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RESEARCH ARTICLE

Impact of modified atmosphere packaging and ozonated water on the shelf life, quality, and safety of vegetables stored at non optimum temperatures

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ABSTRACT

The objective of this paper was to investigate the effects of passive MAP at non-optimum storage temperature, with and without ozonated washing, on the quality, safety, and shelf life of asparagus (*Asparagus officinalis*), broccoli (*Brassica oleracea*) and spinach (*Spinacia oleracea*). Six treatments were examined: unwashed produce stored in produce bags and in MAP bags (washed in ice-cold water stored in produce bags and in MAP bags; and washed in ozonated water stored in produce and in MAP bags. Samples were stored at 13ºC at 95% RH. Product quality was evaluated in terms of overall visual quality, color, and texture. Microbiological analyses included psychrotrophs, total aerobic microorganisms, generic *Escherichia coli*, coliforms, and yeast and mold populations. The MAP bags equilibrated to an atmosphere of 3.5% CO_2 and 8% O_2 for asparagus, 12% CO_2 and 3% O_2 for broccoli, and 7% CO_2 and 4-6% O_2 for spinach. Asparagus, broccoli, and spinach stored in MAP bags had a storage extension of 4, 7 and 7 days, respectively, compared to those in produce bags. The use of MAP prevented yellowing in broccoli and spinach, and preserved visual texture and wilting in spinach. Ozonated water did not significantly affect the quality attributes in any crop. No treatments had a significant effect on native microbial populations. These data indicate that the use of passive MAP bags for storing broccoli and spinach under non-optimum temperatures could be an alternative for extending shelf life for growers without appropriate cold storage access.

Keywords: Yellowing, decay, aerobic plate counts, off-odor, broccoli, spinach, asparagus

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INTRODUCTION

Rapid cooling and proper temperature management are crucial for maintaining the quality of fresh produce during storage (Kader, 2013). Refrigerated storage is the most effective postharvest handling for preserving the quality and increasing the shelf life of fresh produce because temperature controls the rate of the metabolic processes and physiological responses occurring in plant tissues (Prusky, 2011; Kader, 2013). However, achieving and maintaining optimum temperature conditions during the postharvest chain is not always possible. One of the most common challenges for small acreage producers is access to cooling (Watkins and Nock, 2012). For instance, a regional small acreage grower's survey in the Central U.S. indicated that access to refrigerated storage facilities is one of the main barriers for increasing local food

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production in the area (C.L. Rivard, unpublished data). Particularly, only 32% of the growers surveyed had access to rapid cooling facilities, and 6% had access to refrigerated trucks. Moreover, small acreage operations are typically diversified and a typical storage temperature, when cooling is limited, is 13°C in order to commingle chilling sensitive and non-sensitive commodities (Watkins and Nock, 2012).

The use of modified atmosphere packaging (MAP) technology, for maintaining the postharvest quality of fresh produce, is well established in the food industry (Zhang et al., 2015). MAP is creating an atmosphere with elevated CO_2 and reduced O_2 levels, which can extend the shelf life of the packaged crop by reducing the respiration rate, ethylene production and sensitivity, decay, and water loss, among other physiological changes (Wilson et al., 2019). In some cases, MAP has been effective in inhibiting bacterial and mold growth (Caleb et al., 2013). MAP can be achieved actively, by flushing a gas mixture inside a package or passively, through a synergy of the film permeability with the product respiration rate which modifies the gas composition inside the package (Ghidelli and Pérez-Gago, 2018). Active MAP is more effective than passive MAP because the effect of the atmosphere on the product is immediate (Gil, 2016). However, the implementation of active MAP is not always feasible due to high cost of equipment and gasses (Rodriguez-Aguilera and Oliveira, 2009). The effectiveness of passive map depends on the packaging film properties, the commodity itself, and the storage temperature. Specifically, the oxygen transmission rate (OTR) of the packing film should match the respiratory behavior of the commodity, as affected by the storage temperature, to establish a beneficial atmosphere (Lange, 2000). Inadequate internal gas composition can result to anaerobic respiration and off-odour production accompanied by loss of visual appearance (Belay et al., 2019)Passive MAP stored in non-optimum temperatures has demonstrated a beneficial effect for various fruits and vegetables (D'Aquino et al., 2016; Murmu and Mishra, 2017; Batziakas et al., 2020).

Besides temperature control and packaging, washing and sanitizing can positively affect the quality and shelf life fresh produce, by removing dirt and possible pesticide residues, lowering the commodity temperature, and lowering the commodity latent pathogen infection load (Watkins and Nock, 2012). Washing fruits and vegetables in sanitizing agents such as chlorine, is important for reducing the presence of microbial contaminants that can lead to foodborne illness (Ramos et al., 2013). One potential alternative to sanitation with chlorine is the use of ozone as an aqueous disinfectant, which has been classified as generally recognized as safe (GRAS) for food contact surface applications (Piemontese et al., 2018). Due to its strong oxidizing activity, ozonated water has shown promising results in the reduction of microorganisms on fresh whole fruits and vegetables (Klockow and Keener, 2009; Sothornvit and Kiatchanapaibul, 2009) and on fresh-cut produce (Beltrán et al., 2005). One of the greatest benefits of using ozonated water is the absence of residues after treatment, due to ozone's decomposition into oxygen (Pandiselvam et al., 2019).. In addition, ozone does not have a detrimental effect on compounds important to human health, including total phenolic content, antioxidants and vitamins when applied in aqueous solution (Karaca and Velioglu, 2014). Overall, ozone treatments have been found to increase the shelf life of various fruits and vegetables (Pandiselvam et al., 2019).

Knowledge of the effect of the combined use of MAP and ozonated water on produce quality is still limited. A beneficial effect of ozonated water combined with MAP, was observed in controlling browning and maintaining visual quality of shredded lettuce (*Lactuca sativa* L.) (Beltrán et al., 2005); reduced lignification of fresh-cut carrots (*Daucus carota* L., cv. Pusa kesar) (Chauhan et al., 2011)and in shelf life extension of asparagus (Sothornvit and Kiatchanapaibul, 2009) and green chilies (*Capsicum annuum* L.) (Chitravathi et al., 2015), providing a promising sanitizing method before storing in MAP. However, the effect of washing fresh vegetables in ozonated water in combination with non-optimum MAP storage on their quality and shelf life is still unknown.

