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# The effects of active and passive modified atmosphere packaging on the survival of *Salmonella enterica* serotype Typhimurium on washed romaine lettuce leaves

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ABSTRACT

Modified atmosphere packaging (MAP) is commonly used to preserve the quality of ready-to-eat lettuce by inhibiting oxidative browning and growth of microbial populations. The efficacy of MAP is improved by the initial displacement of air with a gas mixture of a desirable composition (active MAP). The present work focused on the effects of MAP on indigenous microbial populations and the survival of *Salmonella enterica* on the surface of the lettuce. Chlorine-washed leaves of romaine lettuce were inoculated with *S. enterica* serotype Typhimurium or not inoculated and packaged in one of the three systems: (a) passive MAP in polyethylene bags; (b) active MAP in the same bags with a gas mixture of 10% $O_2$, 10% $CO_2$, and 80% $N_2$; and (c) control without MAP. Active MAP ensured the more rapid establishment of a steady-state atmosphere and favorable conditions during the transitional period preceding the steady state. The active MAP had an antimicrobial effect on indigenous lettuce microflora, but not on *Salmonella* and even favored the survival of the pathogen, possibly due to the elimination of its natural antagonists. The effects of the passive MAP were less pronounced. The results obtained draw attention to potential safety risks of ready-to-eat fresh produce kept in active MAP and require further investigation.



## 1. Introduction

Ready-to-eat (RTE) packaged lettuce salads were implicated in several salmonellosis outbreaks (Little & Gillespie, 2008). Large-scale industrial processing and distribution of RTE products exacerbate the risk of cross-contamination and can lead to geographically widespread outbreaks. Therefore, the study of the food-safety implications of technologies used in the production and handling of RTE lettuce is of great importance.

Modified atmosphere packaging (MAP) is commonly used to preserve the quality of minimally processed lettuce by inhibiting the oxidative browning and the growth of microbial populations. The antimicrobial effect of MAP is mainly related to the enhanced concentration of carbon dioxide (Bennik, Smid, Rombouts, & Gorris, 1995), whereas the inhibition of browning is due to reduced oxygen level (Smyth, Song, & Cameron, 1998). In passive MAP, generation of modified atmosphere by produce respiration typically takes one to several days. During this transitional period, the produce continues to deteriorate. Initial displacement of air with a gas mixture of desired composition (active MAP) allows the avoidance of the transitional period, thereby improving the quality of the product (Ares, Lareo, & Lema, 2008).

However, a concern exists that under MAP conditions foodborne pathogens like *Salmonella* that are relatively tolerant to high $CO_2$ (Hintlian & Hotchkiss, 1987) and low oxygen (Ryan, 1972) may outgrow spoilage microorganisms, resulting in more visually appealing, but unsafe products (Farber et al., 2003). Indeed, certain enhancement of *Salmonella* growth parallel to inhibition of mesophilic and psychrotrophic microorganisms was observed on shredded romaine lettuce kept in passive MAP (Oliveira et al., 2010). To the best of our knowledge, no data are available concerning the effect of active MAP on the survival of *Salmonella* on RTE lettuce.

The purpose of this work was to study the effects of active and passive MAP on indigenous microflora on washed RTE lettuce leaves and on the survival of *Salmonella enterica* serotype Typhimurium on the surface of these leaves.

## 2. Material and methods

### 2.1. Product preparation and storage

The heads of romaine lettuce were taken from the farm on the day of harvest. The aseptically separated medium-size leaves were soaked for 2 min in sodium hypochlorite solution (Sterosept; Johnson Diversey, Yavne, Israel) containing 200 ppm free chlorine (test kit HI3831F; Hanna Instruments, Padova, Italy), rinsed with tap water and dried in a basket centrifuge K50-7ECO (Kronen GmbH, Kehl am Rhein, Germany). The leaves intended for *Salmonella* inoculation were treated as described in Section 2.2.

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The leaves were placed in bags made out of 35 μm-thick low-density polyethylene (Pol Bag Industries, Yehud, Israel) with an oxygen transmission rate of 5700 $cm^3\ m^{-2}\ day^{-1}\ bar^{-1}$ at 23 °C and a water vapor transmission rate of 2.9 $g\ m^{-2}\ day^{-1}$ at 23 °C and 85% RH (manufacturer's information). Each bag contained about 100 g of lettuce (typically 8 leaves). Thirty-six bags were prepared for a storage trial. Half of the bags included one marked leaf that was inoculated with *Salmonella* ("inoculated packages"). The other half of the bags contained no inoculated leaves ("uninoculated packages"). Both inoculated and uninoculated packages were divided into three packaging subgroups. For the active MAP treatment, the air in the bags was replaced with a mixture of gases (10% $O_2$, 10% $CO_2$, and 80% $N_2$) using the Multivac A300 chamber machine (Multivac, Wolfertschwenden, Germany). For the passive MAP treatment, the bags were sealed without the atmosphere replacement. Picture of the MAP package is presented in Fig. 1. For the perforated control, the bags were sealed as in the passive MAP treatment, but then the two corners on the bottom of the bag were cut off, forming two openings (5–7 mm in diameter) sufficient to cancel out the MAP effect. The packages were stored at either 8 °C (simulated refrigerated display conditions) or 20 °C (simulated non-refrigerated display conditions).

