# Influence of Packaging Films on the Sensory and Microbiological Evolution of Minimally Processed Borage (*Borrago officinalis*)

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**ABSTRACT: The atmospheres prevalent in borage packaged in 5 different films, together with different water-vapor permeabilities of the films, determined the evolution of the visual and microbiological quality of borage. PVC films proved inadequate to extend the sensory quality since the samples packaged with the films were spoiled on day 9, whereas among the P-Plus films, the least permeable film was the most adequate to extend the sensory quality of borage until day 15. For most of the treatments, no correlation between the microbial growth and changes in appearance was found. Thus, some treatments with a fair sensory evaluation had microbial counts higher than those allowed by the European legislation.**

**Keywords: borage, minimally processed vegetables, packaging films, sensory quality, microbial quality**

## Introduction

MINIMALLY PROCESSED FRESH (MPF) VEGEtables or "grade 4" products are ready-to-eat vegetables and include fresh, washed, and chopped vegetables ready for use and packaged with sealed polymeric films or trays. This fourth form of trading (preceded by fresh, canned, and frozen) was developed in the 1980s as an answer to an emerging consumer demand for convenience and for high-quality and preservative-free products with the appearance of fresh products, though less severely processed (Saracino and others 1991).

The extension of the shelf life of MPF products is achieved by means of a combination of correct refrigerated storage throughout the entire cold chain, a Modified Atmosphere Packaging (MAP), and good manufacturing and handling practices. MAP is a food-preservation technology whereby the composition of the atmosphere surrounding the product is different from the composition of air (O'Beirne 1990). In minimally processed vegetables, the gas composition in the package is modified by the respiration of the vegetative tissue (passive modification). After a time, an equilibrium-modified atmosphere (EMA) is created depending on the respiration activity of the product, the storage temperature, and the permeability characteristics of the packaging material. The permeability of the package depends on the characteristics and temperature of the packaging material, the thickness of the material, the type of permeating gas, and the differences in gas concentration across it (Exama and others 1993).

Thus, passive atmosphere modification is a complex process with many interactions among different components. It is important to understand the interrelationships of the different parameters involved to design a suitable MAP system to preserve the quality of a chosen commodity (Chau and Talasila 1994).

Most of the MAP approaches to respiring products are based on a reduction in the $O_2$ and an increase in the $CO_2$ concentration. A low $O_2$ level and a high $CO_2$ concentration may delay the browning and spoilage of the fresh appearance; however, they also can cause off-flavors and flavor losses (Cameron and Smyth 1997).

Many reports on the use of permeable polymeric films to extend the shelf life of minimally processed vegetables by means of the modification of the package atmospheric conditions (MAP) have been published (Priepke and others 1976; Kader and others 1989; Carlin and others 1990; Kwon and Lee 1995; Lee and others 1996; Mannapperuma and Singh 1998; Van de Velde and Hendrickx 2001). The main spoilage mechanisms affecting minimally processed vegetables are microbial growth, oxidation (enzymatic browning), and moisture losses. MAP is effective at inhibiting these spoilage mechanisms, as well as at reducing the respiration rate of vegetables. However, MAP does not eliminate the need of good hygiene practices and refrigeration (Willocx and others 1994).

Apart from the biochemical causes, food spoilage is caused by the growth of microorganisms that render the food unmarketable or inedible. It is characterized by undesirable sensory changes in the color, texture, flavor, or odor.

Microorganisms require certain definable conditions to grow and reproduce. In a food product, these conditions are either intrinsic properties of the product, such as the pH and Aw , or extrinsic factors associated with the storage environment. Some of the most relevant extrinsic factors are the temperature and gaseous composition of the environment. These extrinsic factors can be controlled by means of MAP to delay the spoilage and extend the shelf life. Thus, a $CO_2$ concentration in excess of 5% (v/v) inhibits the growth of most spoilage bacteria, especially psychrotrophic species. In general terms, Gram-negative bacteria are more sensitive than Gram-positive ones. Nevertheless, the survival of some pathogen microorganisms such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, or *Aeromonas hydrophila* in MPF products is a concern studied by many authors (Nguyen-the and Carlin 1994).

Borage (*Borrago officinalis* L.) is a vegetable that is very appreciated in the Ebro valley (in the north of Spain) with an annual production of about 15000 tons, but is less known in other regions. It is an annual herb half a meter high covered in a pubescent and even prickly epidermis that must be removed in the preparation stage. After peeling, the boiled fleshy stems are very pleasant to eat. The presentation of borage as a MPF product, ready to cook, would be very convenient for its commercialization to reduce the transport costs, the storage space and the preparation time.

Borage needs a severe treatment that affects its transpiration activity. In this sense, an important benefit of MAP is a large reduction in the transpiration rate. The package will act as a barrier to the movement of water vapor and will help to preserve a high relative humidity inside, which in turn will reduce the transpiration rate.

The aim of this study was to assess the impact of the preservation technologies on the visual quality and the growth of indicator microorganisms (spoilage) in minimally processed borage packaged with different films.

## Materials and Methods

### Preparation of the samples

Borage was obtained from a local grower in October and November 2001 and directly transported from the fields to the laboratory. After the manual peeling and cutting, the borage stems were quickly washed with 100 ppm free chlorine water (pH 7.0) (sodium phosphate buffer) at 4 ± 2 °C (10 L/K) by immersion for 5 min. After that, the stems were rinsed until the free chlorine levels were below 0.3 ppm. A solution of 2000 ppm ascorbic acid was used for the rinse. Then, the excess water was eliminated by centrifugation.

