Effect of Superatmospheric Oxygen Packaging on Sensorial Quality, Spoilage, and *Listeria monocytogenes* and *Aeromonas caviae* Growth in Fresh Processed Mixed Salads

ANA ALLENDE, 1 LIESBETH JACKSENS, 2 FRANK DEVLIEGHERE, 2 JOHAN DEBEVERE, 2 AND FRANCISCO ARTE S 1

1 Postharvest and Refrigeration Laboratory, Department of Food Science and Technology, CEBAS-CSIC, Murcia, Spain; 2 Laboratory of Food Microbiology and Food Preservation, Department of Food Technology and Nutrition, Ghent University, Gent, Belgium; and 3 Postharvest and Refrigeration Group, Department of Food Engineering, Technical University of Cartagena, Cartagena, Murcia, Spain

MS 01-435: Received 27 November 2001/Accepted 4 May 2002

ABSTRACT

Atmospheres with O<sub>2</sub> levels higher than 70 kPa have recently been suggested as an innovation to modified atmosphere packaging (MAP) for fresh processed vegetables to maintain sensory quality and safety. In the present work, mixed vegetable salad collected from a commercial processing plant and stored with the MAP technique was studied. Two gas mixtures were actively generated by using an initial O<sub>2</sub> concentration of 95 kPa and combined with two plastic films. The low-barrier film permeability for O<sub>2</sub> was 1.629 mO<sub>2</sub>/m<sup>2</sup> × 24 h × atm with 30 μm of thickness (Hyplast, Hoogstraten, Belgium) and the O<sub>2</sub> permeability of the high-barrier film was 2 mO<sub>2</sub>/m<sup>2</sup> × 24h × atm with 150 μm of thickness (Euralpack, Wommelgem, Belgium) at 23°C. As control, active conventional MAP with application of 3 to 5 kPa of O<sub>2</sub> and 6 to 8 kPa of CO<sub>2</sub> was used. Packaged salads were stored up to 8 days at 4°C and at temperatures simulating chilled distribution chain conditions. Microbial safety and sensory quality, as well as the survival of inoculated *Listeria monocytogenes* and *Aeromonas caviae*, were monitored. The effect of superatmospheric O<sub>2</sub> on the growth of aerobic microflora was variable. Under superatmospheric conditions, lactic acid bacteria and members of *Enterobacteriaceae* were inhibited. Nevertheless, growth of yeast and *A. caviae* seem to be stimulated by superatmospheric O<sub>2</sub>, whereas growth of psychrotrophic bacteria and *L. monocytogenes* was not affected. The overall visual appearance (mainly color) of the mixed vegetable salads was better maintained and the shelf life prolonged when packaged under O<sub>2</sub> concentrations greater than 50 kPa.

Several assessments performed on selected fresh processed produce items showed that O<sub>2</sub> concentrations higher than 70 kPa under modified atmosphere packaging (MAP) inhibited microbial growth and decay (2–5, 25, 28, 34). Therefore, it could be considered as an alternative to conventional MAP with moderate-to-low O<sub>2</sub> and elevated CO<sub>2</sub> concentrations (14). Many articles have demonstrated the advantages of conventional MAP using 3 to 5 kPa of O<sub>2</sub> and 5 to 10 kPa of CO<sub>2</sub> (balanced with N<sub>2</sub>) to reduce deterioration of fresh processed vegetables and proliferation of aerobic spoilage microorganisms (1, 6, 21, 27, 31). Nevertheless, it is known that the effect of conventional MAP on aerobic mesophilic microflora is variable. In some fresh processed vegetables packaged under conventional MAP, O<sub>2</sub> levels could decrease rapidly, depending on several factors (respiration activity of the produce, film permeability, surface area, storage temperature) that progressively create anaerobic conditions (e.g., <2 kPa of O<sub>2</sub> and >20 kPa of CO<sub>2</sub>) favorable for growth of anaerobic bacteria and undesirable fermentation reactions (25, 31).

Fresh processed mixed vegetable salad is a perishable product. Current techniques used by the fresh processing vegetables industry have improved the overall quality and extended shelf life of these products, but safety is still an issue of concern. Although conventional MAP helps maintain the quality of fresh processed vegetables, the benefits were not systematically related to a reduction in growth of mesophilic flora (31). In addition, psychrotrophic pathogens, such as *Listeria monocytogenes*, were not inhibited under conventional MAP (9–11, 19, 26).

