 ± 0.67^{a}
Second season			
	0	21.00 ± 0.00^{a}	0.30 ± 0.00^{c}
	6	15.03 ± 1.18^{b}	7.00 ± 1.06^{b}
MAP 2 °C	12	13.30 ± 0.24^{c}	8.20 ± 0.37^{a}
	16	13.43 ± 0.25^{c}	8.80 ± 0.75^{a}
	21	13.28 ± 0.29^{c}	8.80 ± 0.29^{a}
	26	12.90 ± 0.62^{c}	8.93 ± 0.41^{a}

Data shown in %.
[1] The values without the same letter within each storage assay and column represent significant differences ($\alpha = 0.05$).

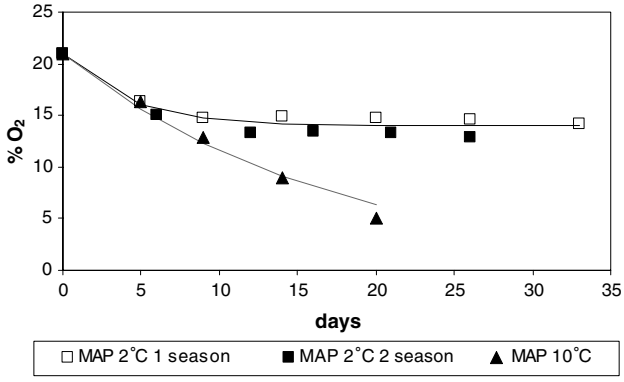

Fig. 1. Kinetic model of oxygen degradation during green asparagus MAP storage.

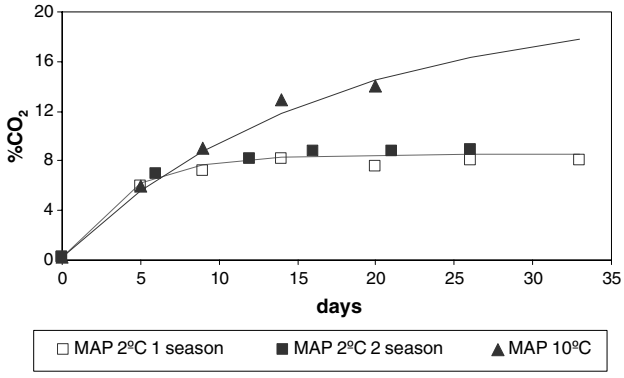

Fig. 2. Kinetic model of carbon dioxide evolution during green asparagus MAP storage.

equilibrium between the second and third sampling dates (5 and 9 days in the first season and 6 and 12 days for the second season) for the storage under MAP at 2 °C. However, for the samples stored under modified atmosphere conditions at 10 °C, no equilibrium was reached for either O_2 or CO_2 during storage. This shows that respiration is a function of storage temperature.

The final concentrations for both gases in the MAP stored at 2 °C are between the tolerance limits for this commodity which are no lower than 10% for oxygen nor higher than 15% for carbon dioxide when the storage temperature ranges between 0 and 3 °C (Berrang, Brackett, & Beuchat, 1990; Salveit, 1993; Thompson, 1996). The O_2 levels detected for the MAP at 10 °C, between the third and fourth sampling dates, were above these limits, with noticeable discoloration of the product, losses in texture quality (turgidity, basal desiccation) and light off-odours and were the most susceptible to decay, as shown by the lower shelf-life when compared with MAP at 2 °C, most likely caused by these O_2 levels (Kader, 1992; Salveit, 1997).

The models that describe the O_2 and CO_2 behaviour over the storage period for each of the conditions tested are represented in the Figs. 1 and 2.

The oxygen variation over time, $O_2(t)$, will be conditioned by two factors: the consumption of this gas by the horticultural product and the exchange of this gas with the exterior through the film, and can be described by the following equation:

$$O_2(t) = C + [C_0 - C]e^{-kt},$$

where $C_0 = 21\%$ and C represents the final concentration. In the case of MAP at 2 °C, reaches equilibrium levels that vary between 14.8% and 13.3%, depending on the season while, for the stalks stored under MAP at 10 °C, since no equilibrium was reached over the storage period, the final oxygen concentration tends towards 0% ($C = 0$).