The borage was packaged using 5 types of films. Two different PVC (polyvinylchlorine) films were provided by FEISA (Madrid, Spain) in 60 cm × 1500 m reels: one of them was a PVC microperforated film (13 mm) (PVC-MP), whereas the other film was a PVC nonperforated film (13 µm) (PVC-NP) with an $O_2$ permeability of 7000 cm$^3$ m$^{-2}$ 24 h$^{-1}$ atm$^{-1}$ at 25 °C, a $CO_2$ permeability of 500000 cm$^3$ m$^{-2}$ 24 h$^{-1}$ atm$^{-1}$ at 25 °C and a water vapor permeability of 200 g m$^{-2}$ 24 h$^{-1}$ atm$^{-1}$ at 25 °C. The other 3 films were 35-µm P-Plus films (made of polypropilene) provided by Danisco (Bristol, U.K.) in 20- × 25-cm bags: film 35PA 120 (P-Plus 120), film 35PA 160 (P-Plus 160), and film 35PA 210 (P-Plus 210) with an $O_2$ permeability of 8000 cm$^3$ m$^{-2}$ 24 h$^{-1}$ atm$^{-1}$ at 25 °C, 15000 cm$^3$ m$^{-2}$ 24 h$^{-1}$ atm$^{-1}$ at 25 °C, 32000 cm$^3$ m$^{-2}$ 24 h$^{-1}$ atm$^{-1}$ at 25 °C, respectively. According to the specifications of the manufacturer, the first 2 numbers stand for the thickness of the film in microns. The set of letters corresponds to the type of film and the last figure is the permeability code. The higher this number is, the more permeable the film is.

The films tested in this work were selected because they are those most widely used nowadays by the industry to package minimally processed vegetables with characteristics similar to those under study.

Seven samples were packaged with each film. When PVC films, both microperforated and nonperforated, were used, 250 g of borage were placed on 140- × 230-mm polystyrene trays. The trays were covered and sealed using a hot plate Hand Wrapper model WS500E (Barcelona, Spain). For films C, D, and E, 250 g of borage were placed into the bags and sealed using a Vaessen-Schoemake machine (Barcelona, Spain). The packaged borage was stored at 4 °C for 15 days, and samples were taken on d 0 and after 1, 2, 4, 7, 9, 11, and 15 d of storage.

The entire experiment was repeated 2 times. The following determinations were made in each replication: gas determination, pH, color, texture, weight loss, ascorbic acid content, microbiological analysis, and sensory evaluation.

All the analyses were performed in duplicate.

### Gas determination

Carbon dioxide and oxygen were determined using an $O_2$ and $CO_2$ head space gas analyzer, Checkmate model 9900 (PBI-Dansensor, Denmark).

### Color determination

The calculation of the color coordinates was carried out from the reflectance spectrum measured with a Photo Research spectroradiometer PR-714 (Photo Research Division of Kolmorgen Instruments Corp., Chatsworth, Calif., U.S.A.) using as blank a reflectance standard plaque with certificate of calibration from Photo Research.

The samples were placed in a Macbeth SpectraLight lighting chamber to ensure the uniformity and stability of the lighting. The geometry used was 0/45 according to Hutchings' recommendations (1999).

For each sample, measures were taken at 10 different points and after that, the mean reflectance spectrum was obtained. From this result, the color coordinates within the CIELAB space were calculated for each sample following the CIE specifications (CIE 1986).

### Texture determination

The texture was measured using a compression press in an Instron Universal Testing Machine (Instron Model 1140, Bucks, U.K.) with a displacement speed of 50 mm min$^{-1}$. The slope of the graph is considered to be the force (in newtons) necessary to obtain a constant deformation of 1 mm. For each sample, the texture of 10 borage stems was determined.

### Other determinations

The pH of the washing and rinsing solutions was measured with a Crison model 2002 pH meter (Crison Instruments, Barcelona, Spain). To measure the pH of the product, 25 g of borage were blended for 2 min with 25 mL of distilled and deionized water (pH 7). The pH of the macerate was determined using the same equipment.

The free chlorine was determined by colorimetric reaction with DPD (N,N-diethyl-1,4-phenylenediamine), (Merk, Darmstadt, Germany).

The weight of the samples was measured with a Sorvall balance model B410 (Sartorius, Barcelona, Spain). The ascorbic acid content was reflectometrically estimated by re-

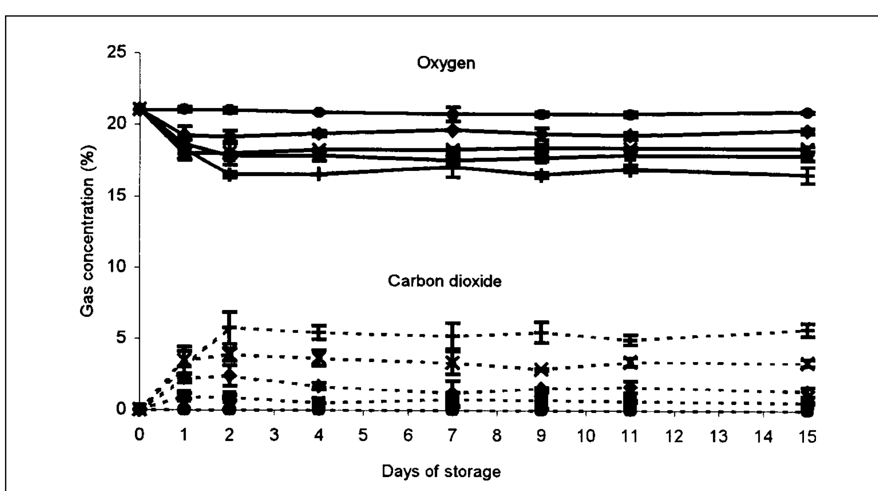


**Figure 1—Oxygen and carbon dioxide concentrations in minimally processed borage. Treatment PVC–MP (●), treatment PVC-NP (■), treatment P-Plus 120 (+), treatment P-Plus 160 (×), and treatment P-Pus 210 (◇).**