The objectives of this study were to determine the effects of MAP and ozonated wash water, alone and in combination, on the produce quality, shelf life, and native microflora of asparagus, broccoli, and spinach stored at non-optimum storage temperature.

MATERIALS AND METHODS

Sample preparation and storage conditions: Spinach, asparagus, and broccoli were harvested from local farms in the region of Kansas City, KS, USA. Produce was acquired on 3 different harvest dates for each crop, with each harvest date creating one replication of the study. Immediately after harvest, produce was brought to the postharvest physiology laboratory at Kansas State University in Olathe, KS and separated into 2 groups – unwashed and to be washed. The unwashed group was split into two experimental groups: one stored in produce bags (CC) and the other stored in passive MAP bags (CM). All the produce to be washed was washed in ice-cold water (approximately 0 ˚C and separated into 2 sub-groups. One of the sub-groups was split into 2 experimental groups, one stored in produce bags (WC) and the other in MAP bags (WM). The remaining produce had an additional wash with ozonated water (1.0-1.5 ppm of ozone; TetraClean^{TM} Systems, LLC, Omaha, NE, USA) at room temperature (around 20 ˚C) for 1 min, and separated into 2 experimental groups, one stored in produce bags (OC) and the other in MAP bags (OM). The passive modified atmosphere bags used for spinach and broccoli where purchased from Chandra Associates (Milford, MA, USA). For asparagus, we used PEAKfresh home use re-usable produce bags (PEAKfresh USA, Lake Forest, CA, USA). Both passive MAP bags are commercially available for consumers and were developed for optimum storage temperatures. All treatment groups were stored at 13 ˚C and 95% RH. This temperature was selected to reflect the storage temperatures used by local growers in the Central U.S. On the day of harvest and during storage, the samples were evaluated for microbiological populations, physical and visual quality.

Microbiological analysis. The microbial populations evaluated include the enumeration of psychrotrophs, total aerobic microorganisms, generic *Escherichia coli*, coliforms, and yeast and mold populations. At each sampling time point, 25 g of product was homogenized with 225 mL of buffered peptone water (BPW; BBL, Franklin Lakes, NJ, USA) at 230 rpm for 2 minutes. Serial dilutions were performed in BPW and the appropriate dilutions were plated in duplicate onto tryptic soy agar (TSA; Remel, Lenexa, KS, USA), potato dextrose agar (PDA; Remel, Lenexa, KS, USA) containing 2.5% lactic acid (Fisher Scientific, Pittsburgh, PA), and *E. coli*/coliform petrifilm (ECC; 3M; St. Paul, MN, USA). Total aerobic plate counts (APC) were enumerated by incubating TSA at 37 ˚C for 18-24 hours while TSA was incubated at 7 ˚C for 7 days to enumerate psychrotroph populations. Two sets of TSA plates were plated in order to enumerate for both APC and pyschrotrophs. Yeast and mold populations were determined by storing PDA plates at 30 ˚C for 3-5 days. The ECC petrifilm were stored at 37 ˚C for 24 $\pm$ 2 hours to quantify coliform populations and then incubated for an additional 24 $\pm$ 2 hours (48 hours total) to quantify populations of generic *E. coli*. Coliform data below the limit of detection (~0.5 Log_{10} CFU/g) were assigned a value of 0.25 Log_{10} CFU/g.

Gas composition. The headspace composition in the MAP and produce bags was determined by measuring CO_2 and O_2 concentrations in the MAP bags daily, with the use of a portable gas analyzer (Bridge analyzer; Bedford Heights, OH, USA).

Physical quality. The surface color of the vegetables was measured using a colorimeter (CR-400 Chroma meter, Konica Minolta, Ramsey, NJ, USA). For asparagus, 10 random spears per group were measured in 2 positions, at 5 cm and at 10 cm from the trimmed end. For broccoli, 5 measurements were taken on each head. For spinach, 10 random leaves from each group, were measured in the middle section of the leaf. Color results are expressed in the CIE L*a*b* color space where h° is hue angle, calculated as $\tan^{-1}$ b*/a* (McGuire, 1992).

Asparagus texture was measured based on Dali et al. (1993) with adaptations using a compression method with a 3 mm cylindrical probe in two areas of 10 spears, at 5 cm and at 10 cm from the trimmed end of the spear. Pre-test speed was 3 mm/s, and test speed 1 mm/s through a distance of 5 mm and a trigger force of 5 g. Results were expressed as force (N)/diameter (cm). The broccoli texture was not measured instrumentally. Spinach Texture was measured for spinach according to Medina et al. (2012) with modifications using a Kramer shear cell with a five-blade probe on a texture analyzer (TA.TX.plus texture analyzer, Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 50 Kg load cell. Twenty grams of leaves without stems were placed in the cell perpendicularly oriented to the blades. The compression test was performed to an initial height of 35mm, with a probe speed of 1.67 mm/s. Leaf texture was expressed as maximum force (N)/weight (g).

Visual quality. For establishing the shelf life of the stored vegetables, a set of quality scales was used. The end of shelf life was determined to be when 30% of each group scored below 5, which was considered the limit of marketability, at the overall visual quality (OVQ) scale. All the visual quality ratings were discussed to reach a consensus between 2 experienced scientists. Random duplicated visual quality evaluations between analysts were performed throughout the experiments in order to assure the veracity of the scores.

For asparagus, the quality scales utilized were based on Li and Zhang (2015) for OVQ with 9 being excellent and 1 very poor and inedible. In addition, ten spears from each group were evaluated for firmness, by pressing the asparagus between the thumb and index finger (9 = tender to 1 = very hard), off-odor (1 = none perceptible odor to 5 = severe off-odor) and decay (1 = none to 5 = severe). For broccoli, the quality rating scales described by Kader and Cantwell (2006) were used. The OVQ scale was from 9 = excellent, bright green and free of defects, to 1 = would not be eaten, complete yellowing, some decay, and objectionable odor. Additionally, off-odors (1 = none to 5 = severe); and decay (1 = none to 5 = severe) were evaluated. For spinach, an OVQ scale described by Kader and Cantwell (2006) was utilized where each group was given a score, with 9 for the best quality and 1 representing the lowest quality. The quality parameters evaluated were for spinach, 50 individual leaves per experimental group were decay (1 = none to 5= extreme soft rot); wilting (1 = none to 9 = extreme) and firmness (9 = very crisp to 1 = soggy); off-odor (1 = none to 5 = very strong off-odor).