The concentration inside the bag of the other respiratory gas $CO_2(t)$ depends on the previous respiratory process, together with the exchange of this gas with the exterior through the film. This gas behaviour can fit the model represented by the following curve:

$$CO_2(t) = ae^{-k_3 t} + b + ce^{-(k_1+k_2)t},$$

where $k_1 + k_2 = k$ represents the kinetic constant of the $O_2(t)$ equation, k_3 describes the constant associated with the exchange rate through the film, and a, b and c are constants related to k_1, k_2 and k_3, to C_0 and C_{CO2}.

$$b = C_{CO_2} + \frac{k_1 k_2}{k_3(k_1 + k_2)} C_0,$$

$$c = \frac{k_1^2 C_0}{(k_1 + k_2)[k_3 - (k_1 + k_2)]},$$

$$a = C_{CO_2} - b - c.$$

CO_2 at equilibrium are 7.14% in the first season, and 8.20% in the second season; internal atmosphere corresponding to the spears storages at 10 °C in MAP experiments a progressive increase of CO_2.

3.2. External appearance

The refrigerated samples showed longitudinal striation, dryness, especially toward the bases, losses in firmness, bract opening (''feathering'') and colour changes (from bright green to dull olive green) earlier than the stalks stored under MAP, even though, at the final sampling dates at 10 °C, off-odours and exudations were detected, as shown in Table 2.

The overall shelf-life was limited by the above sensory qualities, with these changes easily noticeable after 9–12 days for the refrigerated storage samples, depending on the season considered, 14 days for the stalks stored under MAP at 10 °C and 26 days for this same technique stored at 2 °C. The storage time for the MAP at 2 °C was prolonged for one more sampling date during the first season in order to test the changes taking place under these conditions, even though the asparagus was no longer acceptable for selling.

3.3. Weight loss

This parameter was crucial, due to every loss in weight being translated into an economical loss. As shown in Table 3, the weight loss detected, over the 14 days of refrigerated storage, was 11.8% of the initial weight ($p \leqslant 0.001$), with statistical differences at each sampling date. In contrast, the losses detected over the MAP storage were low, only 2.1% at 2 °C and 2.0% at 10 °C along the 33 and 20 storage days, respectively. These losses were not significant ($p > 0.05$) between consecutive days; however, they were significant when the initial and final sampling dates were compared. This was due to the stalks being protected by a low water permeability plastic film.

Table 2
Appearance changes over the storage time in green asparagus stored under Refrigerated and MAP conditions

Storage condition	Days	Turgidity	Colour changes	Longitudinal striation	Basal desiccation	Off-odours	Microorganism spoilage
First season							
Ref 2 °C	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	5	1.7 ± 0.5	1.6 ± 0.5	1.4 ± 0.5	1.6 ± 0.5	1.1 ± 0.4	1.0 ± 0.0
	9	2.6 ± 0.5	1.7 ± 0.5	1.9 ± 0.4	2.6 ± 0.5	1.3 ± 0.5	1.1 ± 0.4
	14	3.6 ± 0.5	2.6 ± 0.5	2.9 ± 0.4	3.7 ± 0.5	1.7 ± 0.5	1.6 ± 0.5
MAP 2 °C	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	5	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	9	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	14	1.3 ± 0.5	1.3 ± 0.5	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.0
	20	1.6 ± 0.5	1.4 ± 0.5	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.4
	26	1.9 ± 0.4	1.6 ± 0.5	1.3 ± 0.5	1.3 ± 0.5	1.6 ± 0.5	1.6 ± 0.5
	33	2.9 ± 0.4	2.6 ± 0.8	1.6 ± 0.5	1.6 ± 0.5	2.6 ± 0.5	2.0 ± 0.6
MAP 10 °C	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	5	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	9	1.6 ± 0.5	1.6 ± 0.5	1.3 ± 0.5	1.7 ± 0.5	1.4 ± 0.5	1.1 ± 0.4
	14	1.9 ± 0.4	1.7 ± 0.5	1.9 ± 0.4	1.7 ± 0.5	1.9 ± 0.4	1.4 ± 0.5
	20	3.3 ± 0.5	2.7 ± 0.5	2.9 ± 0.4	2.4 ± 0.5	2.7 ± 0.5	2.6 ± 0.5
Second season							
Ref 2 °C	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	6	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	1.1 ± 0.4	1.0 ± 0.0
	12	2.6 ± 0.5	2.6 ± 0.5	2.6 ± 0.8	1.7 ± 0.5	1.3 ± 0.5	1.1 ± 0.4
	16	3.7 ± 0.5	2.7 ± 0.5	3.7 ± 0.5	3.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5
MAP 2 °C	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	6	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	12	1.3 ± 0.5	1.3 ± 0.5	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.0	1.0 ± 0.0
	16	1.4 ± 0.5	1.4 ± 0.5	1.3 ± 0.5	1.3 ± 0.5	1.1 ± 0.4	1.0 ± 0.0
	21	1.7 ± 0.5	1.4 ± 0.5	1.4 ± 0.5	1.6 ± 0.5	1.3 ± 0.5	1.1 ± 0.4
	26	1.9 ± 0.4	1.7 ± 0.5	1.4 ± 0.5	1.7 ± 0.5	1.6 ± 0.5	1.6 ± 0.5