**Table 1—Sensory attributes evaluated for borage**

| | |
|---|---|
| Visual appearance | Intensity of green<br>Necrotic ends<br>General browning |
| Texture | Firmness<br>Friability |
| Odor | |
| Mold presence | |

duction of yellow molybdophosphoric acid to blue phosphomolibdenum (Merk, Darmstadt, Germany) (Washko and others 1992).

## Microbiological analyses

Twenty-five grams of borage were aseptically weighed and homogenized in a Stomacher (IUL, Barcelona, Spain) for 2 min with 225 mL of sterile peptone water (0.1% peptone plus 0.5% sodium chloride). Further decimal dilutions were made with the same diluent.

The total number of aerobic mesophilic microorganisms was determined on Plate Count Agar (PCA, Merck) following the pour plate method by incubation at 30 °C for 72 h (ICMSF 1978). The anaerobic mesophilic counts were calculated in the same way but by incubation under anaerobic conditions (ICMSF 1978).

The psychrotrophs were determined on Plate Count Agar (PCA, Merck) with an incubation temperature of 7 °C for 10 d following the pour plate method (ICMSF 1978).

The Faecal Coliforms were determined by the MPN method for a 3-tube series using Brilliant Green Bile Lactose Broth (BGBL, Difco) incubated at 44 °C for 48 h; when gas was formed, subcultures were made onto Levine Agar (Merck) and incubated at 37 °C for 48 h. The plates were then examined for suspected *E. coli* colonies (ICMSF 1978).

The aerobic spores were determined on PCA following the pour plate method and incubated at 30 °C for 72 h after a heat treatment at 80 °C for 10 min to destroy the vegetative cells. The anaerobic spores were determined in the same way but by incubation under anaerobic conditions (ICMSF 1978).

## Sensory evaluation

The sensory evaluation was used to discriminate between the visual appearance, texture, odor, and mold presence of borage packaged with different films. A panel of 7 judges assessed the sensory characteristics of packaged borage. The judges were selected on the basis of their interest, time available, liking for vegetables, aptitude for describing the sensory characteristics of food products, and sensory evaluation experience. The panelists were all members of the Dept. of Agriculture and Food at the Univ. of La Rioja.

The judges were trained in the discriminative evaluation of borage. The borage used in the training sessions had been subjected to various storage times and treatments. Fresh borage from the batch used in the processing was used as a control (score = 5). The training panel observed the effects of storage during 15 d in air against storage in a modified atmosphere. The products were presented on coded plastic dishes. The training sessions were conducted under normal lighting conditions (ISO/DIS 8589).

A simple scorecard was devised to quantify each sensory attribute. The intensity of the attributes evaluated (Table 1) was quantified on a scale from 1 to 5, where 1 to 2 = very poor, 3 to 4 = fair, and 5 = excellent. The judges relied on their training experience to score products. The sensory evaluation was used to determine the shelf life of these products. The scores below 3 for any of the attributes assessed were considered as an indicator of the end of the shelf life.

During the test sessions, the order of presentation of the samples was randomized. The evaluation of the samples was carried out under the same conditions than the training sessions.

## Statistical analysis

The entire experiment was carried out 2 times for each condition tested and for each one all the analyses were performed in duplicate.

The variance analysis was done using the SPSS program for Windows, Statistics version 10.0. A level of $P < 0.05$ was considered as a significance level. Tukey's test for comparison of means and correlation coefficient analyses between different parameters were performed using the same program.

The plate count data were written as logarithms prior to their statistical treatment.

# Results and Discussion

## Atmosphere within the packages

The kinetics of the $O_2$ and $CO_2$ changes within the packages depended on the permeability of the film (Figure 1). In treatment PVC–MP, the atmosphere within the packages had the same composition than the ambient air during the whole storage period. The atmosphere in the rest of the packages changed during the first 2 d, and after that, an EMA was reached.

Significant differences in $CO_2$ and $O_2$ concentrations were found between the borage packaged with different films with the exception of the $O_2$ levels of treatments PVC–NP and P-Plus 160. The consumption of oxygen was linked to the production of $CO_2$ except in treatment PVC–NP due its high $CO_2$ permeability. In fact, apart from treatment PVC–MP, this treatment had the lower levels of $CO_2$.

The oxygen concentration ranged between a minimum of 16.9% for P-Plus 120 and a maximum of 21.0% for treatment PVC–MP, whereas the $CO_2$ levels ranged between 0.0% for treatment PVC–MP and 5.4% for P-Plus 120. These values show a slight respiration rate in the borage packaged and stored at 4 °C. The storage at low temperatures reduces the changes in the atmosphere due to the influence of temperature on the respiration rate of fresh-cut vegetables (Willocx and others 1994).