Data already exist on the sensory quality changes and the growth of different microorganisms associated with the spoilage and safety of fresh processed fruits and vegetables stored under elevated O<sub>2</sub> concentrations (4, 5, 25, 28, 34). Exposure to superatmospheric O<sub>2</sub> may inhibit, have no effect, or even stimulate growth of different microorganisms from the same genus; however, the toxic effect of O<sub>2</sub> on microbial growth due to the formation of superoxide radicals (O<sub>2</sub> <sup>-</sup>) and their effects on cell metabolism have already been explained (5, 20, 22). The fact that some microorganism growth was not affected or even stimulated under superatmospheric O<sub>2</sub> indicated the presence of a defense mechanism, such as O<sub>2</sub>-decomposing enzymes or radical scavengers, to avoid lethal damage by O<sub>2</sub> (5). Zhulin et al. (35) explained that in the presence of superatmospheric O<sub>2</sub>, bacteria mainly used three strategies against reactive O<sub>2</sub> species generated by the partial reduction of O<sub>2</sub>: regulation of the expression of genes involved in aerobic metabolism and in the enzymatic defense, use of noncoupled respiration and mild uncoupling mechanisms to accelerate the respiratory consumption of O<sub>2</sub>, and escape from microenviroment...
ments where the O₂ level is too elevated. Because of the different behavior that microorganisms have under this atmosphere, it is necessary to study the effect of superatmospheric O₂ on the microflora of each product before it is used.

The aims of this study were (i) to evaluate the changes in sensory and microbial quality of mixed lettuce salad packaged under superatmospheric O₂ and under conventional MAP throughout cold storage, (ii) to study the effect of fluctuating temperatures during the chill distribution chain on the microbial and sensory quality of the product, and (iii) to determine the growth of inoculated L. monocytogenes and Aeromonas caviae under superatmospheric O₂ and conventional MAP throughout cold storage compared with that under the same atmosphere conditions of the chill distribution chain.

**MATERIALS AND METHODS**

Mixed vegetable salads packaged under superatmospheric O₂ at 4°C. Fresh processed mixed vegetable salad, including 20% endive (Cichorium endiva), 20% curly endive (Cichorium endiva), 20% radicchio (Cichorium intybus), 20% lollo rosso (Lactuca sativa), and 20% lollo bionda (Lactuca sativa) letsucces, was collected from a vegetable processing plant in Sint-Lievens Houte (Belgium). The processing included receipt, shredding, washing, rinsing, drying, and packaging in bulk at atmospheric conditions. Vegetable salads were transported to the laboratory (30 min) under refrigeration and immediately packaged into individual bags. Each bag (package dimensions, 21 × 17 cm) contained 150 g of mixed vegetable salad. To generate three different MAP treatments, two types of films and two gas concentrations (superatmospheric O₂ and conventional MAP) were applied as follows.

**MAP 1.** The MAP 1 technique consisted of salad packaged initially at approximately 3 to 5 kPa of O₂ and 6 to 8 kPa of CO₂ balanced by N₂, using a low-barrier film. Based on previous studies (24), the respiration rate of the mixed lettuce was calculated at 4°C (RO₂ = 4.52 mLo₂/kg·h). The calculated film permeability, which is necessary to obtain an equilibrium at 3 kPa of O₂, was 1,266 mLo₂/m²·24 h·atm at 4°C. We worked with a low-barrier film with a O₂ permeability of 1.629 mLo₂/m²·24 h·atm and 30 μm of thickness (Hyplast, Hoogstraten, Belgium). The selected atmosphere (3 to 5 kPa of O₂ and 6 to 8 kPa of CO₂ balanced by N₂) was introduced into packages before thermal sealing by a gas-packaging device (gas mixer, Witt M618–3MS0, Air Products, Vilvoorde, Belgium). The bags were sealed for 5 min with the desired atmosphere inside a zip bag and then heat sealed. When bags were sealed, the external zip bags were eliminated.

**MAP 2.** The MAP 2 technique consisted of salad packaged initially at 95 kPa of O₂, balanced by N₂, using the same low-barrier film. This atmosphere was introduced within bags directly from a cylinder with pure O₂ by using a low-barrier film with a O₂ permeability of 2 mLo₂/m²·24 h·atm at 23°C and 150 μm of thickness (Euralpack, Wommelgen, Belgium). The film was selected to maintain the highest possible initial O₂ concentrations within the packages. These bags were filled as described for MAP 2.

The selected MAP techniques were chosen based on results formerly obtained in previous experiments. This assay was performed twice.

**Gas atmosphere.** Changes in O₂ and CO₂ levels within packages throughout the shelf life were monitored. A 40-ml gas sample was taken from the headspace of the packages with a syringe and injected into a gas analyzer (Servomex, Series 1400, Crowborough, United Kingdom) to measure the composition of the gas before packages were opened.

**Microbial quality.** To determine the microbial quality of the mixed vegetable salads, standard enumeration methods were used. The following media and incubation conditions were used: plate count agar (CM325, Oxoid, Unipath, Basingstoke, United Kingdom) for total psychrotrophic counts by pour plating, incubated at 22°C for 72 h; deMan-Rogosa-Sharpe medium (pH 6.2) (CM361, Oxoid) for lactic acid bacteria (LAB) counts on pour plate and overlaid with the same medium, incubated at 30°C for 72 h; yeast glucose chloramphenicol (64894, Diagnostic Pasteur, Marnes-La-Coquette, France) to enumerate yeast by spread plating and incubating for 72 h at 30°C; violet red bile glucose agar (CM485, Oxoid) for Enterobacteriaceae bacteria counts on pour plate and overlaid with the same medium, incubated at 37°C for 24 h; and modified bile-salts-igasan-brillant green agar (30) (pH 8.7) and ampicillin dextrin agar by spread plating for Aeromonas growth, incubated at 30°C for 24 h.