* Scoring scale employed: 1 = very good; 2 = good; 3 = acceptable; 4 = unacceptable.

Table 3
Weight loss, pH and acidity evolution in green asparagus stored under Refrigerated and MAP conditions[1]

Storage condition	Days	Weight loss[2]	pH	Acidity[3]	Vitamin C[4]
First season					
Ref 2 °C	0	499.27 ± 4.13^{a}	6.46 ± 0.04^{a}	0.10 ± 0.01^{c}	209 ± 6.33^{a}
	5	478.88 ± 5.79^{b}	6.35 ± 0.03^{b}	0.11 ± 0.01^{b}	162 ± 4.97^{b}
	9	455.07 ± 4.53^{c}	6.11 ± 0.06^{c}	0.11 ± 0.00^{bc}	97.5 ± 2.87^{c}
	14	440.37 ± 4.57^{d}	6.18 ± 0.08^{c}	0.12 ± 0.00^{a}	84.3 ± 1.47^{d}
MAP 2 °C	0	499.27 ± 4.13^{a}	6.46 ± 0.04^{a}	0.10 ± 0.01^{b}	209 ± 6.33^{a}
	5	495.41 ± 7.63^{ab}	6.32 ± 0.08^{b}	0.10 ± 0.01^{b}	174 ± 7.74^{b}
	9	493.52 ± 7.49^{ab}	6.36 ± 0.01^{b}	0.09 ± 0.00^{b}	157 ± 7.94^{c}
	14	492.47 ± 7.85^{ab}	6.32 ± 0.01^{b}	0.10 ± 0.00^{b}	122 ± 1.52^{d}
	20	490.57 ± 7.50^{ab}	6.16 ± 0.11^{c}	0.10 ± 0.00^{b}	101 ± 4.41^{e}
	26	490.15 ± 7.48^{ab}	6.05 ± 0.05^{d}	0.11 ± 0.01^{a}	95.0 ± 4.21^{e}
	33	488.78 ± 6.99^{b}	5.92 ± 0.08^{e}	0.11 ± 0.01^{a}	70.7 ± 12.40^{f}
MAP 10 °C	0	499.27 ± 4.13^{a}	6.46 ± 0.04^{a}	0.10 ± 0.01^{b}	209 ± 6.33^{a}
	5	495.41 ± 7.63^{ab}	6.32 ± 0.08^{ab}	0.10 ± 0.01^{b}	174 ± 7.74^{b}
	9	491.92 ± 7.30^{ab}	6.15 ± 0.12^{b}	0.09 ± 0.01^{b}	133 ± 7.27^{c}
	14	492.61 ± 6.12^{ab}	5.82 ± 0.27^{c}	0.10 ± 0.01^{b}	103 ± 7.48^{d}
	20	489.24 ± 7.02^{b}	5.53 ± 0.22^{d}	0.13 ± 0.01^{a}	52.5 ± 4.74^{e}
Second season					
Ref 2 °C	0	452.76 ± 10.1^{a}	6.15 ± 0.01^{a}	0.07 ± 0.00^{c}	134 ± 2.87^{a}
	6	425.17 ± 6.14^{b}	5.74 ± 0.19^{b}	0.08 ± 0.01^{bc}	108 ± 4.80^{b}
	12	405.12 ± 6.28^{c}	5.43 ± 0.28^{c}	0.09 ± 0.01^{b}	61.9 ± 5.88^{c}
	16	386.87 ± 4.81^{d}	5.03 ± 0.17^{d}	0.11 ± 0.01^{a}	37.8 ± 4.00^{d}
MAP 2 °C	0	452.76 ± 10.1^{a}	6.15 ± 0.01^{a}	0.07 ± 0.00^{a}	134 ± 2.87^{a}
	6	452.16 ± 10.0^{a}	5.98 ± 0.06^{ab}	0.07 ± 0.00^{a}	121 ± 1.87^{b}
	12	449.96 ± 10.0^{a}	6.08 ± 0.01^{a}	0.07 ± 0.00^{a}	85.2 ± 3.60^{c}
	16	449.31 ± 10.2^{a}	5.84 ± 0.11^{b}	0.07 ± 0.00^{a}	76.8 ± 2.10^{d}
	21	448.47 ± 9.81^{a}	5.80 ± 0.09^{b}	0.07 ± 0.00^{a}	60.5 ± 0.96^{e}
	26	448.09 ± 9.71^{a}	5.42 ± 0.33^{c}	0.08 ± 0.00^{a}	57.5 ± 4.00^{e}

