Microbial and quality changes in minimally processed baby spinach leaves stored under super atmospheric oxygen and modified atmosphere conditions

Ana Allende [a], Yaguang Luo [b,*], James L. McEvoy [b],
Francisco Artés [a], Chien Y. Wang [b]

[a] *Postharvest and Refrigeration Group, Department of Food Engineering, Technical University of Cartagena, Paseo Alfonso XIII, 48 30023 Cartagena, Murcia, Spain*
[b] *Produce Quality and Safety Laboratory, Plant Sciences Institute, Henry A. Wallace Agricultural Research Center, USDA-ARS, Bldg. 002, BARC-West, Beltsville, MD 20705, USA*

Abstract

The effect of super atmospheric O_2 and modified atmosphere packaging (MAP) on plant metabolism, organoleptic quality and microbial growth of minimally processed baby spinach was studied. Packaging film O_2 transmission rates and initial levels of super atmospheric O_2 in the packages significantly affected the changes of in-package atmospheres during storage, and consequently quality of baby spinach leaves. In general, a barrier film maintained a higher O_2 level for both 80 and 100 kPa O_2 treatments during entire storage. Packages with the barrier film also exhibited a more rapid accumulation of CO_2 than those with the permeable film, with CO_2 levels ranging from 16.2 to 22.5 kPa in the barrier film packages, versus 6.1–10.6 kPa in the permeable film packages at the end of 12 days of storage at 5 °C. Packages prepared with the barrier film with an initial O_2 level at 21% accumulated CO_2 during storage and exhibited a significant reduction in aerobic mesophilic bacterial growth compared to the perforated film packages (control). However, this treatment also developed strong off-odor and a loss of tissue integrity. Adding super atmospheric O_2 to the packages alleviated tissue injury in addition to reducing microbial growth and was beneficial in maintaining quality of fresh-cut baby spinach.
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Keywords: High oxygen; Off-odor; Modified atmosphere packaging; Tissue electrolyte leakage

1. Introduction

Packaged fresh-cut (minimally processed) vegetables are becoming more and more popular because they are convenient and ready-to-eat. The fresh-cut produce industry has been on a double-digit growth rate in response to an increased demand by consumers. A major challenge facing the industry, however, is the rapid quality deterioration and reduced shelf-life of fresh-cut products compared with whole vegetables due to physiological disorders and decays (Huxsoll and Bolin, 1989; Jacxsens, 2002). Although modi-

fied atmosphere packaging (MAP) has been successfully used to maintain the quality of fresh-cut fruit and vegetables, new technologies that allow an extension of the shelf-life are still in much demand by producers and distributors (Nguyen-the and Carlin, 1994; Ahvenainen, 1996). Being living materials, fresh-cut produce can modify the atmosphere in their packages as a result of respiratory O_2 consumption and CO_2 evolution (Pirovani et al., 1998). In general, gas compositions inside a MA package are low in O_2 and high in CO_2, depending primarily on temperature, product fill weight and respiration rate, package film O_2 and CO_2 transmission rates and the total respiring surface area. Major problems associated with fresh-cut vegetables are the development of strong off-odors and decay, discoloration, and tissue softening (Zagory and Kader, 1988; Bolin and Husxoll, 1991; Heimdal et al., 1995; Willox, 1995; López-Gálvez et al., 1997). For fresh-cut baby spinach, the major are particularly in strong off-odor and decay. Fresh-cut baby spinach leaves have a very high respiration rate and require high levels of O_2 in the packages to maintain their quality (Gorny, 1997; Wooster, 1998). Since packaging films currently available for fresh-cut produce do not have sufficient O_2 transmission rates to allow adequate O_2 in the packages to maintain product quality, commercially processed baby spinach leaves are currently packaged with perforated films. This practice, however, raises a food-safety concern that human pathogenic bacteria may transfer through the perforations and result in post-processing contamination of the produce. It is desirable that a technology be developed to maintain quality and safety, as well as to extend shelf-life of fresh-cut baby spinach packaged with non-perforated films.

It has been reported that treatment of super atmospheric O_2 is an effective means for both inhibiting microbial growth and enzymatic discoloration and preventing anaerobic fermentation reactions (Day, 1996, 2000, 2001). The improved effect of super atmospheric O_2, when combined with increased CO_2 concentrations on fresh-cut vegetables, was previously demonstrated (Heimdal et al., 1995; Amanatidou et al., 1999; Day, 2001; Allende et al., 2002). Yet, negative impact of super atmospheric O_2 on postharvest physiology and product quality was also reported. Kader and Ben-Yehoshua (2000) reviewed the application of super atmospheric O_2 for quality maintenance of fresh

produce and suggested that more research is needed due to the limited published information and the conflict reports on the effect of super atmospheric O_2. For minimally processed baby spinach leaves, since the traditional MAP technology does not provide the desired package atmospheres due to the limitation in package film permeability and the food safety concerns over the use of perforated film, the application of super atmospheric O_2 atmospheres could be a solution to overcoming these limitations. The main objectives of this research were (1) to evaluate the changes of package atmosphere conditions as influenced by super atmospheric O_2 treatment and packaging film oxygen transmission rate (OTR); and (2) to investigate the effect of packaging atmospheres, particularly elevated O_2 and CO_2 gas compositions, on plant metabolism, organoleptic quality, and microbial growth of fresh-cut baby spinach.