Microbial analyses were performed on days 0 (production day), 3, 5, 6, 7, and 8. Each microbial count was the mean of four packages. All the analyses were performed in duplicate. Thirty grams of lettuce from each bag was mixed with 270 ml of peptone saline solution (8.5 g of NaCl [Vel 8605, Merck, Eurolab, Leuven, Belgium] plus 1 g of peptone [L34, Oxoid]) in a sterile stomacher bag and homogenized for approximately 1 min with a Colworth Stomacher 400 (Steward Laboratory, London, England). Tenfold dilution series were made in peptone saline solution as needed for plating. The microbial quality was evaluated following the French legislation (13) and the microbial criterion proposed by Debevere (17). Microbial counts were expressed as log₁₀ CFU/g.

**Sensory quality.** The sensory quality was assessed by six members of an expert sensory panel on the same days of microbial analysis, using a hedonic scale. All sensory tests were performed in a special tasting room with separated boxes and a red light. Organolectic properties, such as taste, odor, and texture, were evaluated under the red light to exclude the influence of visual characteristics (29). The visual properties (color and overall visual quality) were judged under normal light. The panelists used a descriptive graduated scale to record their perceptions of overall visual quality (excellent or fresh appearance, very good, good, or poor appearance or not fresh), aroma (excellent or fresh; very good; good; fair or slight; or poor, none, or not typical), texture (excellent, crisp, fresh, or succulent; very good; good; fair; or poor or limp), flavor, and color (same scale as for aroma). The scales were transformed to a 10-point scoring system, where 1 and 10 were the best and worst scores, respectively, and 5 was the limit of marketability from the consumer point of view. Results were obtained comparing the evaluations of the three MAP treatments. Appraisal of the quality was performed by applying a Tukey and Duncan multiple range test as implemented in SPSS 9.0 for Windows 95 to determine significant differences (P < 0.05) between the applied atmospheres.

**Shelf life.** Shelf life was determined according to sensory properties and the mentioned microbial criterion. Product was not
any more accepted for each one of the evaluated variables when the mean score was above 5.

Mixed vegetable salads packaged under superatmospheric \(O_2\) throughout the chill chain. A simulation of time and temperature conditions, from production to consumption by the consumer, of fresh processed mixed vegetable salads was performed. The whole chill distribution chain was divided into different consecutive handling and storing steps, including loading and unloading of the trucks and storage periods where the product was subjected to different time-temperature combinations. Taking into consideration previous studies concerning fresh processed vegetables throughout chill distribution chain \((13, 33)\), the steps shown in Figure 1 were followed. After production, fresh processed vegetables were conventionally stored at 4°C at the factory. The whole lot was transported to a central store and afterward to the distribution center, where pallets were loaded onto temperature-controlled trucks and transported to supermarkets and placed in cold rooms until moved to the shopping area. When packets were unloaded in the display cabinet, it was found that packaged fresh processed vegetables commonly stand for more than 1 h at room temperature \((33)\).

A temperature data logger (Escort, Tech Innovators, New Zealand) measured the temperature every 20 min. The data logger sensor was introduced into the package and the bag was mixed with the other prepared packages following the same steps. In this trial, salads were packaged under MAP 3 at two temperatures that simulated two temperatures at the display cabinet in the supermarket \((7/12^\circ C)\) and under MAP 1 stored at 7°C. A display cabinet temperature of 7°C was considered the maximum acceptable temperature, whereas a temperature of 12°C was considered temperature abuse. Comparisons between MAP 3 \((7^\circ C)\) and MAP 3 \((12^\circ C)\) and between MAP 3 \((7^\circ C)\) and MAP 1 \((7^\circ C)\) were made. To evaluate the influence of the temperature throughout the chill distribution chain on the salad quality, changes in gas composition, microbial growth \(\text{total aerobic psychrotrophic count, LAB, Enterobacteriaceae, Aeromonas spp., yeasts, and molds,} \) and sensory quality of the product were monitored. Microbial and gas analyses were performed after 24, 42, 52, 100, 102, 118, 142, and 166 h of storage. Sensory quality was evaluated after approximately 4, 5, 6, and 7 days of storage.