Quality Statistical Analysis: all experiments were conducted in triplicate and the mean values with standard deviations were used in the analysis. Data were evaluated using the PROC MIXED command in Statistical Analysis Software (SAS, Version 9.4, Cary, NC, USA), and significant differences were established as $P \leq 0.05$. To evaluate significant differences ($P \leq 0.05$) between treatments the FIT Model on JMP Software (version 13; SAS Institute, Cary, NC, USA) was used.

Microbiological Statistical Analysis: All experimental procedures were replicated three times. Data were subjected to the MIXED procedure with LSMEANS of SAS and statistical significance was evaluated at the P=0.05 threshold.

RESULTS

Postharvest Quality

Asparagus

The internal concentration of % CO_2 of asparagus stored in passive MAP bags took two days to equilibrate ($P < 0.0001$) (Table 1) at 13°C. The unwashed asparagus control (CM) equilibrated at 3.6%, while the asparagus washed with cold water at 3.6% and ozonated water (OM) at 3.5%, none of which were significantly different. The internal % O2 concentration equilibrated after one day ($P < 0.0001$) at 8.2% (CM); 8% (WM) and 8.9% (OM). (Table 1)

Table 1. Internal gas composition and shelf life of crops stored at 13 °C under different washing treatments and stored with and without commercial passive MAP bags[y]

	Days to reach internal gas equilibrium (% CO_2 / % O_2)	Internal % CO_2	Internal % O_2	Days of shelf life
Asparagus				
CC[z]	-	Atmospheric	Atmospheric	9.7 (1.53)[ab]
WC	-	Atmospheric	Atmospheric	9.7 (1.53)[ab]
OC	-	Atmospheric	Atmospheric	10.3 (0.58)[a]
CM	2/1	3.6 (0.38)	8.19 (0.98)	12.3 (1.53)[ab]
WM	2/1	3.6 (0.39)	8 (0.88)	13.0 (1.73)[ab]
OM	2/1	3.5(0.33)	8.9 (0.62)	12.6 (1.15)[b]
P value	*<0.0001*	*ns*[x]	*ns*	*<0.05*
Broccoli				
CC	-	Atmospheric	Atmospheric	5.7 (0.58)[a]
WC	-	Atmospheric	Atmospheric	5.7 (0.58)[a]
OC	-	Atmospheric	Atmospheric	5.3 (0.58)[a]
CM	1/1	11.3 (0.65)	2.9 (0.58)	12.3 (2.89)[b]
WM	1/1	12.2 (0.53)	2.9 (1.13)	11.7 (3.21)[b]
OM	1/1	11.2 (0.73)	2.9 (0.75)	12.0 (0.00)[b]
P value	*<0.0001*	*ns*	*ns*	*<0.05*
Spinach				
CC	-	Atmospheric	Atmospheric	10.3 (2.06)[a]
WC	-	Atmospheric	Atmospheric	13.0 (2.45)[ac]
OC	-	Atmospheric	Atmospheric	12.0 (0.00)[a]
CM	3/2	7.54 (0.39)[a]	4.54 (0.73)[a]	15.5 (1.73)[bc]
WM	3/2	6.75 (0.35)[b]	6.52 (1.11)[b]	17.5 (1.00)[b]
OM	3/2	7.24 (0.20)[a]	3.74 (1.14)[a]	17.0 (2.00)[b]
P value	0.0013/0.0002	0.0056	0.0120	<0.05

[z] CC: unwashed stored into produce bag; WC: washed in cold water stored into produce bag; OC: washed in cold water plus ozonated water stored into produce bag; CM: unwashed stored into passive MAP bag; WM: washed in cold water stored into passive MAP bag; OM: washed in cold water plus ozonated water stored into passive MAP bag.
[y] Average (SD) of days of storage of three separate trials.
[x] NS indicates not significantly different.

Passive MAP packaging improved the overall quality of the asparagus ($P < 0.0001$) during storage, adding 2-4 days of shelf life when compared to asparagus stored in open produce bags (Figure 1). Washing and washing method did not have an effect on the overall quality of the spears ($P > 0.05$) (Figure 1). Neither packaging nor washing had an effect on the yellowing of the spears as demonstrated by the yellowing scale and hue values ($P > 0.05$; data not shown).

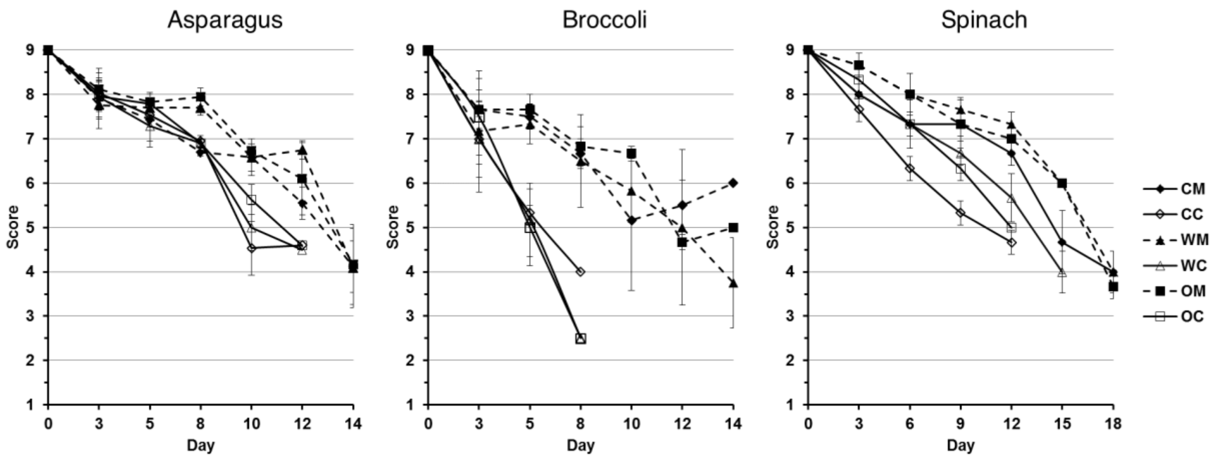

Figure 1 . Overall quality of asparagus, broccoli and spinach during storage at 13 ˚C. Six treatments were applied to every crop: unwashed produce in produce bags (CC) and in MAP bags (CM); washed in cold water (0 °C) stored in produce bags (WC) and in MAP bags (WM); additional wash in ozonated water (1.0 to 1.5 ppm for 1 min) stored in produce bag (OC) and in MAP bags (OM). Each value represents the mean of three trials, and the vertical bars correspond to standard error.