2. Materials and methods

2.1. Sample preparation

Fresh baby spinach (*Spinacia oleracea* L.) leaves were obtained from a local wholesale market in Jessup, MD (USA), on the day of their arrival. The products were transported (within 30 min) under refrigerated conditions to our laboratory and processed immediately as follows. Baby spinach leaves were washed in a $150 \mu L L^{-1}$ (2.0 mM) chlorine (NaOCl) solution at $10°C$ for 1 min, followed by rinsing with $10°C$ tap water. The excess surface water remaining on the leaves of the products was removed by centrifuging for approximately 30 s with a handheld salad spinner (OXO Good Grips, Elmira, NY).

2.2. Packaging

The prepared samples, 200 g each, were packaged in polyethylene film bags (48 cm × 65 cm) with two different O_2 permeabilities: an O_2 permeable film (P) with an OTR at $15 \, pmol \, s^{-1} \, m^{-2} \, Pa^{-1}$ and an O_2 barrier film (B) with an OTR at $0 \, pmol \, s^{-1} \, m^{-2} \, Pa^{-1}$. The permeable film was supplied by Packaging Concept Inc. (Salinas, CA, USA), and the barrier film by Koch Supplies Inc. (North Kansas City, MO, USA). Permeabilities of the films were tested by the manufacturers

at 23 °C and 1 atm condition. Super atmospheric O_2 conditions of the packages were provided by flushing the bags with the desired gas with compositions of 80 and 100 kPa O_2 (Praxair Inc., Danbury, CT, USA). The O_2 levels inside the bags were constantly monitored with an O_2/CO_2 Analyzer (Model Combi Check 9800-1, PBI Dansensor Inc., Denmark) during flushing. The bags were hermetically sealed when the atmosphere inside the bags reached the desired O_2 level. The conventional (passive) MAP bags were prepared with the same permeable and barrier films without gas flushing. The perforated bags were prepared using the permeable film with each bag modified with six perforations (1.0 cm diameter) made with a cork borer. All samples were stored at 5 °C for 12 days for subsequent evaluation on product quality and microbial growth.

2.3. Respiration rate and gas composition

Each 200 g prepared sample was placed in a 2 L glass jar at 5 °C. A continuous flow of CO_2-scrubbed and humidified air was pumped into the jars to avoid dehydration and excessive CO_2 accumulation. The respiration rate as a CO_2 evolution rate was monitored every 6 h for 11 days using a gas chromatograph (HP 5890a, Hewlett Packard Co., Rockville, MD) fitted with a Hayesep Q column (2.4 m × 3 mm) at 60 °C and a thermal conductivity detector. Triplicate samples were prepared and tested.

2.4. Gas composition and product quality evaluation

Headspace gas samples were withdrawn from the packages with a gas-tight syringe on the day of the evaluation. The concentrations of O_2 and CO_2 were analyzed with O_2/CO_2 infrared gas analyzers (Model S-3A/I and Model CD-3A, respectively; Ametek Pittsburgh, PA).

Tissue electrolyte leakage was measured following a modified procedure from Hong et al. (2000). Samples, 100 g each, were submerged in 1 L of deionized water at 5 °C for 30 min. The electrolyte of the solution was measured using a conductivity meter (Model 135A; Orion Research Inc., Beverly, MA, USA). Total electrolyte of the samples was determined after freezing the samples at −20 °C for 24 h and subsequent thawing. Electrolyte leakage was expressed as a percentage of the total electrolyte.

Overall quality of fresh-cut baby spinach leaves was evaluated after 12 days of storage by a six-member expert panel. The members of the panel were trained to recognize and score the quality attributes of baby spinach prior to the test. A 9-point hedonic scale, where 9 = 'like extremely', 7 = "like moderately', 5 = 'neither like nor dislike', 3 = 'dislike moderately', and 1 = 'dislike extremely' was used for the evaluation (Meilgaard et al., 1991). The samples were coded with random three-digit numbers to mask the treatment identity as an effort to minimize subjectivity and to ensure test accuracy.

All quality evaluations were performed in a temperature-controlled room at 5 °C to minimize the effect of temperature variation during testing.