Effect of superatmospheric \(O_2\) on pathogenic microorganisms: inoculation. The inoculated cultures were one stock culture of \(A.\ caviae\) \((HG4)\), previously isolated at the Laboratory of Food Microbiology and Food Preservation (University of Ghent) from fresh commercial spinach, and two strains of \(L.\ monocytogenes\). one from stock culture \((\text{Scott A})\) and another isolated from fresh commercial green and red bell peppers at the same laboratory \((\text{LM LJ1})\) \((26)\). Each strain was consecutively subcultured twice in brain heart infusion broth \((\text{CM225, Oxoid})\) for 24 h at 37°C for \(L.\ monocytogenes\) and 30°C for \(A.\ caviae\). After the cultures were transferred the second time, they were allowed to adapt to the final temperature of 4°C for 6 h. After this period of incubation, concentrations of approximately \(5 \times 10^6\) \(\text{CFU/ml}\) of \(L.\ monocytogenes\) and approximately \(1 \times 10^8\) \(\text{CFU/ml}\) of \(A.\ caviae\) grown in the broth \(\text{(confirmed via plate counts)}\) were found. A final concentration of \(10^3\) to \(10^4\) \(\text{CFU/g}\) of each microorganism was desired in the product. This final concentration was reached by adding 0.5 ml from the adequate dilution of each microorganism inside the bags containing 150 g of mixed lettuces. Product was carefully mixed to ensure a homogenized distribution of the inoculate.

To evaluate the effect of the selected MAP \((\text{MAP 1, MAP 2, and MAP 3)}\) on pathogen microorganisms, 30 bags were stored up to 10 days under each MAP at 4°C and analyzed on days 3, 5, 6, 7, and 10. Another 32 bags were packaged with the MAP 3 method and subjected to time and temperature changes, to simulate a chill distribution chain, and stored at 7 or 12°C \((16 \text{ bags at each temperature})\) in the display cabinets. As control, 16 bags were packaged with the MAP 1 method and subjected to time and temperature changes and stored at 7°C in the display cabinet.

Microbial analysis. Samples were analyzed after 24, 42, 52, 100, 102, 118, 142, and 166 h. To determine the survival or growth of \(L.\ monocytogenes\), the following media and incubation conditions were used: \(Listeria\) selective agar base \((\text{Oxoid formulaion: CM856 [Oxoid]}) + \text{Listeria\ selective supplement and Oxford formulation: SR40E [Oxoid]}\) was used and plates were incubated at 37°C for 6 h. The strains of \(A.\ caviae\) were surface plated on modified bile-salts-irgasan-brillant green agar \((\text{pH 8.7)}\) and on ampicillin dextrin agar \((30)\), and the plates were incubated at 30°C for 24 h.

**RESULTS**

Gas composition changes. When MAP 3 was applied, \(O_2\) concentration within the bags always remained higher than 60 kPa at any storage temperature \(\text{(Fig. 2)}\). But using MAP 2, the \(O_2\) level decreased to approximately 12 kPa. Under an initial active high \(O_2\) level, no optimal equilibrium atmosphere was attained. Final concentrations were similar to those obtained by Heimdal et al. \((23)\) by applying...
Comparison growth of psychrotrophic bacteria under superatmospheric O$_3$

**TABLE 1. Growth of psychrotrophic bacteria, lactic acid bacteria, and Enterobacteriaceae (log$_{10}$ CFU/g) on mixed vegetable salad packaged under three MAP techniques**

<table>
<thead>
<tr>
<th>Day</th>
<th>MAP 1</th>
<th>MAP 2</th>
<th>MAP 3</th>
<th>MAP 1</th>
<th>MAP 2</th>
<th>MAP 3</th>
<th>MAP 1</th>
<th>MAP 2</th>
<th>MAP 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.98</td>
<td>5.78</td>
<td>5.78</td>
<td>3.48</td>
<td>3.34</td>
<td>3.34</td>
<td>4.08</td>
<td>4.08</td>
<td>4.08</td>
</tr>
<tr>
<td>3</td>
<td>6.58</td>
<td>7.10</td>
<td>6.61</td>
<td>4.63</td>
<td>4.38</td>
<td>3.91</td>
<td>4.28</td>
<td>4.19</td>
<td>3.93</td>
</tr>
<tr>
<td>5</td>
<td>7.08</td>
<td>7.52</td>
<td>7.16</td>
<td>5.25</td>
<td>4.50</td>
<td>4.76</td>
<td>5.49</td>
<td>4.91</td>
<td>4.43</td>
</tr>
<tr>
<td>6</td>
<td>7.27</td>
<td>8.02</td>
<td>7.80</td>
<td>5.34</td>
<td>4.87</td>
<td>5.03</td>
<td>5.54</td>
<td>5.51</td>
<td>5.26</td>
</tr>
<tr>
<td>7</td>
<td>7.46</td>
<td>8.11</td>
<td>7.80</td>
<td>5.43</td>
<td>4.93</td>
<td>5.50</td>
<td>5.57</td>
<td>4.98</td>
<td>5.41</td>
</tr>
<tr>
<td>8</td>
<td>7.76</td>
<td>8.18</td>
<td>7.70</td>
<td>5.92</td>
<td>5.10</td>
<td>6.06</td>
<td>5.34</td>
<td>5.28</td>
<td>5.37</td>
</tr>
</tbody>
</table>

* MAP techniques included conventional (MAP 1) and superatmospheric O$_3$ (MAP 2 and MAP 3) at 4°C. Values between parentheses indicate a 95% confidence interval. n = 4.