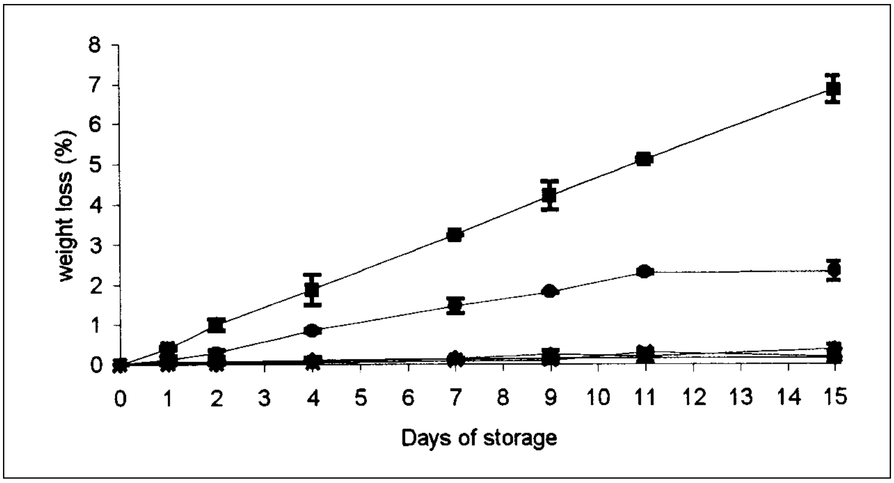


**Figure 2—Effect of packaging films on the weight losses in minimally processed borage. Treatment PVC–MP (●), treatment PVC–NP (■), treatment P-Plus 120 (+), treatment P-Plus 160 (×), and treatment P-Pus 210 (◇).**

## Weight losses

As shown in Figure 2, the weight losses in the different treatments under study were conditioned by the permeability to water vapor of the film used in the packaging. None of the three treatments where P-Plus films were used suffered substantial changes during the storage. However, the treatments with PVC films showed considerable losses. Thus, treatment PVC–MP showed losses of about 3% with respect to its initial weight after 15 days of storage, and these losses reached a level of about 7% of the initial weight in treatment PVC–NP. This film proved to be the most permeable to water vapor under the conditions tested.

## Evolution of the ascorbic acid content

Chemical preservatives may be useful to extend the shelf life of some minimally processed products. When chemical treatments are used, they must be effective to preserve 1 or more quality factors without degrading other quality factors. For example, a chemical additive to improve or preserve the color should not adversely affect the flavor. Moreover, chemical additives should not impair, or be perceived to impair, the product wholesomeness. Ideally, the chemicals applied should be perceived as useful and nutritional additives. Ascorbic acid is considered as a useful nutrient (Huxsoll and Bolin 1989).

Ascorbic acid is used in minimally processed fruits and vegetables to improve the appearance since it prevents the browning and other oxidative reactions (Bauernfeind and Pinkert 1970). This effect can also be ascribed to the addition of citric acid (Dziezak 1986). These 2 acids are often used jointly (Whitaker 1972).

In prior tests, the effect of the immersion of borage in solutions of ascorbic acid, citric acid, and combinations of both after its disinfection with chlorine was studied. It was verified that the borage immersed in 0.2% ascorbic acid solutions for 5 min showed a better appearance than that rinsed only with water. The rinse using citric acid solutions or a combination of citric acid with ascorbic acid was not so useful to improve the visual quality. The use of ascorbic acid solutions with a higher concentration led to the appearance of off-flavors in borage.

The immersion in 0.2% ascorbic acid solutions caused an increase in the levels of ascorbic acid of the borage of about 40% (90 to 110 ppm and 140 to 160 ppm before and after rinse, respectively). The levels reached were not similar to those recommended by Cort (1982) to inhibit effectively the browning (about 2000 ppm) but were over the 100 ppm level reported by the same author as an inductor of pro-oxidative effects. Sapers and others (1990) found that the immersion in ascorbic acid solutions delayed the browning in apples and potatoes stored at 4 °C, although these authors used 2.25% ascorbic acid solutions, which gave levels over 2000 ppm of this acid in the final product. However, the authors did not describe the flavor of these products after the treatment.

The stability of ascorbic acid during the storage of borage was studied (Figure 3). This acid has a high chemical instability to oxygen, temperature, humidity, and light and its degradation is associated with organoleptic deterioration caused by browning (Lee and Nagy 1988; Wong and Stanton 1989). In the presence of oxygen, the ascorbic acid destruction occurs simultaneously by oxidative and anaerobic mechanisms, the latter pathway being slower than the former. The oxidative degradation pathway occurs under neutral or alkaline conditions, reducing its speed with a pH lower than 6 (Rojas and Gerschenson 2001).

In all the treatments under study, the pH was between 6 and 7 during the whole storage period, and no significant differences among the treatments were identified. Within this pH range, the degradation of ascorbic acid takes place basically by an oxidative pathway. Indeed, in our case, the oxygen concentration in the packages determined the differences found in the degradation rate of ascorbic acid (r = –0.922, significance level of 99%). Thus, the degradation of ascorbic acid reached a value of 66% in treatment PVC–MP after 15 d0 of storage and a value of 55% in treatment P-Plus 210, whereas the losses in treatments PVC–NP, P-Plus120, and P-Plus 160 were around 26%.