2.5. Enumeration of microorganisms

Baby spinach leaf samples of 30 g each were macerated in 270 mL sterile peptone water with a 400 Lab Stomacher (Seward Medical, London, UK) and filtered with sterile glass wool. A 50 μL sample of each filtrate or its appropriate dilution was logarithmically spread on agar plates with an automatic spiral plater (Autospiral[TM] DW, Don Whitley Science Ltd., West Yorkshire, UK). Enumeration of the selected microorganisms was performed with the following culture media and conditions: (1) Tryptic Soy Agar (Difco Lab, Sparks, MD, USA) incubated aerobically at 30 °C for 24–48 h for total mesophilic aerobic microorganisms, and anaerobically at the same conditions for total mesophilic anaerobic microorganisms; the anaerobic condition was provided with BBL[TM] GasPak[TM] pouches (B-D Microbiology Systems, Sparks, MD, USA); (2) Potato Dextrose Agar with 100 μg mL^{-1} chloramphenicol incubated at 30 °C for 48 h for yeast; (3) Lactobacilli Man-Rogosa-Sharpe agar (Difco Lab) incubated at 30 °C for 72 h under 20 kPa CO_2 and 5 kPa O_2 provided with a water-jacketed incubator with automatic gas control (Forma Scientific Inc., Marjetta, OH, USA) for lactic acid bacteria (LAB); (4) McConkey Agar (Difco Lab) incubated at 37 °C for 24 h for enteric bacteria; (5) Pseudomonas Selective Agar (Difco Lab) incubated at 30 °C for 24–48 h for Pseudomonads spp.; (6) Erwinia Selective Medium (Atlas, 1997) incubated at 30 °C for 48 h for Erwinia spp. Microbial colonies were counted using a Protos Colony Counter (Model

50000; Synoptics Ltd., Cambridge, UK) and reported as $\log \text{cfu} \, \text{g}^{-1}$ of tissue.

2.6. Experimental design and statistical analysis

Experimental units were bags and there were three replications per treatment per evaluation period. Statistical analysis of the data was carried out using a SAS procedure (SAS Version 8.2, SAS Institute Inc., Cary, NC, USA). Significant differences among treatments were determined using the general linear model. Prior to the final experiment, a preliminary experiment was conducted with limited treatments. Both preliminary and final experiment yield a similar trend.

3. Results and discussion

3.1. Respiration rate and gas composition

The respiration rate as CO_2 evolution of fresh-cut baby spinach ranged from 146.3 ± 1.2 to $202.0 \pm 7.1 \, \text{nmol} \, \text{kg}^{-1} \, \text{s}^{-1}$ during testing, which is higher than other fresh-cut vegetables (Barth et al., 2002).

Oxygen levels inside the packages of MAP and super atmospheric O_2 treatments significantly decreased over time except those in the perforated packages (Fig. 1A). Both film OTR and initial O_2 level in the packages significantly affected O_2 reduction rates and thus the final O_2 levels in the packages. For barrier packages initially filled with ambient air (21-B), O_2 level decreased rapidly, reaching $0.07 \, \text{kPa} \, O_2$ on day 6. The O_2 level in permeable packages (21-P) decreased at a slightly slower rate, with $2.5 \, \text{kPa} \, O_2$ on day 6 and $0.10 \, \text{kPa} \, O_2$ on day 9. The differences between these two treatments are primarily attributed to their film permeabilities, as expected. The changes in O_2 levels in the super atmospheric O_2 treatments followed a reversed trend seen in the 21-P and 21-B treatments with respect to film permeability. With super atmospheric O_2 treatments (both 80 and 100 kPa treatments), there were faster reductions in O_2 levels in all of the permeable films than in barrier films. This is because the function of film barrier properties here is to prevent the O_2 from being transmitted outward rather than inward, contrary to the passive MAP treatment. The barrier film was able to maintain higher O_2 levels inside the packages while permeable film allowed O_2 to
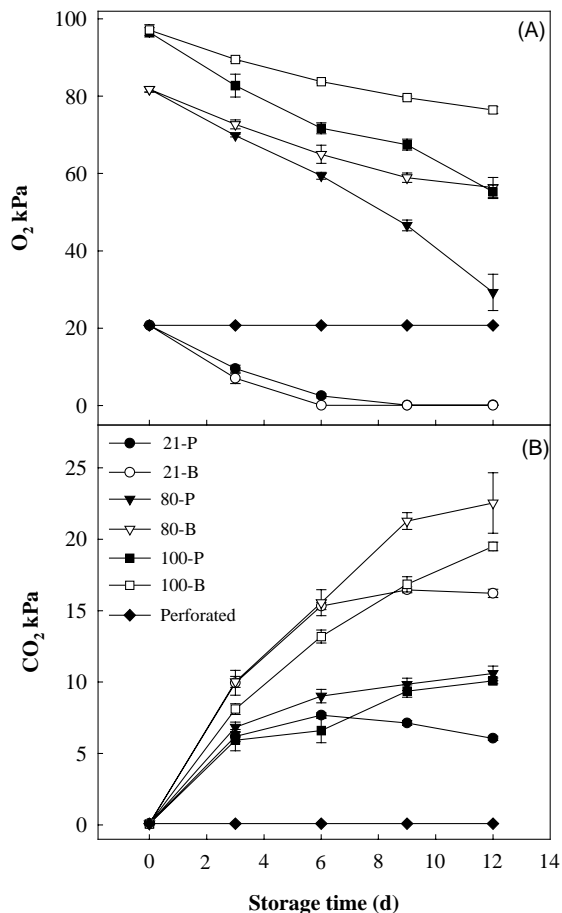