A 59-μm multilayer coextruded film and initial concentrations of 80 kPa of O$_2$ and 20 kPa of CO$_2$. When MAP 1 was applied, equilibrium concentrations were kept at approximately 3 to 4 kPa of O$_2$ and 7 to 8 kPa of CO$_2$. The storage temperature in the display cabinet (7 or 12°C) provoked slight differences in the atmospheric composition within the bags. As expected, the O$_2$ level decreased faster at 12 than 7°C, and the final CO$_2$ level was higher in bags stored at 12°C due to higher respiration rates.

**Effect of superatmospheric O$_3$ on microbial quality.**

Comparison growth of psychrotrophic bacteria under superatmospheric O$_2$ (MAP 2 and MAP 3) and conventional MAP (MAP 1) after 7 days at 4°C showed no significant difference (Table 1). French legislation allows a maximum of 4.7 log CFU/g (10$^5$ CFU/g) to guarantee the sensory quality (17, 26). Several in vitro studies were performed on yeast growth under superatmospheric O$_2$ atmospheres. Jacxsens et al. (25) reported that growth of Candida lambica was reduced by superatmospheric O$_2$; however, Amanatidou et al. (4) observed that the growth rate of Candida guilliermondii and Candida sake was stimulated by approximately 80 kPa of O$_2$. On the other hand, the combined application of 80 kPa of O$_2$ and 20 kPa of CO$_2$ almost completely prevented growth of Candida guilliermondii and C. sake. The effect of high O$_2$ on yeast growth was studied on fresh processed vegetables as well. Jacxsens et al. (25) reported that superatmospheric O$_2$ prolonged shelf life 3 more days when yeast growth was evaluated in chicory endive and grated celeriac (Apium graveolens var. rapaceum L.). In the present work, high initial yeast counts (close to 5 log CFU/g) were found in all experiments. Bags stored at 4°C exceeded this limit level after 3 days of storage by all applied MAPs (Fig. 3A). No difference was observed among the different MAPs before day 6. At that point, a difference of approximately 2-log CFU/g was observed between MAP 2 and MAP 3 and conventional MAP (MAP 1), which showed the lowest count. Yeast counts for MAP 1 and MAP 3 were similar on day 7; however, the same difference of 2 log CFU/g was kept to MAP 2. Equal tendency was observed on day 8. In storing the product at variable temperatures, the limiting level for yeast count was reached more quickly when prod-
Effect of superatmospheric O$_2$ on pathogenic microorganisms. An experimental study was performed to evaluate the survival or growth of inoculated *L. monocytogenes* on fresh-processed mixed vegetable salads. Product

---

**TABLE 2. Growth of psychrotrophic bacteria, lactic acid bacteria, and Enterobacteriaceae (log$_{10}$ CFU/g) on mixed vegetable salad packaged under conventional MAP (MAP 1) and superatmospheric O$_2$ (MAP 3) at variable temperatures, simulating the chill chain.**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Psychrotrophic bacteria</th>
<th>Lactic acid bacteria</th>
<th>Enterobacteriaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.33 (6.16–6.51)</td>
<td>7.16 (6.94–6.38)</td>
<td>6.08 (5.82–6.33)</td>
</tr>
<tr>
<td>24</td>
<td>6.82 (6.63–6.71)</td>
<td>7.36 (7.12–7.60)</td>
<td>6.39 (6.24–6.54)</td>
</tr>
<tr>
<td>42</td>
<td>7.06 (6.66–6.66)</td>
<td>7.56 (7.32–7.80)</td>
<td>6.69 (6.53–6.84)</td>
</tr>
<tr>
<td>52</td>
<td>7.07 (6.76–7.75)</td>
<td>7.57 (7.33–7.81)</td>
<td>6.70 (6.54–6.85)</td>
</tr>
<tr>
<td>100</td>
<td>7.49 (7.33–7.70)</td>
<td>7.58 (7.34–7.82)</td>
<td>6.80 (6.64–6.95)</td>
</tr>
<tr>
<td>102</td>
<td>7.82 (7.78–7.87)</td>
<td>7.57 (7.33–7.81)</td>
<td>6.80 (6.64–6.95)</td>
</tr>
<tr>
<td>118</td>
<td>8.04 (7.90–8.08)</td>
<td>7.81 (7.57–8.05)</td>
<td>6.90 (6.74–7.09)</td>
</tr>
<tr>
<td>142</td>
<td>8.06 (7.92–8.10)</td>
<td>7.83 (7.59–8.11)</td>
<td>6.90 (6.74–7.09)</td>
</tr>
<tr>
<td>166</td>
<td>8.08 (7.94–8.20)</td>
<td>7.83 (7.59–8.11)</td>
<td>6.90 (6.74–7.09)</td>
</tr>
</tbody>
</table>