[1] The values without the same letter within each storage assay and column represent significant differences ($\alpha = 0.05$).
[2] Data shown in g fresh weight.
[3] Data shown in g/100 g citric acid.
[4] Data shown in mg/100 g vitamin C.

The same tendency was detected for the second season stalks, with significant losses ($p \leqslant 0.001$) between sampling dates and total losses of 14.6% over the 16 refrigerated storage days. This was slightly greater than those detected in the first season's refrigerated samples but markedly higher than those non-significant losses ($p > 0.05$) experienced by the MAP stalks of only 1.0% over the 26 storage days.

3.4. Texture

This parameter is, along with the external appearance, the factor that determines the acceptance or rejection by the consumer (Rodrıguez, Jiménez, Guillén, Heredia, & Fernández-Bolaños, 1999; Siomos, Sfakiotakis, & Dogras, 2000; Zurera, Muñoz, Moreno, González, & Amaro, 2000).

Due to the tissue differences of the three parts analysed, the initial shear forces were markedly different among them. The base showed the greatest force required and the apex the least.

The initial texture determined for both seasons, as shown in Table 4, was higher for the middle and base section in the second season, due to the later harvest date of that season. This is because the fibre increases over the harvesting season, even though there are other factors that can influence this, such as climate and especially temperature in the 24 h prior to harvesting (Clore, Carter, & Drake, 1976; Powers & Drake, 1980; Sosa, Vest, & Herner, 1976).

The texture measurements of the stalks in both seasons showed a lignification process, which was affected by the storage conditions and the stalk portions (base, middle or apex). This increase in the shear force is due mainly to lignin development and the activity of the enzyme primarily responsible for these changes, the phenylalanine–amonialyase (PAL), which decreases in concentration from the base to the apex (Guillén, Sánchez, Jimenez, & Heredia, 1995; Lau, Tang, & Swanson, 2000; Lipton, 1990). This is the reason why the base experiences the greatest increase in shear force among all the portions while, among the treatments, the refrigerated storage samples experienced an in-

Table 4
Texture evolution in green asparagus stored under Refrigerated and MAP conditions[1]