Fig. 1. Effects of super atmospheric oxygen treatments and package film oxygen transmission rate on the changes of O_2 (A) and CO_2 (B) gas compositions inside packages containing fresh-cut baby spinach leaves during storage. Bars represent $\pm$ S.E.

transmit outward. In addition, the level of initial super atmospheric O_2 also had a significant effect on the final O_2 levels. Treatments with $100 \, \text{kPa} \, O_2$ (100-P and 100-B) maintained higher O_2 levels inside the bags than those with $80 \, \text{kPa} \, O_2$ treatments (80-P and 80-B).

All treatments except perforated bags accumulated CO_2 over time (Fig. 1B). Package film permeability had a more pronounced effect on CO_2 accumulation than the initial O_2 levels did. For both passive MAP and super atmospheric O_2 treatments, there was a stronger accumulation of CO_2 within the barrier film bags than their corresponding O_2 treatments inside the permeable film bags. There were significant inter-

actions among package film permeability, initial O_2 treatment and the storage time, with the lowest CO_2 accumulation in 21-P treatment, and the highest in 80-B treatment on day 12. Contrary to O_2 changes, the function of film permeability was to transmit CO_2 outward. Since the barrier film has a low CO_2 transmission rate, it did not allow CO_2 evolved from spinach to be transmitted out and the accumulation of CO_2 inside the package may have led to increased production of CO_2 due to anaerobic respiration. Although there was a slower accumulation of CO_2 with the permeable films, the accumulation of CO_2 and depletion of O_2 (Fig. 1A) indicated that the permeability of the package films was insufficient for the spinach leaves.

3.2. Product quality

The measurement of tissue electrolyte leakage has been used as an indicator for tissue and membrane integrity in various studies (Murata, 1989; Marangoni et al., 1996). We have previously observed that electrolyte leakage was closely related to the quality and shelf-life of fresh-cut cilantro leaves (Kim et al., 2004). In this study, tissue electrolyte leakage increased over time (Fig. 2). There was a sharp increase in electrolyte leakage in the samples with 21-B treatment starting on day 6, coinciding with rapid quality deterioration (Fig. 3). This may indicate the onset of anaerobic
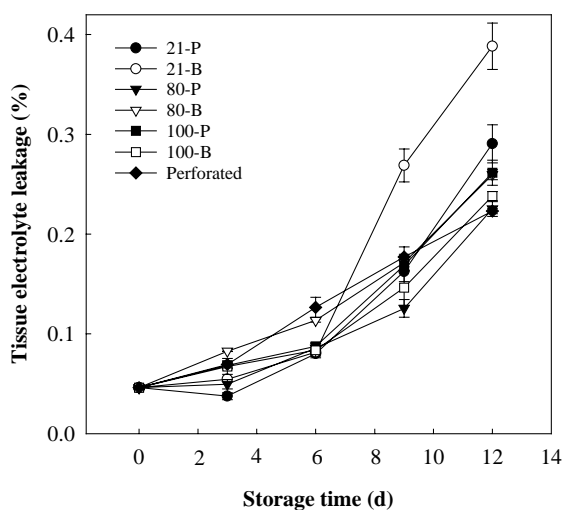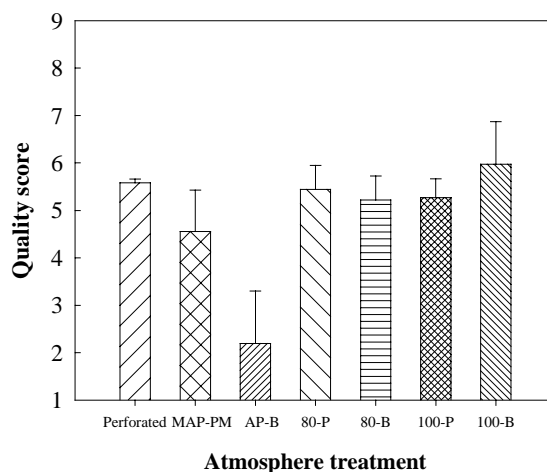
Fig. 3. Overall quality of fresh-cut baby spinach evaluated at the end of storage. Bars represent $\pm$ S.E.

respiration and CO_2 injury, result from rapid accumulation of CO_2 and depletion of O_2 (Fig. 1A, B). Interestingly, both 100-B and 80-B treatments had greater accumulations of CO_2 inside the packages, but the electrolyte leakage was much lower in those two treatments than that in 21-B. Significant differences were found between 21-B and the rest of the treatments ($P < 0.01$), among the control and 100-P, 80-B and 21-P bags ($P < 0.01$), and a slight difference ($P = 0.04$) was found between perforated and 100-B bags at the end of the shelf life. This suggests that adding high O_2 inside the packages of super atmospheric O_2 treatment contributed to lower tissue electrolyte leakage (Fig. 2) and higher product quality scores (Fig. 3) than without the addition of high O_2.