* Bags stored under MAP 3 were subjected to 12 and 7°C at the display cabinet. Values between parentheses indicate a 95% confidence interval. n = 4.
was packaged under superatmospheric O₂ (MAP 2 and MAP 3) and conventional MAP (MAP 1) at 4°C and analyzed to evaluate supporting growth of *L. monocytogenes*. Carlin et al. (12) reported that in several types of fresh processed green salads stored at 10°C for 7 days, *L. monocytogenes* showed a small growth increase. Various types of salad, stored at 4°C for 4 days, supported growth of *L. monocytogenes*, indicating that it can survive and multiply during storage of chilled precut salads, and, therefore, it is of concern as a potential contaminant of this type of product (32). Additionally, Farber et al. (18) revealed that the growth of this organism was less on cabbage packaged in high-permeable film, implying that in some cases the aerobic microflora, like the LAB, can compete successfully with *Listeria* spp. Supporting this statement, Aytac and Gorris (7) reported that although *L. monocytogenes* was unable to grow on air-stored chicory endive at 6.5°C, it did grow on the vacuum-packaged product stored at the same temperature. Results obtained in the present work on *L. monocytogenes* growth after 10 days of storage, when conventional MAP or superatmospheric O₂ was used, did not show any significant change, except on day 5. On that day, *L. monocytogenes* counts were 0.85 ± 0.62 log CFU/g higher by using MAP 1 than MAP 2. Nevertheless, the rest of the changes were not significant (Tables 3 and 4), and consequently, *L. monocytogenes* only survived on mixed salads without any influence of the gas atmosphere within the bags. It was reported (18) that *L. monocytogenes* levels slightly increased or remained constant on various vegetable products (Caesar salad, coleslaw, onion, rutabaga, and stir-fry) stored at 4°C for 9 days. Our present results confirm these data. It is obvious that temperature has a strong effect on *L. monocytogenes* growth. Therefore, the influence of temperature changes along the chill distribution chain on mixed vegetable salads packaged under superatmospheric O₂ (MAP 3) was evaluated and compared with *L. monocytogenes* growth on conventional MAP (MAP 1) stored at the same temperature (Table 4). As expected, *L. monocytogenes* grew better when an abusive temperature (12°C) was applied; but again, the detected increase or decrease in *L. monocytogenes* growth under different MAP was not significant (Tables 3 and 4). Only survival of *L. monocytogenes* on lettuce was observed throughout the chill distribution chain.

There are not many studies on *A. caviae* on fresh processed vegetables. It was reported that *A. caviae* (HG4) was inhibited under 95 kPa of O₂ at in vitro conditions (25). In this case, *A. caviae* (HG4) growth was studied when inoculated in mixed vegetable salad stored following the chill distribution chain. Table 3 shows that *A. caviae* grew better under superatmospheric O₂ conditions (MAP 2 and MAP 3) when the product was stored continuously at 4°C. Similar results for product stored at 4°C were found at 7°C (Table 4). In this case, between days 2 and 5, stimulation of *A. caviae* growth was found. Finally, *A. caviae* counts were similar for all MAP after day 5.

**Sensory quality.** When comparing the evaluated sensory variables, color was the most determinative attribute for the overall visual quality of the product. Tissue browning due to oxidation of phenolic compounds, mainly by polyphenol oxidase enzyme (27), was also included in the color variable. No significant differences were obtained when the other sensory attributes were evaluated. Table 5 illustrates changes of the mean score for color and overall visual quality of mixed vegetable salads. Taking into account those sensory attributes, MAP 1 and MAP 2 exceeded the limit of marketability sooner than MAP 3 for both variables. This means that products kept until day 10 under superatmospheric O₂ (>60 kPa) conditions have a prolonged shelf life. On day 8 the product was not accepted any longer for all the studied MAPs. Off-odors were observed after 7 days of storage at 4°C, without any difference among treatments. Correlation of off-odors and aroma, measured sensorially with ethanol and acetaldehyde concentrations, was shown by López-Gálvez et al. (29). Heimdal et al. (23) reported that fresh processed iceberg lettuce...
packaged under 80 kPa of O$_2$ and 20 kPa of CO$_2$ showed less browning that those packaged in passive MAP. Instead of browning, a yellowing of leaves occurred in those bags, which was interpreted as a result of chlorophyll loss. No browning or yellowing of leaves occurred in MAP 2 and MAP 3 because of higher CO$_2$ levels. Jacxsens et al. (25) reported that superatmospheric O$_2$ was particularly effective in inhibiting enzymatic browning of different fresh processed vegetables, including shredded chicory endive. Beneficial effects of superatmospheric O$_2$ in sensory quality of different vegetable products sensitive to enzymatic discoloration (e.g., radicchio lettuce, lollo rossa lettuce) have already been reported (15, 16).