## Color

Among the color coordinates determined (a*, b*, and L*), the most significant differences were found for coordinate a* (Figure 4). This coordinate shows a tendency toward red color (a* positive) or green color (a* negative). Coordinate a* increased during the storage in all the treatments under study due to the browning of the borage samples, and a correlation between the evolution of the values of coordinate a* and the oxygen concentrations in the different treatments under study was identified (r = 0.794, significance level of 99%).

The highest values were obtained in the samples from treatment PVC–MP, and the lowest ones in those from treatment P-Plus 120.

On the other hand, coordinate L* (brightness indicator) decreased during the storage in all the treatments, and no significant differences among them were found. No considerable changes in the values of coordinate b* (indicator of the tendency toward yellow for b* positive or blue for b* negative) were identified in the treatments during the storage.

These values are in line with the scores given by the panelists in the sensory evaluation of color for the different treatments during the storage period. The correlation found between the sensory and instrumental measurements was r = –0.838, with a significance level of 99%. Values of a* above –2 corresponded to sensory scores below of 3. This correlation allowed us to dispose of a

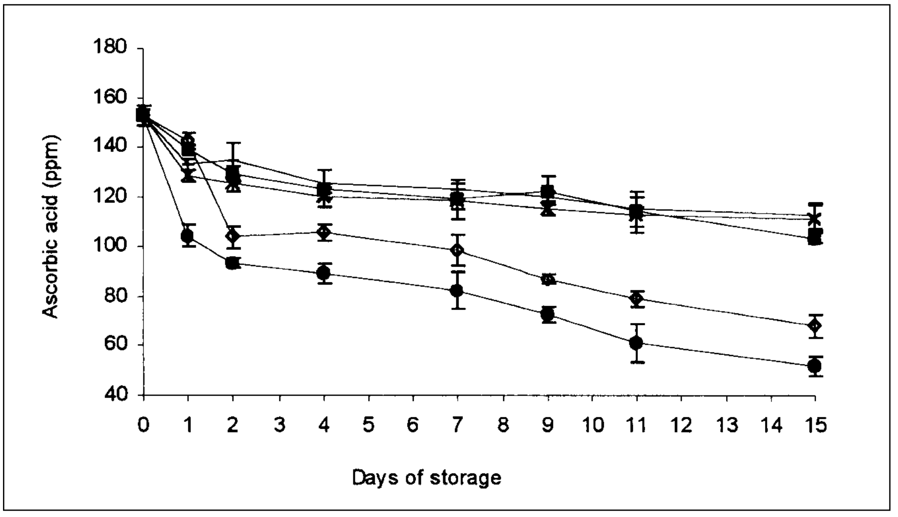


**Figure 3—Influence of packaging films of the ascorbic acid content. Treatment PVC–MP (●), treatment PVC–NP (■), treatment P-Plus 120 (+), treatment P-Plus 160 (×), and treatment P-Pus 210 (◇).**

standard to determine the critical sensory value of 3.

Thus, treatment PVC–MP received low scores for the color attributes after 7 d of storage due to the browning and presence of necrotic ends. On the other hand, treatments PVC–NP and P-Plus 160 showed color intensity losses and necrotic areas began to appear after 9 d of storage. Treatments P-Plus 210 and P-Plus 120 were not rejected by the panelists until d 11 and 15, respectively.

Hence, color seems to be one of the most important attributes to determine the sensory quality of packaged products (Hutchings 1999).

### Texture

The firmness changes during the storage may be a consequence of water losses and cellular breakdown due to both microbial growth and biochemical activity (Barry-Ryan and others 2000).

The impact of MAP packaging on texture depends on the type of vegetable and the packaging conditions. Thus, Barry-Ryan and others (2000) and Bolin and Huxsoll (1991) reported an increase in firmness for shredded carrots and lettuce, respectively. According to these authors, firmness increases during the storage due to the drying out of the vegetables and may be partially caused by lignin production. However, Ponting and others (1972) for apples, and Varoquaux and others (1990) for pineapple slices, reported rapid texture decreases during the storage ascribable to the release of proteolitic and pectolitic enzymes caused by cellular breakdown. Besides, a microbial increase may have an impact on the changes in texture. In this sense, pectolitic bacteria have been identified by different authors as responsible for the texture losses in vacuum-packaged carrot slices (Buick and Damoglou 1987) and in a number of other minimally processed vegetables (Manvell and Ackland 1986).

In our case, the texture measurements showed a firmness decrease during the storage period for all the treatments (Figure 5). After 15 d of storage, the firmness measurements were higher in the treatments with P-Plus films (about 9.5 N). No significant differences among the P-Plus films were found. The fastest texture decrease was detected for borage packaged with PVC-films, especially in treatment PVC–NP (6.2 N on d 15). A correlation between the texture evolution and the water vapor permeability in the different films tested was found (r = –0.912, with a significance level of 99%). Thus, the quicker firmness losses in treatment PVC–NP can be ascribed to the higher permeability to water vapor of the film, whereas the treatments with P-Plus films that did not suffer weight losses showed a slower firmness decrease.

Similar results were found in the sensory evaluation of texture. Hence, the treatments with P-Plus films reached the highest scores during the storage period. A correlation between the texture measurements and the sensory evaluation of the same parameter was identified (r = 0.802, with a significance level of 99%). Thus, the treatments that obtained scores of 3 or lower from the panelists in the sensory evaluation of texture corresponded to measures of 9 N or below. As in the color evaluation, this correlation allowed us to dispose of a standard to determine the critical sensory value of 3.