Sensory quality of baby spinach during storage was significantly affected by the package film oxygen permeability and the initial super atmospheric O_2 treatments (Fig. 3). The 21-B treatment exhibited the lowest overall quality at the end of storage followed by 21-P samples, primarily due to the development of a strong off-odor and loss of freshness. The quality deterioration of baby spinach leaves in MAP treatment was probably caused by anaerobic respiration and low O_2/high CO_2 injury. As reported by Gorny (1997) and Wooster (1998) and confirmed in our experiment, baby spinach leaves have a high respiration rate. Ko et al. (1996) also reported that the O_2 extinction point (EP) of spinach is 0.2–0.4 kPa; and quality deterio-

Fig. 2. Changes on the tissue electrolyte leakage of fresh-cut baby spinach leaves during storage. Bars represent $\pm$ S.E.

rated when the O_2 level in MAP dropped below this EP. In this study, O_2 level fell below EP starting on day 6 (Fig. 1A), which probably provoked anaerobic respiration, resulting in the development of an off-odor and loss of freshness. Spinach leaves treated with super atmospheric O_2 showed acceptable quality scores. The sensory results agreed well with those from tissue electrolyte leakage. Beneficial effects of super atmospheric O_2 in regard to the sensory quality of other vegetable products have been reported (Allende et al., 2001; Day, 2000, 2001; Jacxsens et al., 2001). The improved quality in super atmospheric O_2 treatment suggests that the inclusion of super atmospheric O_2 in the bags helped to maintain quality under extremely high CO_2 conditions.

3.3. Microbial growth

The initial microbial load on fresh processed baby spinach leaves after washing was $7.2 \pm 0.1 \log \mathrm{cfu\,g}^{-1}$ for aerobic mesophilics (Fig. 4A). This result is consistent with the findings previously reported by Babic et al. (1996) for fresh-cut spinach. Initial population of anaerobic mesophilics was $5.8 \pm 0.1 \log \mathrm{cfu\,g}^{-1}$ (Fig. 4B), $5.9 \pm 0.1 \log \mathrm{cfu\,g}^{-1}$ for total *Enterobacteriaceae* (Fig. 4C) and $6.1 \pm 0.1 \log \mathrm{cfu\,g}^{-1}$ for yeast (Fig. 4D). Those values were higher than those obtained by Babic et al. (1996) which ranged from 3 to $4 \log \mathrm{cfu\,g}^{-1}$ for total *Enterobacteriaceae* and yeast, respectively. In the preliminary test, we also examined numbers of *Erwinia* spp. and *Pseudomon-*
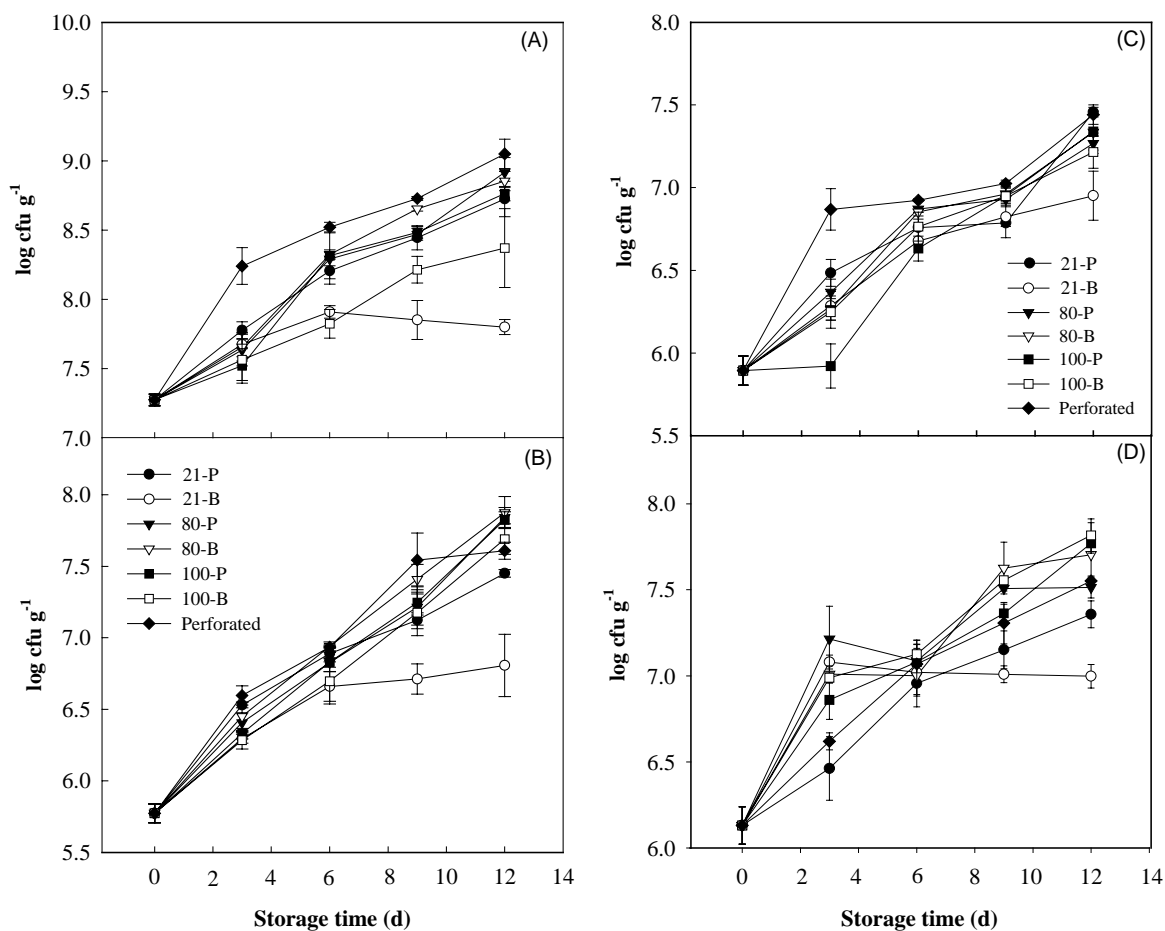