### CONCLUSIONS

It has been confirmed that superatmospheric O$_2$ does not affect all microorganisms in the same way. From our work, it can be concluded that psychrotrophic bacteria growing in mixed vegetable salad were not affected by use of different MAP techniques. Yeast growth was stimulated when superatmospheric O$_2$ was applied; however, LAB and Enterobacteriaceae showed growth inhibition when elevated O$_2$ and CO$_2$ concentrations were applied by MAP 2 and MAP 3. As expected, all bacteria counts were higher when the product followed the chill distribution chain, and the same happened when an abusive temperature (12°C) was

### TABLE 3. Growth of Listeria monocytogenes and Aeromonas caviae (log$_{10}$ CFU/g) on mixed vegetable salad packaged under three MAP techniques

| Day | Listeria monocytogenes | | Aeromonas caviae | |
|-----|------------------------|------------------|------------------|
|     | MAP 1                  | MAP 2            | MAP 3            | MAP 1                  | MAP 2            | MAP 3            |
|     |                        |                  |                  |                        |                  |                  |
| 0   | 2.64 (2.55–2.75)       | 2.64 (2.55–2.74) | 2.64 (2.55–2.74) | 4.40 (4.22–4.58)       | 4.40 (3.79–5.01) | 4.40 (3.79–5.01) |
| 3   | 2.53 (2.13–2.90)       | 2.71 (2.48–2.85) | 2.66 (1.93–3.39) | 4.41 (4.20–4.62)       | 6.05 (5.75–6.05) | 5.90 (5.75–6.05) |
| 5   | 2.85 (2.56–3.14)       | 2.52 (2.46–2.58) | 2.39 (2.21–2.55) | 4.88 (4.47–5.29)       | 5.89 (4.98–5.94) | 6.48 (6.34–6.61) |
| 6   | 2.84 (2.54–3.13)       | 2.41 (2.31–2.51) | 2.54 (2.53–2.55) | 4.78 (4.65–4.91)       | 7.29 (7.04–7.53) | 7.66 (7.57–7.75) |
| 7   | 2.83 (2.47–3.23)       | 2.83 (2.72–2.95) | 2.68 (2.59–2.77) | 4.48 (4.48–4.88)       | 7.53 (7.37–7.64) | 7.72 (7.58–7.86) |
| 10  | 2.94 (2.68–3.20)       | 2.84 (2.76–2.92) | 2.24 (1.76–2.70) | 3.95 (3.70–4.20)       | 8.08 (7.71–8.44) | 7.75 (7.36–8.15) |

*MAP techniques included conventional (MAP 1) and superatmospheric O$_2$ (MAP 2 and MAP 3) at 4°C. Values between parentheses indicate a 95% confidence interval. n = 4.

### TABLE 4. Growth of Listeria monocytogenes and Aeromonas caviae (log$_{10}$ CFU/g) on mixed vegetable salad packaged under conventional MAP (MAP 1) and superatmospheric O$_2$ (MAP 3) at variable temperatures, simulating the chill distribution chain

<table>
<thead>
<tr>
<th>Hour</th>
<th>Listeria monocytogenes</th>
<th>Aeromonas caviae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7°C, MAP 1</td>
<td>7°C, MAP 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.14 (1.95–2.31)</td>
<td>2.14 (1.85–2.44)</td>
</tr>
<tr>
<td>24</td>
<td>2.30 (2.02–2.58)</td>
<td>2.66 (2.47–2.88)</td>
</tr>
<tr>
<td>42</td>
<td>2.69 (2.67–2.71)</td>
<td>2.29 (2.14–2.44)</td>
</tr>
<tr>
<td>52</td>
<td>2.70 (2.50–2.88)</td>
<td>2.54 (2.42–2.65)</td>
</tr>
<tr>
<td>100</td>
<td>2.46 (2.35–2.56)</td>
<td>2.59 (2.46–2.70)</td>
</tr>
<tr>
<td>102</td>
<td>2.49 (2.30–2.70)</td>
<td>2.52 (2.43–2.61)</td>
</tr>
<tr>
<td>118</td>
<td>2.43 (2.36–2.49)</td>
<td>2.64 (2.55–2.74)</td>
</tr>
<tr>
<td>142</td>
<td>2.16 (2.03–2.29)</td>
<td>2.48 (2.47–2.49)</td>
</tr>
<tr>
<td>166</td>
<td>2.31 (2.18–2.44)</td>
<td>2.00 (1.80–2.20)</td>
</tr>
</tbody>
</table>

* Bags stored under MAP 3 were subjected to 12 and 7°C at the display cabinet. Values between parentheses indicate a 95% confidence interval. n = 4.
used in the display cabinets. The challenge test showed that 
*L. monocytogenes* survived when it was inoculated in fresh
processed mixed vegetable salads and there was no effect of
gas atmosphere. However, superatmospheric O$_2$
stimulated *A. caviae* growth. The general appearance was main-
tained longer, and the shelf life of the product was pro-
longed by using O$_2$ concentrations higher than 60 kPa
throughout the storage period. Color was the most impor-
tant parameter that affected general appearance, and mixed
vegetable salad stored under elevated O$_2$ and CO$_2$
concentrations obtained the best score for this parameter.