The subjective firmness decreased with time for all treatments but was more prominent on the spears stored in produce bags (P < 0.0001) (Figure 2). In addition, the puncture test at 10 cm showed that the asparagus stored in produce bags had also a more hardened core (P < 0.0001) compared to the samples stored in passive MAP bags, which were more tender (Figure 2). Asparagus stored in passive MAP bags affected the production of off-odors (P < 0.0001), with odors taking longer to develop and being less intense than samples in produce bags (Figure 3). MAP storage also affected visual decay (P < 0.0001) (Figure 4), since after the eighth day of storage, the spears in produce bags presented a rapid increase in decay, while the samples in MAP had a less pronounced effect The ozonated water treatment did not have a significant effect (P > 0.05) on any of the parameters analyzed under the conditions of this study.

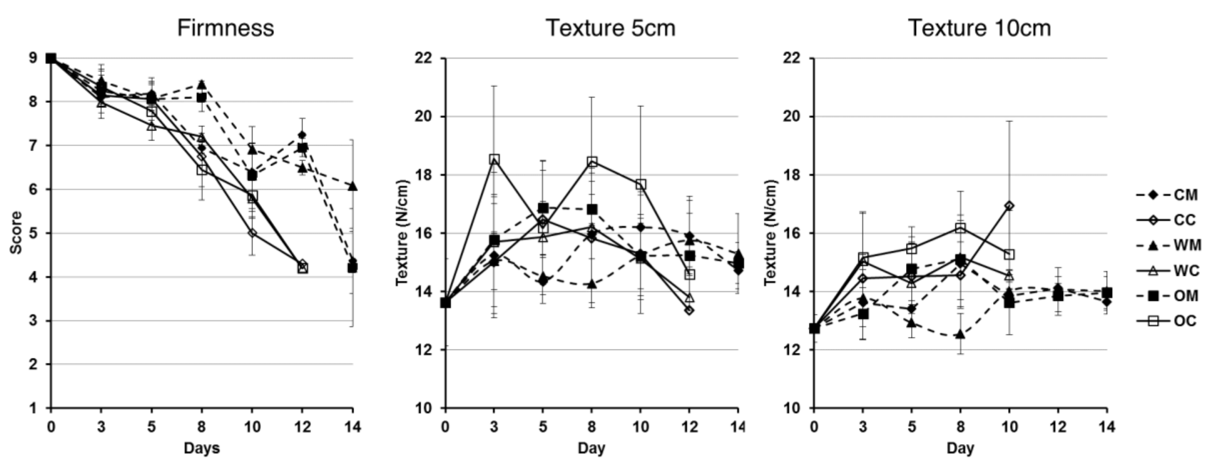

Figure 2. Qualitative firmness and analytical texture at 5 and 10 cm from the cut end of asparagus spears stored at 13 °C, in produce bags (CC) and in MAP bags (CM); washed in cold water (0 °C) stored in produce bags (WC) and in MAP bags (WM); additional wash in ozonated water (1.0 to 1.5 ppm for 1 min) stored in produce bags (OC) and in MAP bags (OM). Each value represents the mean of three trials, and the vertical bars correspond to standard error.

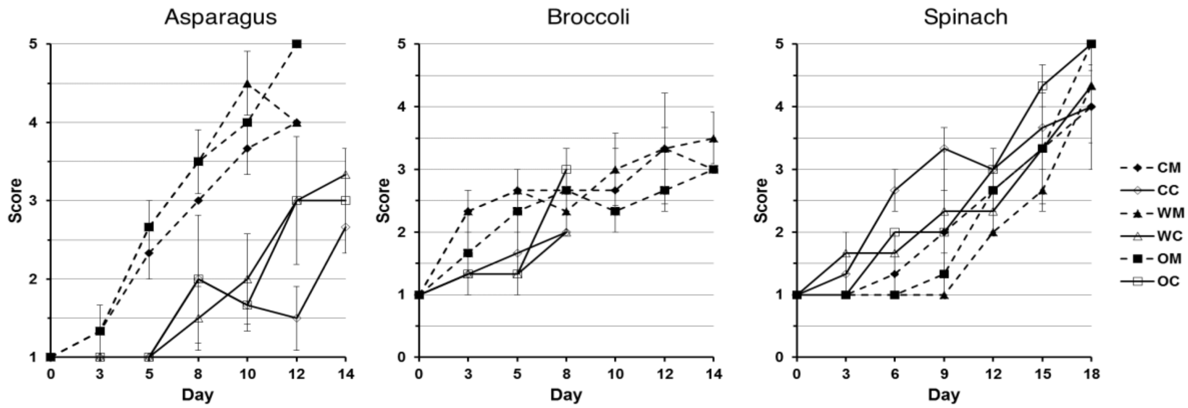

Figure 3. Qualitative off-odor analysis of asparagus, broccoli and spinach during storage at 13 °C in produce bags (CC) and in MAP bags (CM); washed in cold water (0 °C) stored in produce bags (WC) and in MAP bags (WM); additional wash in ozonated water (1.0 to 1.5 ppm for 1 min) stored in produce bags (OC) and in MAP bags (OM). Each value represents the mean of three trials, and the vertical bars correspond to standard error.