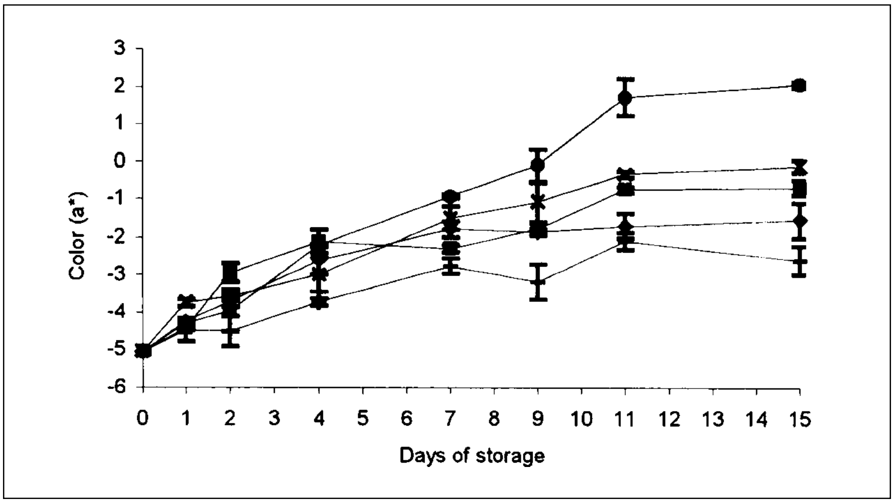


**Figure 4—Influence of packaging films on the color (a* values) in minimally processed borage. Treatment PVC–MP (●), treatment PVC–NP (■), treatment P-Plus 120 (+), treatment P-Plus 160 (×), and treatment P-Pus 210 (◇).**

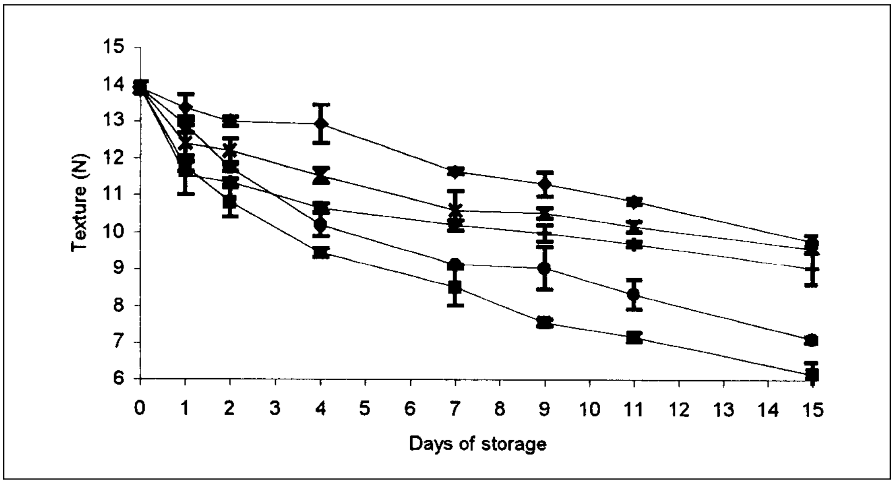


**Figure 5—Effect of packaging films on the texture in minimally processed borage. Treatment PVC–MP (●), treatment PVC–NP (■), treatment P-Plus 120 (+), treatment P-Plus 160 (×), and treatment P-Pus 210 (◇).**

### Microbiological analysis

The mesophilic counts of the unprocessed borage were of 7.6 log CFU/g. After peeling the borage, the counts were reduced to 7.0 log CFU/g. Sanitation with a 100 ppm chlorine solution reduced the mesophilic presence to 5.0 log CFU/g. These results are in line with those reported by other authors for similar vegetables and treatments (Adams and others 1989; Garg and others 1990; Nguyen and Calin 1994).

Although some authors had reported some antimicrobial activity of ascorbic acid (Adams and others 1989), the mesophilic counts did not show further reductions after rinse with a 0.2% ascorbic acid solution. This is in line with the results obtained by Priepke and others (1976) who concluded that the addition of ascorbic acid to water is ineffective to cause a reduction in the microbial load of the vegetable. Nevertheless, other authors (Burham and others 2001) proved the effectiveness of ascorbic acid to destroy pathogen microorganisms such as *E. coli* O157:H7 in apples, though in concentrations much higher than those used in this study.

Figure 6 shows the evolution of the aerobic mesophilic counts during the storage for the different treatments. With the exception of treatment PVC–MP, all the treatments showed a 1-day lag phase. This adaptation phase was not identified in the samples from treatment PVC–MP, since their $O_2$ concentration was similar to that found in the atmosphere during the storage period.

After this lag phase, the mesophilic counts increased in all the treatments. Until d 7, the evolution was similar in all of them. After d 9, significant differences were identified among treatments PVC–NP, P-Plus 120, and P-Plus 160 compared with treatments PVC–MP and P-Plus 210.

The legal regulations on minimally processed fresh vegetables establish a maximum total limit for microbial counts of $5 \times 10^7$ CFU/g (7.7 log CFU/g) (Francis and others 1999). According to this, the samples from treatments PVC–MP and P-Plus 210 exceeded the legal limit at about d 5 and 6, respectively. Treatments PVC–NP, P-Plus 120, and P-Plus 160 only reached the maximum legal limit after d 8.