### 2.2. Inoculation

The laboratory strain *Salmonella enterica* Typhimurium SL1344 (Kroupitski, Pinto, Brandl, Belausov, & Sela, 2009) was used in this work. For lettuce inoculation, the bacteria were grown in LB broth for 18–20 h at 37 °C with shaking (150 rev $min^{-1}$) to obtain stationary phase cultures. For bacterial enumeration, serial dilutions of the culture were plated in duplicate on xylose lysine deoxycholate (XLD) selective agar (Acumedia, Baltimore, MD, USA) and the plates were then incubated at 37 °C. Black colonies were considered to be *Salmonella*, and the number of colony-forming units (CFU) was determined by plate counts following incubation at 37 °C for 24 h. The bacteria were centrifuged at 1900 *g* for 15 min and the pellet was washed twice with sterile deionized water (SDW). Inocula were prepared by resuspending the pellet in 10 ml SDW. We inoculated the chlorine-washed leaves by placing 200-μL droplets of the *Salmonella* suspension (7 $\log_{10}$ CFU $ml^{-1}$) onto the center of the adaxial surface of each leaf and then let the leaves dry for ca. 30 min under a sterile air flow in a biosafety cabinet. The *Salmonella*-contaminated leaves were marked and added without an additional decontamination step to "inoculated packages" as described in Section 2.1, one contaminated leaf per package.

**Fig. 1.** Appearance of a MAP package of romaine lettuce leaves. The white sticker near the lower right corner is an atmosphere-sampling septum.

### 2.3. Sampling and analyses

Samples for microbiological analysis were taken at Day 0 and after 7 days of storage at 8 °C or 3 days at 20 °C. Individual leaves from "uninoculated" and "inoculated" packages were weighed and transferred into sterile Stomacher bags that each contained 100 ml of sterile 0.1% peptone water and homogenized for 2 min at high speed in a Stomacher 400 circulator (Seward, Worthing, UK). Only marked inoculated leaves were taken for analysis from the "inoculated packages". Test samples were serially diluted in 0.1% peptone water and aerobic plate counts were determined by surface inoculation of plate count agar (PCA) (Oxoid, Basingstoke, UK). The plates were incubated at 30 °C for 48 h and the number of colony-forming units (CFU) per gram of plant material was calculated. The enumeration of *Salmonella* was performed by dilution plating on XLD selective agar as described in Section 2.2. The data were transformed into logarithmic form as decimal logarithms of the CFU per gram for the statistical analysis.

Prior to microbiological analysis, atmospheric samples were collected from the uninoculated packages. This was done with an airtight syringe that entered and exited the package through a silicon rubber septa taped on the film surface. The partial pressures of oxygen and carbon dioxide in the samples were determined using the Servomex 1450B gas analyzer (Servomex, Crowborough, UK). An additional independent 1-week storage trial was conducted without inoculation, in order to characterize the development of the atmosphere in the packages at the two storage temperatures.

The trials were performed at least in triplicate and repeated twice demonstrating similar trends. The results of one characteristic trial are presented in this paper. Microsoft Office Excel spreadsheets were used to calculate means, standard deviations, and 95% *t* confidence intervals. Means were separated using Duncan's Multiple Range Test, following one-way analysis of variance (ANOVA) using NisuyWin, version 4.0 (Prof. A. Marani, Hebrew University of Jerusalem).

## 3. Results and discussion

### 3.1. Atmosphere composition

At 8 °C, passive and active MAP systems demonstrated opposite trends of atmosphere changes during the transitional period, but by Day 7 reached similar steady-state levels of oxygen and carbon dioxide (13 and 2–3 kPa, respectively). During storage at 20 °C, the partial pressure of oxygen decreased in both the active and passive MA packages, eventually reaching the level of 2–3 kPa (Fig. 2). In the active MAP treatment, this value was approached by Day 3. In contrast, it took as long as 7 days for this value to be reached in the passive MAP treatment. The in-package $CO_2$ concentration in the active MAP treatment was stable at 20 °C at the optimal level of 9–10 kPa, indicating equilibrium between the respiration of the lettuce and the diffusion of $CO_2$ through the film. In the passive MAP treatment, the steady-state level of 8–9 kPa was reached only by the end of storage (Day 7). No atmosphere modification was observed in the control non-hermetic packages at 8 °C or 20 °C.

The results obtained confirmed the thesis that while steady-state $O_2$ and $CO_2$ levels are determined by film characteristics and the produce respiration rate, the amount of time needed to achieve these levels depends on the initial atmospheric composition (Rodov, Horev, Goldman, Vinokur, & Fishman, 2007). The produce in active MAP at both storage temperatures was continuously exposed to lower concentrations of oxygen and higher concentrations of carbon dioxide than the produce in the corresponding passive MAP treatments. In this way, the conditions in the active MAP system were closer to the recommended MA composition (Gorny, 1997).