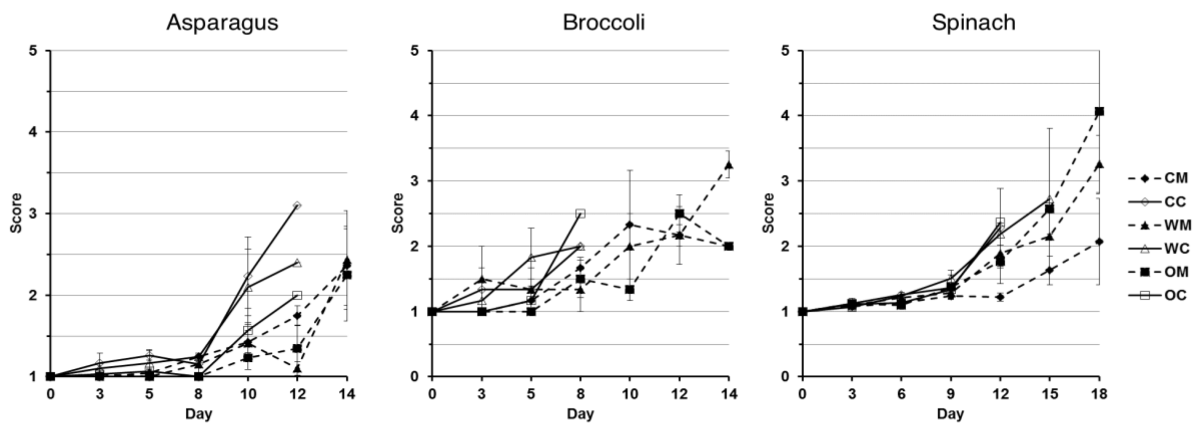

Figure 4. Qualitative decay analysis of asparagus, broccoli and spinach, during storage at 13 °C in produce bags (CC) and in MAP bags (CM); washed in cold water (0 °C) stored in produce bags (WC) and in MAP bags (WM); additional wash in ozonated water (1.0 to 1.5 ppm for 1 min) stored in produce bags (OC) and in MAP bags (OM). Each value represents the mean of three trials, and the vertical bars correspond to standard error.

Broccoli

None of the treatments affected the internal gas composition of the broccoli stored in passive MAP bags, and therefore not differing between each other (P > 0.05) (Table 1). Internal % CO_2 of samples stored in passive MAP bags equilibrated after one day of storage at 13 ˚C (Table 1). The unwashed control broccoli (CM) equilibrated at 11.3%; broccoli washed with cold water (WM) equilibrated at a slightly higher concentration of 12.2%, which was not significant; and broccoli additionally washed with ozonated water equilibrated at 11.2% (Table 1). Internal % O_2 concentration also equilibrated after one day of storage at 2.9% for CM, 2.9% for WM and 2.9% for OM (Table 1).

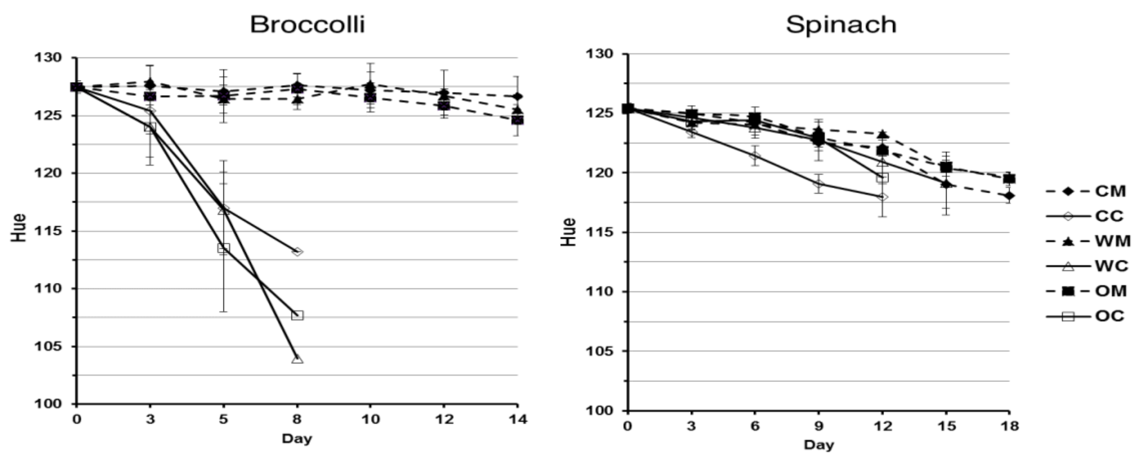

Figure 5. Hue angle of spinach and broccoli stored at 13 °C in produce bags (CC) and in MAP bags (CM); washed in cold water (0 °C stored in produce bags (WC) and in MAP bags (WM); additional wash in ozonated water (1.0 to 1.5 ppm for 1 min) stored in produce bags (OC) and in MAP bags (OM). Each value represents the mean of three trials, and the vertical bars correspond to standard error.

The use of passive MAP bags for storing broccoli had a highly significant effect on overall quality scores ($P < 0.0001$) (Figure 1). Broccoli stored in produce bags had a rapid decrease on OVQ and had, on average, a 5 day shorter shelf life (Figure 1), which was primarily due to yellowing of the florets (Figure 5). Samples stored in passive MAP bags had higher overall quality scores throughout storage and had an average shelf life of 12 days (Figure 1). However, ozonated water treatment did not affect the overall quality of broccoli in either package (Figure 1). Yellowing scores rapidly increased in samples stored in produce bags ($P < 0.0001$) since the hue angle for samples CM, WM and OM remained nearly constant during storage (Figure 5). The ozonated water treatment did not have a significant effect ($P > 0.05$) on the color of broccoli (Figure 5).

Packaging with passive MAP affected visual decay ($P < 0.0001$) (Figure 4), off-odor scores ($P < 0.01$) (Figure 3). When comparing the effect of packaging on subjective decay scores, produce bags had higher scores than those observed for samples stored in passive MAP bags (Figure 4). Off-odor, on the other hand, was higher throughout storage in passive MAP samples than those stored in produce bags (Figure 3). The ozonated water treatment did not have a significant effect ($P > 0.05$) on any of the quality parameters when stored under the conditions of this study.