Storage condition	Days	Apical section	Middle section	Basal section
First season				
Ref 2 °C	0	49.20 ± 3.97^{c}	54.68 ± 4.05^{d}	151.77 ± 27.12^{c}
	5	50.87 ± 3.47^{bc}	64.14 ± 3.41^{c}	189.69 ± 45.49^{b}
	9	55.63 ± 5.91^{ab}	69.64 ± 5.07^{b}	201.77 ± 20.45^{b}
	14	58.20 ± 3.25^{a}	82.05 ± 5.16^{a}	258.31 ± 23.50^{a}
MAP 2 °C	0	49.20 ± 3.97^{b}	54.68 ± 4.05^{c}	151.77 ± 27.12^{a}
	5	51.16 ± 3.88^{ab}	57.73 ± 4.25^{c}	156.83 ± 25.09^{a}
	9	51.22 ± 1.61^{ab}	58.57 ± 2.48^{bc}	157.84 ± 16.17^{a}
	14	52.10 ± 2.19^{ab}	60.99 ± 2.90^{abc}	158.71 ± 33.89^{a}
	20	51.37 ± 1.31^{ab}	64.95 ± 2.57^{ab}	160.61 ± 29.48^{a}
	26	53.90 ± 4.22^{a}	66.55 ± 3.00^{a}	168.42 ± 25.16^{a}
	33	53.89 ± 2.87^{a}	67.79 ± 11.80^{a}	166.80 ± 31.50^{a}
MAP 10 °C	0	49.20 ± 3.97^{ab}	54.68 ± 4.05^{ab}	151.77 ± 27.12^{b}
	5	51.16 ± 3.88^{a}	57.73 ± 4.25^{ab}	156.83 ± 25.09^{b}
	9	50.99 ± 5.14^{a}	59.75 ± 5.47^{a}	161.31 ± 10.21^{ab}
	14	45.23 ± 4.77^{b}	53.60 ± 5.45^{b}	160.28 ± 15.68^{ab}
	20	44.73 ± 1.23^{b}	53.90 ± 3.63^{ab}	174.20 ± 19.82^{a}
Second season				
Ref 2 °C	0	51.61 ± 5.00^{b}	61.17 ± 3.66^{c}	188.29 ± 26.11^{c}
	6	54.32 ± 5.76^{ab}	74.37 ± 2.94^{b}	222.49 ± 25.52^{b}
	12	55.45 ± 3.31^{ab}	76.32 ± 6.79^{b}	235.37 ± 29.88^{b}
	16	58.71 ± 6.87^{a}	82.37 ± 5.20^{a}	271.91 ± 14.53^{a}
MAP 2 °C	0	51.61 ± 5.00^{a}	61.17 ± 3.66^{b}	188.29 ± 26.11^{a}
	6	51.80 ± 1.90^{a}	69.87 ± 6.24^{ab}	209.16 ± 38.96^{a}
	12	49.94 ± 3.74^{a}	70.73 ± 6.87^{ab}	203.73 ± 37.29^{a}
	16	52.82 ± 4.14^{a}	71.70 ± 6.82^{a}	210.94 ± 61.79^{a}
	21	52.28 ± 4.80^{a}	72.30 ± 8.47^{a}	215.32 ± 25.03^{a}
	26	50.77 ± 3.35^{a}	73.93 ± 12.66^{a}	219.19 ± 33.68^{a}

Data shown in N.
[1] The values without the same letter within each storage assay and column represent significant differences ($\alpha = 0.05$).

crease markedly greater than those stalks under MAP storage for both seasons. The observed increase in the basal maximum shear force at the end of the first season's storage period was highly significant ($p \leqslant 0.001$) in the refrigerated samples, representing an increase of 70.2%, followed by the stalks stored under MAP at 10 °C ($p \leqslant 0.05$), with an increase of 14.8% and finally the MAP samples at 2 °C showed minor non-significant increases ($p > 0.05$) of 4.28%. The increases detected for the second season showed a similar tendency, with the same level of significance for the assays.

This same tendency was detected in the other two sections (middle and apex), though neither the increases over the storage time nor the differences between treatments were as marked. There was, however, a tendency toward a decrease in the shear force for the apex section during the MAP storage at 10 °C, which would be due to the lower PAL activity in conjunction with the softening effect of CO_2, detected in higher amounts during this storage period (Lipton, 1990; Sánchez Pineda de las Infantas, Cano Muñoz, & Cruz Ramírez, 2000).

3.5. pH and acidity

As reflected in Table 3, the pH decreases significantly ($p \leqslant 0.001$) over the storage time for each storage experiment, decreasing to levels of 6.2 over the 14 days of refrigerated storage, 5.9 and 5.5 after 33 and 20 days of MAP storage at 2 and 10 °C, respectively. The same tendency was detected during the second season, even though the initial values were lower than those of the first season, due to the different harvesting season (Hernández Méndez, Bernalte García, & Carballo García, 1993; Simon & Cerrolaza, 1993).

The acidity underwent a parallel increase over the storage time with the three storage techniques tested in the first season ($p \leqslant 0.01$), being more pronounced in the refrigerated storage samples, where significant differences were detected between sampling days. This was due to the higher water loss and solute concentrations, while the stalks stored under MAP showed stability until the 20 or 14th storage days at 2 or 10 °C, respectively. The second season follows the same trend, with pronounced increases over the refrigerated storage period

($p \leqslant 0.01$). However these were not significant ($p > 0.05$) for those detected under MAP 2 °C storage.