Fig. 4. Changes in the populations of aerobic mesophilic bacteria (A), anaerobic mesophilic bacteria (B), *Enterobacteriaceae* (C), and yeast (D) on fresh-cut baby spinach during storage. Bars represent ± S.E.

ads spp., with the initial loads being 3.5 ± 0.2 and $6.1 \pm 0.1 \log \text{cfu g}^{-1}$, respectively. The initial counts of lactic acid bacteria were below the detection limit of 60cfu g^{-1}. Microbial populations increased during storage of all treatments. Samples in both 21-B and 100-B treatments displayed significant reductions in aerobic mesophilic growth compared to those in perforated film. The inhibition of aerobic bacterial growth in 21-B treatment was probably attributed to the accumulation of high CO_2 levels in this treatment. The antimicrobial activity of CO_2 at high concentration has been well established (Devlieghere et al., 2000; Hendricks and Hotchkiss, 1997; Bennik et al., 1998). Reports on the effect of conventional and super atmospheric O_2 MAP on microbial growth vary considerably in the literature. Jacxsens et al. (2001) found no difference in aerobic psychrotrophic growth in chicory endives between conventional (3 kPa O_2 and 5 kPa CO_2) and superatmospheric O_2 (95 kPa O_2 and 5 kPa N_2) MAP, yet a significant growth reduction in packaged celeriac under the same superatmospheric O_2 MAP condition. Amanatidou et al. (1999) found a higher inhibitory effect on bacterial growth when high O_2 concentrations (80 and 90 kPa) were combined with high CO_2 concentrations (10 and 20 kPa). Our previous work on fresh-cut mixed salad found no difference between conventional (3–5 kPa O_2 and 6–8 kPa CO_2) and super atmospheric O_2 (95 kPa O_2 and 5 kPa CO_2) MAP (Allende et al., 2002). Our current results with higher inhibition with MAP-B were probably due to the higher accumulation of CO_2 inside the packages.

Anaerobic mesophilic growth (Fig. 4B) was less responsive to the passive MAP and super atmospheric O_2 treatments than growth of aerobic bacteria. Only the 21-B treatment affected anaerobic bacterial growth with a $0.8 \pm 0.3 \log \text{cfu g}^{-1}$ decrease compared to the samples of perforated film after 6 days of storage. Super atmospheric O_2 treatments had no effect on anaerobic bacterial growth. Experiments on anaerobic bacteria of mushroom slices yielded the same results (Jacxsens et al., 2001).

For total *Enterobacteriaceae*, passive MAP and especially super atmospheric O_2 MAP (100-P) significantly ($P < 0.01$) inhibited its growth compared to perforated bags (Fig. 4C) on day 3, which agrees with our previous report on mixed salad (Allende et al., 2002). No significant differences were found among

treatments after 6 and 9 days of storage. On day 12, the only significant difference was found between perforated and 21-B treatment. Babic and Watada (1996) also reported that *Enterobacteriaceae* were not greatly affected by two different controlled atmosphere treatments (0.8 kPa O_2, and 0.8 kPa $O_2 + 10$ kPa CO_2) baby spinach. However, Amanatidou et al. (2000) found that *Enterobacteriaceae* were inhibited under 50 kPa O_2 and 30 kPa CO_2 but stimulated under 80 or 90 kPa O_2.