Spinach

The internal concentration of carbon dioxide (% CO_2) of the samples stored in passive MAP bags (CM, WM and OM) reached equilibrium after three days of storage at 13 ˚C ($P < 0.01$) (Table 1). Washing with cold water (WM group) significantly lowered the % CO_2 ($P < 0.01$) to an average of 6.8% compared to the unwashed spinach (CM) at 7.5% and the ozonated water (OM) at 7.2% (Table 1). The internal concentration of oxygen (% O_2) in these samples took two days of storage to equilibrate ($P < 0.001$) (Table 1). The WM group had higher % O_2 ($P < 0.05$), with an average of 6.5 % compared to the unwashed control (CM) at 4.5 % and ozonated water (OM) at 3.7 % (Table 1).

Overall quality decreased during storage on all samples, but at different rates (Figure 1). Washing ($P < 0.01$) and packaging ($P < 0.0001$) positively affected overall spinach quality scores throughout storage (Figure 1). After nine days of storage, all samples packed in passive MAP bags (WC, WM and OM) had higher quality scores than samples packed in produce bags

(CC, WC and OC) (Figure 1). WM and OM had the highest scores throughout storage, and, as a result, had a longer shelf life of 17.5 and 17.0 days, respectively (Table 1). However, they did not differ from each other, demonstrating a stronger influence of the passive MAP on preserving the quality of spinach than the ozonated water treatment. The shortest shelf life was of the unwashed control group packed in the produce bags (CC), with the lowest overall quality scores and shelf life of 10.3 days (Table 1). According to the leaf color measurements, spinach stored in passive MAP developed yellow leaves later than the spinach stored in open produce bags (Figure 5). The leaves stored in MAP preserved their texture for a longer time with higher firmness scores throughout storage and had less wilting, although the same was not observed through the analytical texture measurement (Figure 6). The texture of spinach showed no differences between treatment groups, only a decline in texture throughout storage (Figure 6).

Decay occurrence was minimal in samples stored in produce bags (CC, WC and OC) until the end of the shelf life (10 to 13 days) (Figure 4), but off-odor developed after three days of analysis in these groups and was highest in the unwashed control group (CC) (Figure 3).

The ozonated water treatment did not have a significant effect ($P > 0.05$) on any of the quality parameters when stored under the conditions of this study. For all quality parameters, washing with cold water had better results for spinach quality than washing with ozonated water.

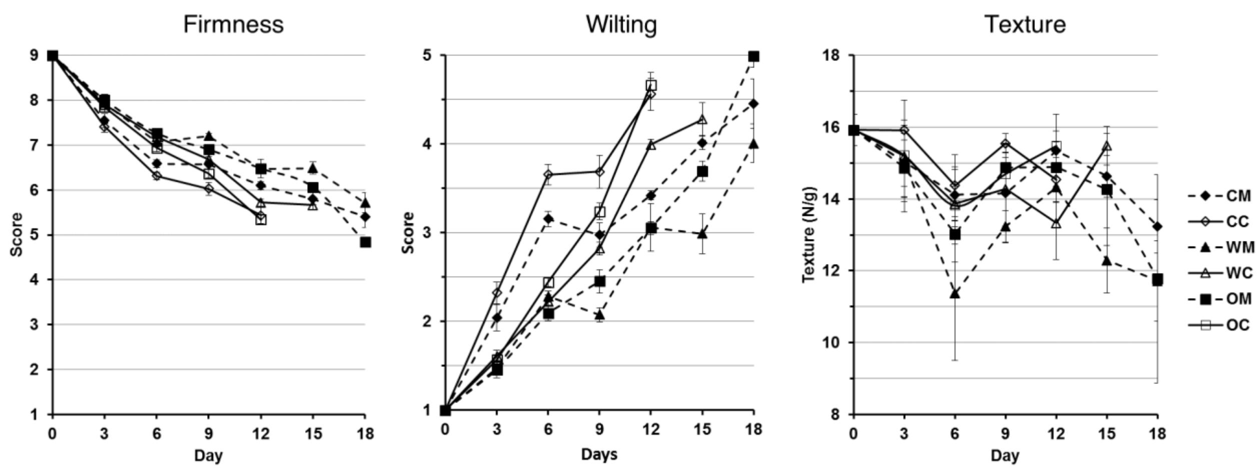

Figure 6. Qualitative firmness, wilting and texture of spinach stored at 13 °C in produce bags (CC) and in MAP bags (CM); washed in cold water (0 °C) stored in produce bags (WC) and in MAP bags (WM); additional wash in ozonated water (1.0 to 1.5 ppm for 1 min) stored in produce bags (OC) and in MAP bags (OM). Each value represents the mean of three trials, and the vertical bars correspond to standard error.

Microbiological Quality

Generic *E. coli* was not detected in this study; therefore, populations were below the limit of detection (~0.5 Log_{10} CFU/g) (data not shown). A treatment × time interaction was not observed ($P > 0.05$) for any microorganism population on any of the crops and a treatment effect was not observed ($P > 0.05$) for spinach, asparagus or broccoli (Figure 7). For this reason, data are presented across time and are independent of treatment. In general, APC, psychrotroph, yeast, and mold populations gradually increased throughout the shelf life for all commodities. With the exception of coliforms on asparagus ($P = 0.1646$)

and broccoli (P = 0.7541), sampling time was a statistically significant ($P \leq 0.05$) variable. Although sampling time associated with coliforms on spinach was calculated as a statistically significant variable ($P \leq 0.05$), the greatest difference in populations between any of the sampling days was 0.38 Log_{10} CFU/g. Coliform populations were consistently low on spinach; thus, the standard errors of the means were also low.