**ACKNOWLEDGMENT**

We acknowledge the concession of an FPI Spanish grant to A.
Allende, related to the CICYT ALI-98-1006 project.

**REFERENCES**

1. Ahvenainen, R. 1996. New approaches in improving the shelf life
   of minimally processed fruit and vegetables. *Trends Food Sci.
   2000. Improving modified atmosphere packaging system for keeping
   Gil, and M. A. Conesa (ed.), Improving postharvest technologies of
   fruits, vegetables and ornamentals. IIR Conference, Murcia, Spain.
   Microbial and sensorial quality of fresh processed lettuce salad under
   high O$_2$ atmosphere throughout the distribution chain. Proceedings of
   the eighth International Controlled Atmosphere Research Conference,
   2000. High oxygen and high carbon dioxide modified atmospheres
   for shelf-life extension of minimally processed carrots. *J. Food Sci.
   65*:61–66.
   elevated oxygen and carbon dioxide on the surface growth of vegeta-
   29*:664–668.
   hydrophila* and *Listeria monocytogenes* on fresh vegetables stored
8. Banks, N. H., D. J. Cleland, A. C. Cameron, R. M. Beaudry, and A.
   A. Kader. 1995. Proposal for a rationalized system of units for
   postharvest research in gas exchange. *J. Am. Soc. Hort. Sci.* 30:
   1129–1131.
   1995. Growth of psychrotrophic foodborne pathogens in solid sur-
   face model system under the influence of carbon dioxide and oxygen.
    *L. monocytogenes* on fresh vegetables stored at controlled atmo-
    minimally processed refrigerated fruits and vegetables, p. 209–312.
    In R. C. Wiley (ed.), Minimally processed refrigerated fruits and
    Effects of carbon dioxide on the fate of *Aeromonas hydrophila*, of aerobic
    bacteria and on the development of spoilage in minimally processed
    Jouve (ed.). La qualité microbiologique des aliments (maîtrise et
    critères), 2nd ed. Polytechnica, Paris, France.
    and non-sulphite dipping, p. 76. In Guideline No. 31. Campden &
    Chorleywood Food Research Association Group, Chipping Camp-
    den, Gloucester, UK.
    van de houdbaarheidsdatum in de etikettering, p. 37–64, Die Keure,
    Brugge, Belgium.
    populations of *Listeria monocytogenes* inoculated on prepackaged
    antimicrobial dip and temperature on the fate of *Listeria innocua*
    and *Listeria monocytogenes* on minimally processed lettuce. *Int. J.
    phere and vacuum packaging to extend the shelf life of respiring
    ical changes and sensory quality of shredded and MA-packaged ice-

---

**TABLE 5. Score means for color and visual appearance of mixed vegetable salad under three MAP techniques**

<table>
<thead>
<tr>
<th>Day</th>
<th>MAP 1</th>
<th>MAP 2</th>
<th>MAP 3</th>
<th>MAP 1</th>
<th>MAP 2</th>
<th>MAP 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>(0.98–2.00)</td>
<td>(1.21–2.04)</td>
<td>(1.39–1.61)</td>
<td>(1.05–1.94)</td>
<td>(0.98–2.00)</td>
<td>(0.93–1.06)</td>
</tr>
<tr>
<td>5</td>
<td>2.60</td>
<td>4.00</td>
<td>2.50</td>
<td>2.80</td>
<td>4.00</td>
<td>2.70</td>
</tr>
<tr>
<td></td>
<td>(2.02–3.16)</td>
<td>(3.49–4.48)</td>
<td>(2.10–2.89)</td>
<td>(2.32–3.27)</td>
<td>(3.55–4.43)</td>
<td>(2.46–2.93)</td>
</tr>
<tr>
<td>6</td>
<td>3.30</td>
<td>5.00</td>
<td>3.00</td>
<td>4.00</td>
<td>5.40</td>
<td>3.30</td>
</tr>
<tr>
<td></td>
<td>(2.62–4.02)</td>
<td>(4.60–5.39)</td>
<td>(2.91–3.29)</td>
<td>(3.50–4.49)</td>
<td>(4.50–6.29)</td>
<td>(3.27–3.33)</td>
</tr>
<tr>
<td>7</td>
<td>6.00</td>
<td>5.50</td>
<td>5.30</td>
<td>7.00</td>
<td>5.70</td>
<td>4.90</td>
</tr>
<tr>
<td>8</td>
<td>6.25</td>
<td>6.00</td>
<td>5.40</td>
<td>6.50</td>
<td>7.00</td>
<td>5.40</td>
</tr>
</tbody>
</table>

* MAP techniques include conventional (MAP 1) and superatmospheric O$_2$ (MAP 2 and MAP 3) at 4°C. Values between parentheses indicate a 95% confidence interval, $n = 4$. 