3.6. Vitamin C

The detected differences between the initial vitamin C levels for the two analysed seasons are due to different factors such as preharvest climatic conditions and cultural practices (rate and composition of fertilisers and irrigation), maturity and harvesting period (Lee & Kader, 2000).

The results reflect a clearly significant decrease for the vitamin C content over time in the refrigerated samples and smaller decreases in the stalks stored under MAP, both at 2 and 10 °C ($p \leqslant 0.001$).

The percentage of vitamin C retention after the 14 days of storage, for the refrigerated, first season samples at 2 °C, was 40.3%. After this same storage period, the percentages detected for the MAP samples at 2 and 10 °C were 58.3% and 49.1%, respectively. This means that the vitamin C concentration was highly conserved in these last two assays when compared with the refrigerated one. The retention levels detected at the end of storage time (33 and 20 days) for the trials carried out under MAP conditions, were 33.8% and 25.1% for the storage at 2 and 10 °C, respectively.

The percentage of retention at the end of the storage time (16 days) by the refrigerated, second season samples was 28.1%, a date where the stalks under MAP 2 °C presented much higher values (57.2%), this percentage decreasing over the rest of the storage time until day 26, when the retention level was only 42.6%.

The differential equation that describes the ascorbic acid degradation kinetics over the storage period is as follows: $\mathrm{dVit\ C}/\mathrm{d}t = -k\mathrm{Vit\ C}$, which represents an exponential decline

$$\mathrm{Vit\ C}(t) = C_0 \mathrm{e}^{-kt},$$

where C_0 represents the initial concentration and k is the coefficient that represents the ascorbic acid degradation rate (Fig. 3).

The deterioration rate varied, depending on the storage method. The highest constant (0.065/days) was for the refrigerated samples, followed by the stalks stored under MAP at 10 °C (0.052/days) and, finally, those asparagus placed under MAP at 2 °C (0.035/days), which underwent the lowest rate of degradation.

3.7. Microscopy analysis

Microscopy analysis, showed meristematic cells in the apex with organelles not very specialised, typical of young tissue in division while, along the stalk, toward the base, the cells were not meristematic.

As described above, it was observed initially that the degree of lignification in the xylem vessels increased from the apex to the base of the stalk, due to the greater degree of cellular differentiation in the base. Moreover, the supporting tissue observed was cellulosic in the apex and lignified toward the base, named colenchyma and sclerenchyma, respectively. These observations are consistent with the shear force analytical results obtained for the texture.

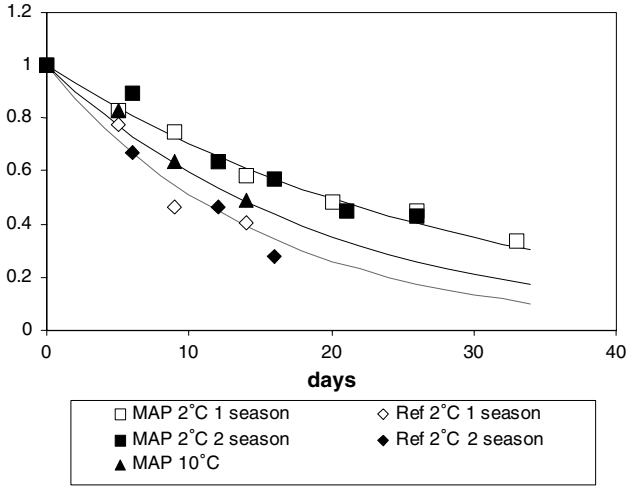

Fig. 3. Kinetic model of vitamin C degradation during green asparagus storage.

Over the storage period, there was a tendency toward lignification of the tissue, which was observed to a greater degree and appeared earlier in the refrigerated storage samples than in the MAP samples, as observed in Figs. 4(a) and (b).

The morphological characteristics of the epidermal cells and stomas did not change over the storage time. However, the loss in cell turgidity (an indication of senescence), the other major change besides lignification, appeared earlier and to a greater degree in the samples under refrigerated storage (Figs. 5(a) and (b)). The plasmolysed cell numbers for each storage technique increased over time. These characteristics were accentuated in the stalks placed only under refrigerated storage when compared to the MAP ones (Figs. 6(a) and (b)).