After 15 d of storage, the highest mesophilic counts corresponded to treatment PVC–MP (11.4 log CFU/g), followed by treatment P-Plus 210 (10.4 log CFU/g). The lowest counts were found in treatments PVC–NP, P-Plus 120, and P-Plus 160 (9.2; 9.0, and 9.0 log CFU/g, respectively). The growth of mesophilic microorganisms was correlated with the level of oxygen in the packages: treatments PVC–MP and P-Plus 210 showed the highest content of oxygen whereas the content of treatments PVC–NP, P-Plus 120, and P-Plus 160 was lower ($r = 0.988$, with a significance level of 99%). These results are in line with those obtained by Barry-Ryan and others (2000) for sliced carrots packaged with P-Plus.

Figure 7 and 8 show the psychrotrophic and anaerobic counts, respectively, during the storage period for the different treatments. The psychrotrophs evolution was very similar to the mesophilic evolution, although the psychrotrophic counts were around 0.5 log CFU/g lower than those found in mesophiles. The same differences in the mesophilic counts among the treatments were observed for psychrotrophs. According to the study carried out by Garg and others (1990) on the microflora of fresh-cut vegetables, in most vegetables, the psychrotrophic and mesophilic counts are comparable, and the shelf life of refrigerated vegetables is mainly affected by the psychrotrophic population. In our case, this correlation was particularly evident in treatment PVC–MP that had the highest psychrotrophic counts and a shelf life of only 7 d.

After a 1-d lag phase, a slight increase in the anaerobic counts was identified during the storage (Figure 8). No significant differences in the counts obtained for the different treatments during the storage were identified, and final values lower than 6 log CFU/g after 15 d of storage were obtained for all the treatments. The composition of the atmosphere obtained inside the packages did not show clear anaerobiosis conditions. Thus, the growth observed in the different treatments proved the facultative character of the anaerobic microorganisms detected.

In turn, the population of sporulated microorganisms remained virtually constant during the whole storage period in all the treatments (around 3 and 2 log CFU/g sporulated aerobic and anaerobic microorganisms, respectively). The low storage temperature (4 °C) seemed to be the most evident cause of the absence of germination of this kind of microorganisms.

The faecal coliforms counts were < 0,47 log/g for all the treatments tested. This finding might be related to the hygienic handling conditions. *Escherichia coli* was not isolated in any sample. Besides, the coliform growth could be inhibited by the high populations reached by other competitors.

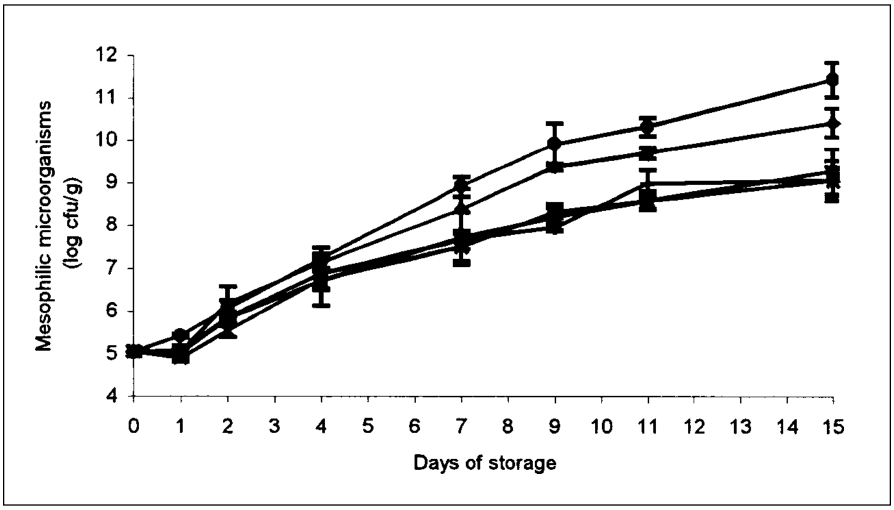


**Figure 6—Effect of packaging films on the mesophilic counts in minimally processed borage. Treatment PVC–MP (●), treatment PVC–NP (■), treatment P-Plus 120 (+), treatment P-Plus 160 (×), and treatment P-Pus 210 (◇).**

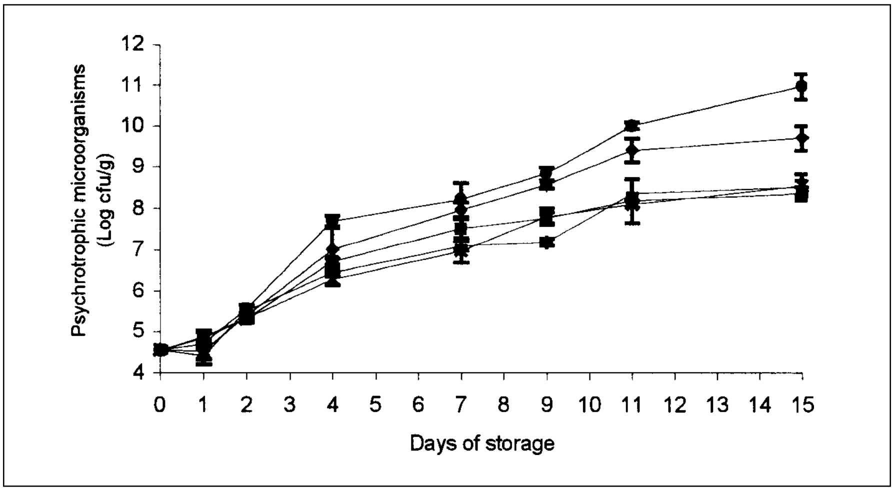


**Figure 7—Effect of packaging films on the psychrotrophic counts in minimally processed borage. Treatment PVC–MP (●), treatment PVC–NP (■), treatment P-Plus 120 (+), treatment P-Plus 160 (×), and treatment P-Pus 210 (◇).**