The effect of yeast on spoilage seems to be commodity dependant. It was reported that yeast growth might be a limiting factor of shelf-life for fresh-cut celeriac, mushroom slices and chicory endive (Jacxsens et al., 2001); but not for baby spinach (Babic and Watada, 1996). Yeast growth was not inhibited by 0.8 kPa O_2, or a combination of 0.8 kPa O_2 and 10 kPa CO_2 treatment (Babic and Watada, 1996) or a super atmospheric O_2 (95 kPa) treatment (Allende et al., 2002) of mixed salad. In this experiment, there was no significant difference on yeast growth among the control and all super atmospheric O_2 treatments. However, significant differences were found when samples from perforated film were compared with passive MAP (21-P and 21-B), with a reduction of $0.6 \pm 0.1 \text{cfu g}^{-1}$ between 21-B and the perforated film after 12 days of storage.

The growth of *Pseudomonads*, *Erwinia*, and lactic acid bacteria was evaluated in our preliminary study (data not shown). During the entire storage period only slight differences in the growth of *Pseudomonads* were observed among the treatments, with final counts ranging from $7.3 \log \text{cfu g}^{-1}$ in 100-B treatment to $7.9 \log \text{cfu g}^{-1}$ in the perforated control. Similar results were found when *Erwinia* growth was evaluated. No significant difference was found among treatments except for 21-B, with a final count $0.7 \log \text{cfu g}^{-1}$ lower than the rest of the treatments, probably due to the low O_2 content and very high CO_2 concentration in the 21-B treatment. Lactic acid bacteria population remained low during the entire storage period, and the same tendency for *Erwinia* growth was observed among the treatments.

4. Conclusions

Film OTR and super atmospheric O_2 treatment in the packages containing fresh-cut baby spinach sig-

nificantly affected the changes of O_2 and CO_2 compositions during storage. Significant impacts resulted in tissue electrolyte leakage, sensory quality and microbial growth of fresh-cut baby spinach when different atmosphere treatments were used. The 21-B treatment significantly reduced aerobic mesophilic bacterial growth, but induced a strong off-odor and loss of tissue integrity due to a combination of extremely low O_2 and high CO_2. Although the CO_2 concentrations inside 100-B and 80-B were greater than those in 21-B, the tissue electrolyte leakage of the samples was lower and the sensory quality of the product was better than samples stored under passive MAP. Additionally, samples in 100-B MAP displayed a significant reduction in aerobic mesophilics growth compares to those stored in perforated film. The super atmospheric O_2 treatments were also advantageous to the perforated packages in reducing aerobic mesophilic growth and eliminating the possibility of post-processing contamination.

Acknowledgements

References

Ahvenainen, R., 1996. New approaches in improving the shelf life of minimally processed fruit and vegetables. Trends Food Sci. Technol. 7, 179–187.

Allende, A., Jacxsens, L., Devlieghere, F., Debevere, J., Artés, F., 2002. Effect of super atmospheric oxygen packaging on sensorial quality, spoilage, and *Listeria monocytogenes* and *Aeromonas caviae* growth in fresh processed mixed salads. J. Food Protect. 65, 1565–1573.

Allende, A., Jacxsens, L., Devlieghere, F., Debevere, J., Artés, F., 2001. Microbial and sensorial quality of fresh processed lettuce salad under high O_2 atmosphere throughout the distribution chain. In: Proceedings of the Eighth International Controlled Atmosphere Research Conference. Acta Horticulturae 600, vol. II. Rotterdam, The Netherlands, pp. 629–635.

Amanatidou, A., Smid, E.J., Gorris, L.G.M., 1999. Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated microorganisms. J. Appl. Microbiol. 86, 429–438.

Amanatidou, A., Slump, R.A., Gorris, L.G.M., Smid, E.J., 2000. High oxygen and high carbon dioxide modified atmospheres for shelf-life extension of minimally processed carrots. J. Food Sci. 65, 61–66.

Atlas, R.M., 1997. Erwinia Selective Media. In: Atlas, R.M. (Ed.), Handbook of Microbial Media, second ed. University of Louisville, Louisville, Kentucky, USA, pp. 519.

Babic, I., Watada, A.E., 1996. Microbial populations of fresh-cut spinach leaves affected by controlled atmospheres. Postharv. Biol. Technol. 9, 187–193.

Babic, I., Roy, S., Watada, A.E., Wergin, W.P., 1996. Changes in microbial populations on fresh cut spinach. Int. J. Food Microbiol. 31, 107–119.

Barth, M.M., Zhuang, H., Saltveit, M.E., 2002. Fresh-cut vegetables. In: Kenneth C. Gross, Chien Y. Wang, Mikal E. Sattveit (Eds.), The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Crops. An Adobe Acrobat pdf of a Draft Version of the Forthcoming Revision to U.S. Department of Agriculture, Agriculture Handbook 66 on the Website of the USDA, Agricultural Research Service, Beltsville Area <http://www.ba.ars.usda.gov/hb66/index.html>.