3.8. Microbial quality

Fresh vegetables normally have an elaborate spoilage microflora, due to intense contact with various types of microorganisms during growth and post-harvest handling, and therefore the numbers of microorganisms found on vegetables are highly variable. Initial mesophilic counts of all of the samples fell within the range 10^4–10^5 cfu g^{-1} which agrees with those found by Zagory (1999).

The microbial growths at the end of shelf-life of the refrigeration assay (14–16 days) and MAP 2 °C assay (33–26 days) were of the same order. Taking into

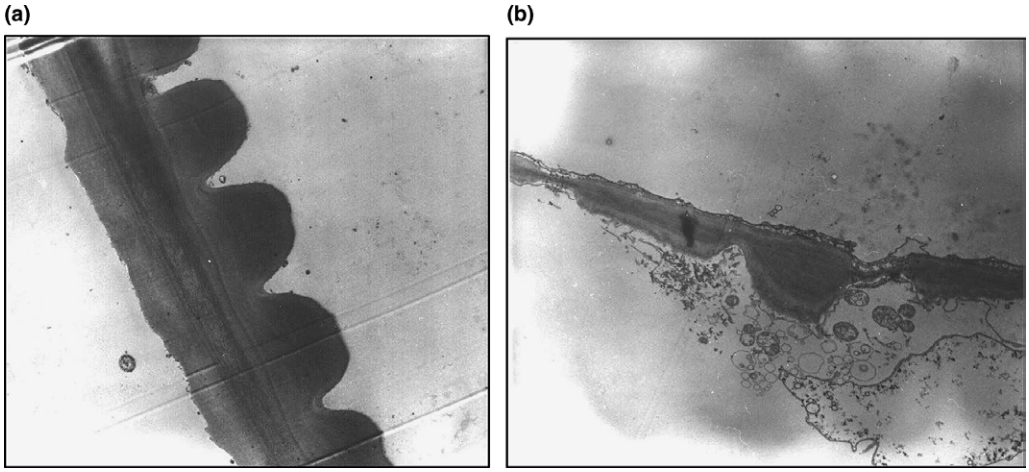

Fig. 4. Lignified cell walls of the green asparagus spear: xylem in the longitudinal section of the vascular bundle. Transmission electronic microscopy: (a) Day 16. Refrigeration storage (3000×); (b) Day 26. MAP at 2 °C storage (3000×).

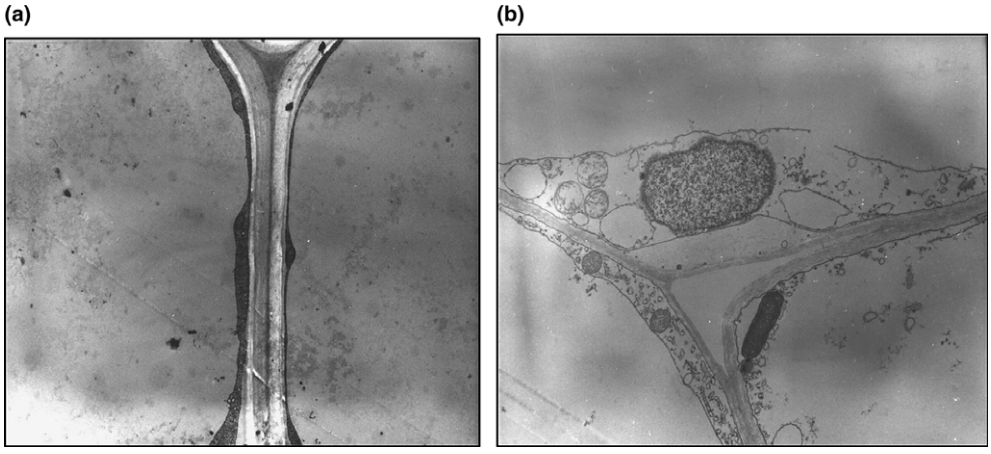

Fig. 5. Base of the asparagus. Transversal section of central parenquimatic cells. Transmission electronic microscopy: (a) Day 0 (4400×); (b) Day 16. Refrigeration storage (7000×).