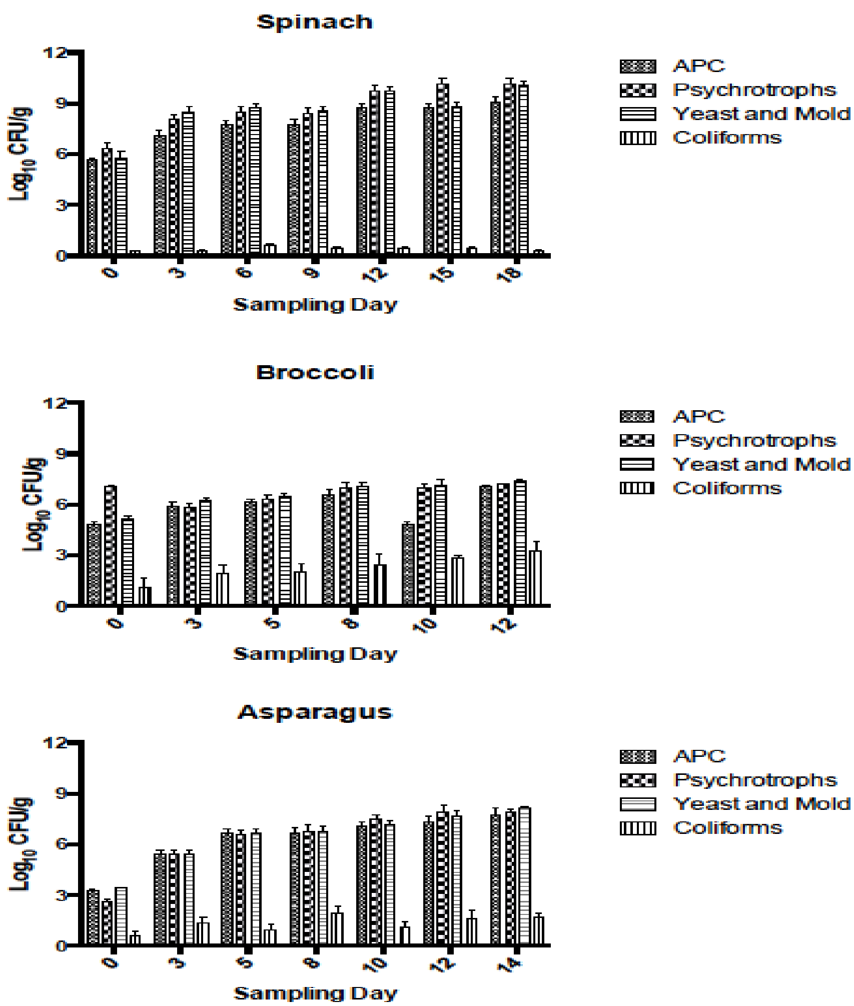

Figure 7. Microbial analysis of asparagus, broccoli and spinach during storage at 13 °C in produce bags (CC) and in MAP bags (CM); washed in cold water (0 °C) stored in produce bags (WC) and in MAP bags (WM); additional wash in ozonated water (1.0 to 1.5 ppm for 1 min) stored in produce bags (OC) and in MAP bags (OM). A treatment × time interaction was not observed ($P > 0.05$) for any microorganism population on any of the crops and a treatment effect was not observed ($P > 0.05$). For this reason, data are presented across time and are independent of treatment. Bars are the means of three trials, and error bars represent the standard error of the mean

DISCUSSION

In the present study, the use of commercially available passive modified atmosphere bags for storing produce under the non-optimum storage temperature of 13 ˚C resulted in the extension of shelf life for asparagus, spinach, and broccoli in comparison to storage in produce bags. Asparagus shelf life was extended by approximately 4 days; an extension of 7 days was observed for broccoli, and spinach shelf life was extended by 5 to 7 days. Storage at the non-optimum temperature of 13 ˚C reflects the produce storage conditions of growers who lack access to adequate cold storage. Under these storage conditions, passive MAP bags extended the shelf life by 34%, 117% and 70% for asparagus, broccoli and spinach, respectively, when compared to the control group. The commercially available MAP bags used in this study were intended for storage at approximately 0 ˚C. Despite the fact that the MAP headspace composition was not optimum for the stored vegetables, it positively affected the quality of the vegetables tested.

Specifically the passive MAP headspace composition for asparagus equilibrated at around 3.5% CO_2 and 8% O_2, while the recommended conditions are approximately 10% of CO_2, with atmospheric O_2 (~21%) (Saltveit, 2020). This indicates that non-optimum gas compositions were used for the preservation of asparagus spears stored in the passive MAP bags, which requires higher concentrations of O_2 due to the higher respiration rate of the crop. However, in this study, the asparagus stored in MAP still had 4 days longer shelf life than those stored in produce bags.

Washing with ozonated water in the conditions used in this study, 1.0-1.5 ppm of ozone for 1 min, was not effective. In studies conducted by An et al. (2007) and Sothornvit and Kiatchanapaibul, (2009), asparagus was subjected to MAP and ozonated water, with both studies storing their samples near optimum temperatures (3 ˚C and 4 ˚C), and both studies reported an improvement in asparagus quality due to combined implementation of these postharvest techniques. An et al. (2007) observed an improvement in enzyme activity of scavenger antioxidant enzymes, which can slow the senescence process and, therefore, extend shelf life. The authors also reported retardation of cell wall component formation, which improved textural attributes of asparagus. Sothornvit and Kiatchanapaibul, (2009), did not find any significant difference in fiber content but saw a reduction in microbial load and growth. The results of the present study also did not reflect an improvement of texture due to ozone or the combined use of ozone and passive MAP. This divergence of results might be due to a difference in the washing protocol used. An et al. (2007) dipped asparagus for 30 min in a 1 mg/L solution of ozonated water, while Sothornvit and Kiatchanapaibul, (2009) washed for 15 min in 0.1 mg/L, whereas the present study washed asparagus in 1.5 mg/L of ozone for one minute, according to the equipment instructions.

Broccoli had pronounced quality maintenance and shelf life extension. The internal gas composition of broccoli stored in passive MAP equilibrated at around 12% CO_2 and 3% O_2. Controlled atmosphere recommendations for broccoli are 5-10% CO_2 and 1-2% O_2 when stored in the temperature range of 0-5 ˚C. Temperature fluctuations above this range will result in off-odor production by the broccoli. To diminish this undesirable effect, the recommendation is to use 7-10% CO_2 and 3-10% O_2 when storing broccoli (Cantwell and Suslow, 2005). The gas composition obtained in our experiment was in the range recommended for O_2 and slightly higher for CO_2. This atmosphere resulted in increasing the shelf life of broccoli stored at 13ºC for 7 days when compared to the samples stored in produce bags. This result is comparable to those reported by Paulsen et al. (2018), which found that broccoli florets stored at 1% O_2 and 15% CO_2 at 8 ˚C exhibited a storage time of 15 days. Even though a storage time of 12 or 14 days is shorter than what can be achieved in optimum storage conditions (21-28 days) (Cantwell and Suslow, 2005), this extra week of storage can be extremely beneficial for small acreage farmers.