Bennik, M.H.J., Vorstman, W., Smid, E.J., Gorris, L.G.M., 1998. The influence of oxygen and carbon dioxide on the growth of prevalent *Enterobacteriaceae* and *Pseudomonas* species isolated from fresh and controlled-atmosphere-stored vegetables. Food Microbiol. 15, 459–469.

Bolin, H.R., Husxoll, C.C., 1991. Control of minimally processed carrot (*Daucus carota*) surface discoloration caused by abrasion peeling. J. Food Sci. 56, 416–418.

Day, B., 1996. High oxygen modified atmosphere packaging for fresh prepared produce. Postharv. News Inform. 7, 31–34.

Day, B., 2000. Novel MAP for freshly prepared fruit and vegetable products. Postharv. News Inform. 11, 27–31.

Day, B., 2001. Fresh Prepared Produce: GMP for High Oxygen MAP and Non-Sulphite Dipping. Guideline No. 31, Campden and Chorleywood Food Research Association Group, Chipping Campden, Gloshister, UK, pp. 76.

Devlieghere, F., Debevere, J., Van Impe, J., 2000. Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. Int. J. Food Microbiol. 43, 105–113.

Gorny, J.R., 1997. A summary of CA and MA requirements and recommendations for fresh-cut (minimally processed) fruits and vegetables. In: Proceedings of the Seventh International Conference on Cont. Atmos., vol. 5. Davis CA, pp. 30–66.

Heimdal, H., Kuhn, B.F., Poll, L., Larsen, L.M., 1995. Biochemical changes and sensory quality of shredded and MA-packaged iceberg lettuce. J. Food Sci. 60, 1265–1268.

Hendricks, M.T., Hotchkiss, J.H., 1997. Effect of carbon dioxide on the growth of *Pseudomonas fluorescens* and *Listeria monocytogenes* in aerobic atmospheres. J. Food Protect. 60, 1548–1552.

Hong, J.H., Mills, D.J., Coffman, C.B., Anderson, J.D., Camp, M.J., Gross, K.C., 2000. Tomato cultivation systems affect subsequent quality of fresh-cut fruit slices. J. Am. Soc. Hort. Sci. 125, 729–735.

Huxsoll, C.C., Bolin, H.R., 1989. Processing and distribution alternatives for minimally processed fruits and vegetables. Food Technol. 43, 124–128.

Jacxsens, L., 2002. Influence of Preservation Parameters on the Quality of Fresh-Cut Vegetables. Doctoral Thesis. University of Ghent, Ghent, Belgium.

Jacxsens, L., Devlieghere, F., Van der Steen, C., Debevere, J., 2001. Effect of high oxygen modified atmosphere packaging on packaging on microbial growth and sensorial qualities of fresh-cut produce. Int. J. Food Microbiol. 71, 197–210.

Kader, A.A., Ben-Yehoshua, S., 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. Postharv. Biol. Technol. 20, 1–13.

Kim, J.G., Luo, Y., Gross, K.C., 2004. Effect of packaging film on the quality of fresh-cut salad savay. Postharv. Biol. Technol. 32, 99–107.

Ko, N.P., Watada, A.E., Schlimme, D.V., Bouwkamp, J.C., 1996. Storage of spinach under low oxygen atmosphere above the extinction point. J. Food Sci. 61, 398–400, 406.

López-Gálvez, G., Peiser, G., Nie, X., Cantwell, M., 1997. Quality changes in packaged salad products during storage. Z. Lebensm Unters Forsch. 205, 64–72.

Marangoni, A.G., Palma, T., Stanley, D.W., 1996. Membrane effects in postharvest physiology. Postharv. Biol. Technol. 7, 193–217.

Meilgaard, M., Civille, G.V., Carr, B.T., 1991. Sensory evaluation techniques, second ed. CRC Press Inc., Boca Raton, pp. 213–215.

Murata, T., 1989. Relation of chilling stress to membrane permeability. In: Wang, C.Y. (Ed.), Chilling Injury of Horticultural Crops. CRC Press, Boca Raton, FL, pp. 201–209.

Nguyen-the, C., Carlin, F., 1994. The microbiology of minimally processed fresh fruits and vegetables. CRC Crit. Rev. Food Sci. Nutr. 34, 371–401.

Pirovani, M.E., Piagentini, A.M., Güemes, D.R., Di Pentima, J.H., 1998. Quality of minimally processed lettuce as influenced by packaging and chemical treatments. J. Food Quality 22, 475–484.

Willox, F., 1995. Evaluation of Microbial And Visual Quality of Minimally Processed Foods: a Case Study on the Product Life Cycle of Cut Endive. Doctoral Thesis. Catholic University of Leuven, Leuven, Belgium.

Wooster, J.J., 1998. New resins for fresh-cut produce packaging. J. Plast. Film Sheet. 14, 76–89.

Zagory, D., Kader, A., 1988. Modified atmosphere packaging of fresh produce. Food Technol. 42, 70–77.