### Sensory evaluation

The scores of visual appearance, texture, and odor decreased during the storage in all the treatments under study. Treatment PVC–MP showed a quicker deterioration, since after 7 d of storage, the score obtained for visual appearance was lower than 3, thus implying the end of its shelf life. The low scores obtained were basically due to the generalized browning and to the presence of necrotic ends in the stems. After 9 d of storage, the samples from treatment PVC–MP lost the typical odor of the vegetal.

The samples from treatment PVC–NP obtained scores above 3 in all the parameters evaluated until d 9, and after this moment, they were spoiled. The dehydrated appearance of the samples and the lost of intensity of green in the samples from this treatment was particularly significant, mainly as a consequence of a higher moisture loss. The samples from treatment PVC–NP lost the typical odor of the vegetable after 7 d of storage.

The evolution of the sensory characteristics of the treatments where a P-Plus was used was different in each one of them. The best results were obtained for the samples from treatment P-Plus 120, which received the highest scores for their texture and color intensity. The browning and the appearance of necrotic areas did not take place until d 15. With this treatment, the typical odor of borage was preserved during the whole period of storage, although an intensity loss was detected. Like treatment PVC–NP, treatment P-Plus 160 was rejected after 9 d of storage, although in this particular case, it was due to a loss of intensity in color, the apparition of necrotic areas, and the development of off-odors. The samples from treatment P-Plus 210 did not suffer color losses, browning, or apparition of necrotic areas until d 11, although the texture values were only fair (scores higher than 3) until d 9, when it was spoiled.

### No molds were detected in any of the treatments

The limited respiratory activity showed by borage at storage temperatures of 4 °C suggests that the permeability of the films used did not determine marked differences in the composition of the atmospheres inside the packages after reaching an equilibrium. Nevertheless the impact of this factor on the development of microorganisms caused differences in the sensory quality of the treatments of up to 3 d, according to the limits established.

The different atmospheres obtained, together with the differences in the permeability to water vapor, also had an influence on the visual quality. The PVC films proved to be inadequate to extend the sensory quality, whereas among the P-Plus films, the least permeable film (P-Plus 120) was the most adequate one to extend the sensory quality of packaged borage.

Except for treatments PVC–NP and P-Plus 160, where the shelf life determined from a sensory point of view coincides with the microbiological limits established, the other treatments did not show any correlation between the development of microorganisms and the changes in the appearance. This lack of correlation was particularly evident in treatment P-Plus 120, rejected for microbiological reasons after d 8 but which had nevertheless a sensory quality score above 3 until d 15.

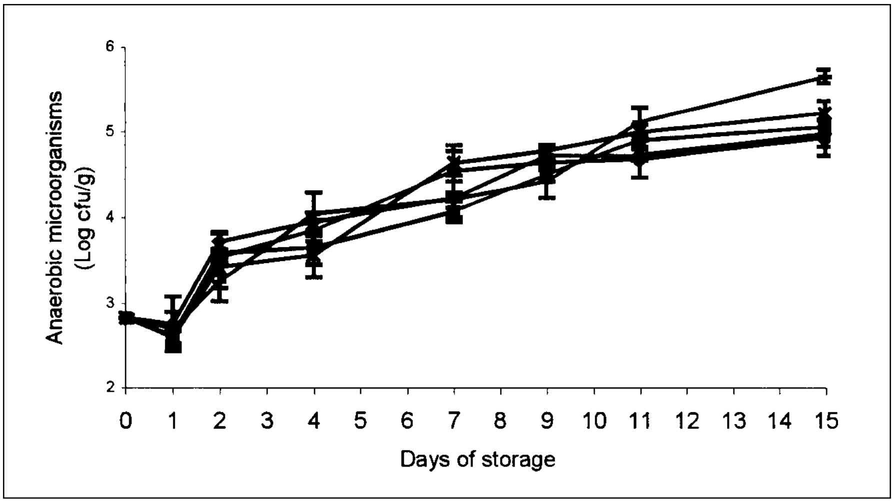


**Figure 8—Effect of packaging films on the anaerobic microorganisms growth in minimally processed borage. Treatment PVC–MP (●), treatment PVC–NP (■), treatment P-Plus 120 (+), treatment P-Plus 160 (×), and treatment P-Pus 210 (◇).**

## Conclusions

Considering the evolution of the a* values and the mesophiles counts during the storage period, the best film to package borage proved to be P-Plus 120. Moreover, the borage packaged with this film showed low weight losses and preserved its texture within levels above 3. The borage packaged with film P-Plus 120 increased its shelf life by around 8 d.

The possibility to apply mixed gases inside the packages according to the permeability of the films used to reach low $O_2$ levels and higher $CO_2$ levels, seems to be an interesting alternative worthy of study as a method to extend the shelf life of minimally processed borage.

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