Passive MAP prevented yellowing of the broccoli heads stored at 13 ˚C, while broccoli stored at produce bags were yellow on day 5 of storage. Paulsen et al. (2018) reported the same effect in broccoli florets stored in MAP at 8-15 ˚C. Besides refrigeration, the yellowing of broccoli caused by chlorophyll breakdown, and induced by ethylene, can be inhibited by increased CO_2 concentrations and the use of 1-methylcyclopropene (Fernández-León et al., 2013). Color preservation of broccoli stored at low temperatures under adequate MAP conditions is well studied (Rakotonirainy et al., 2008; Gao et al., 2018). However, studies storing broccoli under non-optimum temperatures are still limited, yet are of great importance, because yellowing of broccoli can be more prominent after temperature changes during storage, as in the simulation of transport (Ihringer et al., 2010). Schouten et al. (2009) evaluated how controlled atmospheres (CA) at different temperatures affected the color in broccoli. They reported green color preservation after 10 days of storage at 18 ˚C and 1.5 kPa O_2/15 kPa CO_2, and discussed that color retention was better at low levels of O_2 when in combination with higher levels of CO_2 because of the energy provided by the gas exchange. Even though low temperature storage is the most efficient method for dark green color retention in broccoli (Cantwell and Suslow, 2005), passive MAP can effectively retain the color when product is stored at higher temperatures.

Washing broccoli in ozonated water using the conditions described herein did not have a significant effect on any of the quality attributes evaluated. As previously discussed, this could be due to the short exposure time of the one-minute ozonated wash. Lima et al. (2014) employed a longer ozonated wash treatment in broccoli and reported it better-preserved chlorophyll content after 7 days of storage when compared to other sanitation methods. Therefore, the green color could be preserved in broccoli following a longer exposure to ozonated water.

Spinach internal gas concentrations in samples stored in passive MAP at 13 ˚C equilibrated at around 7% of CO_2 and 4% O_2 while recommended conditions are 5-10% CO_2 and 1-3% O_2 for pre-washed spinach leaves at optimum storage temperatures (Saltveit, 2020). Although the atmospheric conditions achieved by the passive MAP were not ideal, the spinach had a shelf life extension of 7 days compared to the leaves stored in produce bags, which is a 70% increase of storage time. Batziakas et al. (2020) noticed that spinach leaves stored at higher temperatures benefited from lower O_2 atmospheres when compared to normal atmospheric conditions. Spinach stored at 12 ˚C in ready-to-eat packages with micro-perforations had demonstrated a shelf life of 6 days (Kou et al., 2014). In the present study, passive MAP bags also significantly decreased yellowing of the leaves, preserved visual firmness, and demonstrated less wilting, compared to samples stored in produce bags. Washing with ozonated water had less pronounced effects on quality when compared to washing with cold water. While, the process of washing had significant effects on overall quality, on wilting, on hue, and off-odor development. However, washing with ozonated water was not better than the cold-water treatment in regards to any of the parameters evaluated. Similarly, Karaca and Velioglu (2014) did not find any changes in quality, such as color or phytochemical content, in lettuce, spinach and parsley (*Petroselinum crispum* L.) treated with aqueous and gaseous ozone.

This study indicates that MAP, with or without a 1.0-1.5 ppm postharvest ozone wash, was not effective at extending the lag phase of microbial growth when spinach, asparagus and broccoli are stored at the non-optimum 13 ˚C temperature. While the ozone wash did occasionally achieve slight reductions in microbial populations on day 0 in comparison to the controls, this reduction was not significant nor was it maintained beyond the day 0 sampling time point. This is not altogether surprising, as ozone does not leave a residue in foods and is known for being unstable when in aqueous and gaseous states (Joshi et al., 2013).

Fresh-cut produce is more at risk for foodborne pathogen growth, as these products have essentially been injured by processing (Francis et al., 2012). When these products are stored at non-refrigerated temperatures the risk is further increased (Francis et al., 2012). It is well documented that bacteria grow rapidly in temperatures between 5 ˚C to 57 ˚C, although their growth is also affected by other extrinsic factors, such as nutrient availability, pH and water activity (McSwane et al., 2015). In the present study, all produce samples were stored at 13 ˚C, a temperature conducive for rapid bacterial growth.

While this study did not specifically evaluate the presence of foodborne pathogens, indicator microbial populations were enumerated to provide evidence as to the potential for foodborne pathogens to be present and survive. The microbial data presented herein suggest that the treatments evaluated would not reduce, or inhibit, the ability of foodborne pathogens to survive or possibly grow. Any initial reduction in microbial populations achieved by ozonated water was not maintained with storage in MAP. With regards to bacteria, it is important to note that some require oxygen for survival, while other bacteria can thrive in the absence of oxygen (McSwane et al., 2015), which is why a reduced oxygen atmosphere cannot be expected to control all bacterial populations. In this study, MAP samples equilibrated environments of reduced oxygen and increased carbon dioxide. Therefore, it is likely that employing MAP selected for the growth of bacteria that can adapt to carbon dioxide and/or reduced oxygen environments. It is also worth noting that the inhibitory effects of MAP towards bacteria increase as the temperature declines (Oliveira et al., 2015). Thus, the ability of MAP to inhibit bacterial growth at 13 ˚C was not as effective as expected at optimum storage conditions.

While the ozonated water and MAP treatments evaluated did not reduce microbial populations, it is also noteworthy that they did not promote excessive microbial growth. The lack of a treatment effect on microbial populations indicates that control and experimental treatments were statistically the same. Because MAP was beneficial for extending shelf life and maintaining product quality, it is important to recognize that the microbial indicator data collected suggest that MAP also did not accelerate or enhance the potential for bacterial spoilage or foodborne pathogenic growth in comparison to control samples. However, as previously mentioned, this study did not enumerate the presence of specific foodborne pathogens or spoilage microorganisms, which would be a recommendation for future research endeavors.

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