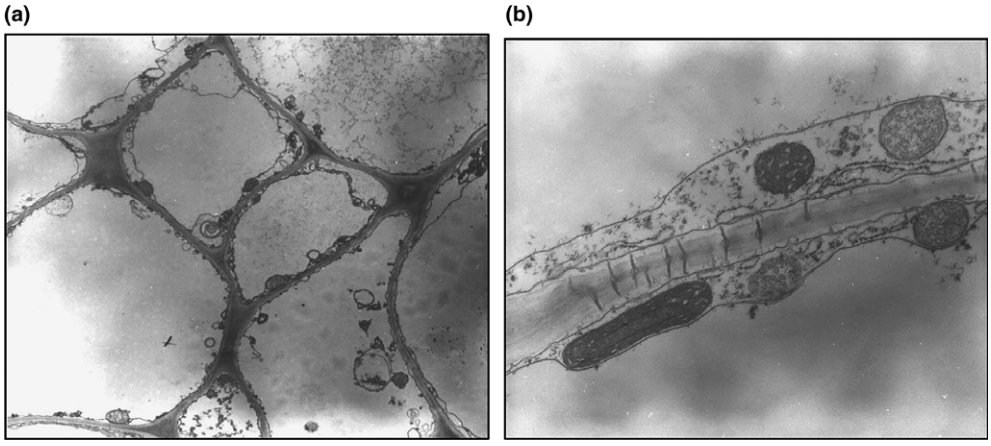

Fig. 6. Plasmolysed basal cells on the asparagus spears. Transmission electronic microscopy: (a) Day 15. Refrigeration storage (3000×); (b) Day 26 MAP at 2 °C storage (12000×).

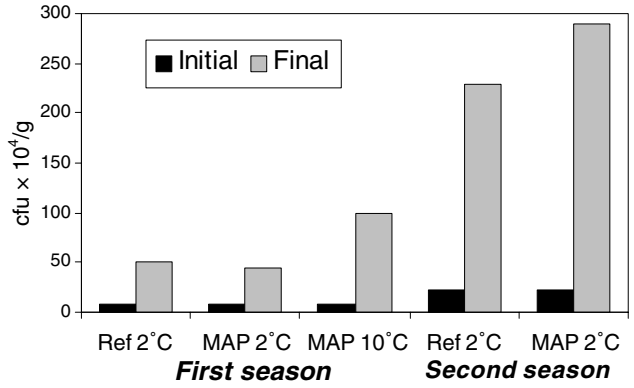

Fig. 7. Microorganism aerobics in green asparagus stored under refrigerated and MAP conditions.

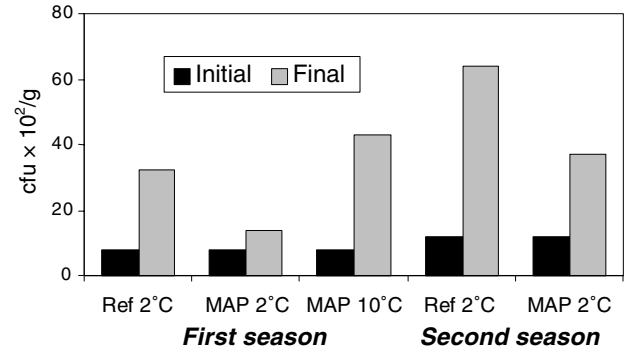

Fig. 8. Yeasts and moulds in green asparagus stored under refrigerated and MAP conditions.

account that storage period is much longer in MAP at 2 °C, this circumstance shows the positive effects of this conservation technique. The highest levels of aerobic microorganisms were detected in those samples stored under MAP at 10 °C ($p \leqslant 0.001$), which implies that temperature influences the growth of mesophiles (Figs. 7(a) and (b)).

Due to the risk of anaerobic growth caused by the low O_2 levels, this type of microorganism was tested for presence in the stalks toward the end of the MAP storage period. However, no anaerobic growth of microorganisms was found in any of the samples tested.

Yeasts and moulds, the main limiting microorganisms of shelf-life, increased from the first date until the end of each assay. There were detectable differences between the levels at the end of the shelf-life between the MAP (33–26 days) and the refrigerated (14–16 days) samples. This indicates better results for the MAP conditions (Figs. 8(a) and (b)).

4. Conclusions

Results showed that, for green asparagus, the different quality attributes (sensory, nutritive and hygienic) were best maintained using MAP storage at 2 °C. This storage system was shown to be the most suitable, increasing the shelf-life of green asparagus by 12 days when compared with refrigerated storage and 6 days when compared with MAP at 10 °C (after 5 days at 2 °C). This is a great advantage for fresh asparagus commerce.

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