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

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Abstract

The effect of super atmospheric O2 and modified atmosphere packaging (MAP) on plant metabolism, organoleptic quality and microbial growth of minimally processed baby spinach was studied. Packaging film O2 transmission rates and initial levels of super atmospheric O2 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 O2 level for both 80 and 100 kPa O2 treatments during entire storage. Packages with the barrier film also exhibited a more rapid accumulation of CO2 than those with the permeable film, with CO2 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 O2 level at 21% accumulated CO2 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 O2 to the packages alleviated tissue injury in addition to reducing microbial growth and was beneficial in maintaining quality of fresh-cut baby spinach.

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-

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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 O₂ consumption and CO₂ evolution (Pirovani et al., 1998). In general, gas compositions inside a MA package are low in O₂ and high in CO₂, depending primarily on temperature, product fill weight and respiration rate, package film O₂ and CO₂ 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 O₂ in the packages to maintain their quality (Gorny, 1997; Wooster, 1998). Since packaging films currently available for fresh-cut produce do not have sufficient O₂ transmission rates to allow adequate O₂ in the packages to maintain product quality (Gorny, 1997; Wooster, 1998). Since packaging films currently available for fresh-cut produce do not have sufficient O₂ transmission rates to allow adequate O₂ in the packages to maintain product quality. Since packaging films currently available for fresh-cut produce do not have sufficient O₂ transmission rates to allow adequate O₂ in the packages to maintain product quality. Since packaging films currently available for fresh-cut produce do not have sufficient O₂ transmission rates to allow adequate O₂ in the packages to maintain product quality.

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/IMᵀ ᵋ/LL (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 x 65 cm) with two different O₂ permeabilities: an O₂ permeable film (P) with an O₂ transmission rate (OTR) of 15 pmol s⁻¹ m⁻² Pa⁻¹ and an O₂ barrier film (B) with an O₂ transmission rate (OTR) of 0 pmol s⁻¹ m⁻² Pa⁻¹. The O₂ 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 O₂ conditions of the packages were provided by flushing the bags with the desired gas with compositions of 80 and 100 kPa O₂ (Praxair Inc., Danbury, CT, USA). The O₂ levels inside the bags were constantly monitored with an O₂/CO₂ 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 O₂ 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 CO₂-scrubbed and humidified air was pumped into the jars to avoid dehydration and excessive CO₂ accumulation. The respiration rate as a CO₂ 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 O₂ and CO₂ were analyzed with O₂/CO₂ 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 mL 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™ 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; (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 CO₂ and 5 kPa O₂ 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 cfu 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 ± 7.1 nmol kg\(^{-1}\) 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 kPa O\(_2\) on day 6. The O\(_2\) level in permeable packages (21-P) decreased at a slightly slower rate, with 2.5 kPa O\(_2\) on day 6 and 0.10 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 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 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 kPa O\(_2\) (100-P and 100-B) maintained higher O\(_2\) levels inside the bags than those with 80 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 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 ± S.E.](image1)

![Fig. 3. Overall quality of fresh-cut baby spinach evaluated at the end of storage. Bars represent ± S.E.](image2)
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; Jacxens 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 cfu g$^{-1}$ for aerobic mesophiles (Fig. 4A). This result is consistent with the findings previously reported by Babic et al. (1996) for fresh-cut spinach. Initial population of anaerobic mesophiles was $5.8 \pm 0.1$ log cfu g$^{-1}$ (Fig. 4B), $5.9 \pm 0.1$ log cfu g$^{-1}$ for total Enterobacteriaceae (Fig. 4C) and $6.1 \pm 0.1$ log 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 cfu g$^{-1}$ for total Enterobacteriaceae and yeast, respectively. In the preliminary test, we also examined numbers of Erwinia spp. and Pseudomon-
Amanatidou et al. (1999) found that packaged celeriac under the same superatmospheric O₂ treatments had no effect on aerobic bacterial growth. Anaerobic mesophilic growth among the control and all super atmospheric O₂ treatments was evaluated in our preliminary study (data not shown). During the entire storage period only slight differences in the growth of *Enterobacteriaceae* were observed among the treatments, with final counts ranging from 7.3 log cfu g⁻¹ in 100-B treatment to 7.9 log cfu g⁻¹ in 100-B treatment. Similar results were found when *Erwinia* growth was evaluated. No significant difference was found among treatments except for 21-B, with a final count of 0.7 log cfu g⁻¹ lower than the rest of the treatments, probably due to the low O₂ content and very high CO₂ 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 O₂ treatment in the packages containing fresh-cut baby spinach sig-

ads spp., with the initial loads being 3.5 ± 0.2 and 6.1 ± 0.1 log cfu g⁻¹, respectively. The initial counts of lactic acid bacteria were below the detection limit of 60 cfu 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 CO₂ levels in this treatment. The antimicrobial activity of CO₂ at high concentrations 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 O₂ 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 O₂ and 5 kPa CO₂) and superatmospheric O₂ (95 kPa O₂ and 5 kPa N₂) MAP, yet a significant growth reduction in packaged celeriac under the same superatmospheric O₂ MAP condition. Amanatidou et al. (1999) found a higher inhibitory effect on bacterial growth when high O₂ concentrations (80 and 90 kPa) were combined with high CO₂ concentrations (10 and 20 kPa). Our previous work on fresh-cut mixed salad found no difference between conventional (3-5 kPa O₂ and 6-8 kPa CO₂) and super atmospheric O₂ (95 kPa O₂ and 5 kPa CO₂) MAP (Allende et al., 2002). Our current results with higher inhibition with MAP-B were probably due to the higher accumulation of CO₂ inside the packages.

Anaerobic mesophilic growth (Fig. 4B) was less responsive to the passive MAP and super atmospheric O₂ treatments than growth of aerobic bacteria. Only the 21-B treatment affected anaerobic bacterial growth with a 0.8 ± 0.3 log cfu g⁻¹ decrease compared to the samples of perforated film after 6 days of storage. Super atmospheric O₂ 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 O₂ 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 O₂ and 0.8 kPa O₂ + 10 kPa CO₂) baby spinach. However, Amanatidou et al. (2000) found that *Enterobacteriaceae* were inhibited under 50 kPa O₂ and 30 kPa CO₂ but stimulated under 80 or 90 kPa O₂.

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 O₂, or a combination of 0.8 kPa O₂ and 10 kPa CO₂ treatment (Babic and Watada, 1996) or a super atmospheric O₂ (95 kPa O₂) 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 O₂ 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 ± 0.1 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 from 7.3 log cfu g⁻¹ in 100-B treatment to 7.9 log cfu g⁻¹ in 100-B treatment. Similar results were found when *Erwinia* growth was evaluated. No significant difference was found among treatments except for 21-B, with a final count of 0.7 log cfu g⁻¹ lower than the rest of the treatments, probably due to the low O₂ content and very high CO₂ 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.
significantly affected the changes of O₂ and CO₂ 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 O₂ and high CO₂. Although the CO₂ 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 compared to those stored in perforated film. The super atmospheric O₂ treatments were also advantageous to the perforated packages in reducing aerobic mesophilic growth and eliminating the possibility of post-processing contamination.

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