Influence of Modified Atmosphere Packaging on Postharvest Quality of Baby Spinach (*Spinacia oleracea* L.) Leaves

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Abstract. The aim of the current study was to determine the influence of modified atmospheric pressure on the quality of baby spinach during storage. Treatments consisted of control [(normal air) (78% N₂; 21% O₂)], modified atmosphere (MA) (5% O₂; 15% CO₂; balance N₂), storage temperature (4, 10, and 20 °C), and number of days after storage (0, 3, 6, 9, and 12). Parameters recorded are gas composition, weight loss, sensory quality, minerals and trace elements (Mg, Fe, and Zn), flavonoids, and antioxidant activity. The results of this study demonstrated that in the headspace gas there was overall reduction in O₂ and increase in CO₂ levels over the storage period. After 6 days of storage, all samples in normal air irrespective of the storage temperature were found to fall short of acceptable marketability with regard to visual appearance. The total antioxidant activity and flavonoids were well maintained under controlled atmosphere (CA) at 4 °C when stored for 9 days.

The biggest problem faced by the food industry in South Africa is the maintenance of quality of fresh produce during postharvest storage (Munhuweyi et al., 2016). Attempts to reduce losses and maintain quality of fresh food, primarily fruits and vegetables, during postharvest storage has been a challenge and priority for the food industry (Velickova et al., 2013). Strategies to prolong the shelf life of fresh-cut vegetables during storage are necessary to preserve their nutritional value and to assure food safety (Conte et al., 2008; Ninfali and Bacchiocca, 2004).

Modified atmosphere packaging (MAP) is the technique of sealing actively respiring produce, fresh fruit and vegetables, in polymeric

film packages to achieve the modification of the O₂ and CO₂ levels within the package atmosphere (Mir and Beaudry, 2004). The MA packaging technology is said to be efficient in maintaining shelf life of fresh produce during storage (Sandhya, 2010). Church and Parsons (1995) reported that the combination of oxygen (O₂), nitrogen (N₂), and carbon dioxide (CO₂) is frequently used when operating the MAP during storage, based on its efficiency and specific effect it has in slowing down the metabolism process of the produce. The high CO₂ or low O₂ concentrations diminish respiration rate, thus, reduces the occurrence of aerobic bacteria and deterioration rate of the stored product (Babic and Watada, 1996).

The concentrations of O₂ in MAP during storage of fresh-cut fruits and vegetables for both quality and safety should range between 1% and 5% (Sandhya, 2010), whereas CO₂ concentrations should be above 10% (Zagory and Kader, 1988). Izumi et al. (1997) demonstrated the benefits of low O₂ atmospheres on spinach during storage. In addition, Gil et al. (1999) and Ko et al. (1996) reported

reduced respiration rate, weight loss, and antioxidant loss on spinach stored in low O₂ concentrations. There is limited data available describing storage requirement of baby spinach using MAP. Thus, the objective of this study was to determine the effect of CA on postharvest quality and shelf life of baby spinach (*Spinacia oleracea* L.).

Materials and Methods

Experimental site. The trial was carried out at the Agricultural Research Council Vegetable and Ornamental Plant Institute situated ≈25 km north of Pretoria on the KwaMhlanga Road (R573), Gauteng Province, South Africa, at lat. 25°59'S; long. 28°35'E. The study was initiated during mid-May to early July 2014 and repeated in June to late Aug. 2015, which was suitable for baby spinach production in South Africa.

Plant materials. Ohio, a baby spinach cultivar (Hygrotech, Pretoria, South Africa) was used for the study. Seedlings of baby spinach (*S. oleracea* L.) were thinned after 10 d of days after planting (DAP). Plants were fertilized with a balanced nutrient solution that contained all nutrients as described by Nematodzi et al. (2017) and Zikalala et al. (2016). The initial soil chemical analyses were determined using a procedure described by Hanlon et al. (1994). The field soil conditions were light sandy clay soils, with a pH ranging from 7.3 to 7.7. All plants were irrigated by using drip irrigation system. The trial was ended 37 DAP.

Treatment and experimental design. Treatments consisted of control (78% N₂; 21% O₂), MA (5% O₂; 15% CO₂; balance N₂), at temperatures (4, 10, and 20 °C) and number of days (0, 3, 6, 9 and 12) arranged in a randomized block design with five replicates. Parameters recorded were gas composition, weight loss, sensory quality, minerals and trace elements (Mg, Fe, and Zn), flavonoids and antioxidant activity.

Weight loss. Baby spinach leaves were weighed using a digital electronic balance [Model MK-500C, DENVER instrument (±0.001 g); Sigma-Aldrich, St Louis, MO] at the beginning and end of each storage period expressed as percentage weight loss.

Gas composition. Headspace O₂ and CO₂ concentrations in packages of baby spinach were measured using a PBI Dansensor CO₂/O₂ gas analyzer (Checkmate 9000, Ringsted, Denmark) by inserting the needle of the measuring assembly through a septum adhered to the packaging container during every storage interval (Mampholo et al., 2013). At each sampling interval, five packages from atmosphere conditions were measured and thereafter, the mean value was determined.

Sensory analysis (visual appearance and off odor). Sensory attributes were scored based on the methods described by Medina et al. (2012) with slight modifications. Product quality was evaluated in fresh material and after 3, 6, 9, and 12 d of storage. Overall acceptance and odor evaluation was carried

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Table 1. Percentage cumulative weight loss of packaged baby spinach stored under modified atmosphere packaging (5% O₂; 15% CO₂; balance N₂) and under untreated atmosphere (78% N₂; 21% O₂) at 4, 10, and 20 °C for 12 d.

Storage duration (d)	Wt loss (%) ^z					
	Modified atmosphere packaging			Untreated atmosphere		
	4 °C	10 °C	20 °C	4 °C	10 °C	20 °C
0	0.00 b	0.00 d	0.00 c	0.00 bc	0.00 c	0.00 d
3	0.39 a	1.59 c	3.39 b	0.06 c	3.14 b	4.21 c
6	0.55 a	2.93 b	3.60 b	0.61 b	3.33 b	6.23 b
9	0.89 a	3.08 b	5.33 a	1.19 a	5.23 a	6.76 b
12	0.94 a	5.03 a	5.79 a	2.24 a	5.29 a	7.56 a

^zWeight loss (%) is presented as the mean of five replicates. Means in columns with different letters are significantly different ($P \leq 0.05$) using Duncan's multiple range test.

Fig. 1. Changes in headspace gas composition [% O₂ (A) and % CO₂ (B)] of baby spinach stored at 4, 10, and 20 °C for 12 d in modified atmosphere (5% O₂; 15% CO₂; balance N₂) and untreated atmosphere (78% N₂; 21% O₂). Each value represents the mean of five determinations at each temperature $\pm$ SE. Data points indicate mean gas composition SE.

Fig. 2. Influence of modified atmosphere and normal air conditions on overall appearance in baby spinach leaves stored at 4, 10, and 20 °C for 12 d. Data points indicate mean score SE.

out by 10 trained panelists between 23 and 45 years of age (70% females and 30% males). In an assessment of postharvest decay condition, the samples were individually scored in hedonic scale arranged as 1 to 9 [where 9 = excellent, 7 = good (some leaves slightly yellowed or decayed), 5 = fair (with acceptable marketability), 3 = poor (dominated by yellow or decayed leaves), 1 = extremely poor (inedible)]. With regard to odor, a scale of 1 to 9 was applied: where 9 = excellent, 7 = slightly off-odor, 5 = moderate off-odor, 3 = strong off-odor, and 1 = intolerable off-odor.

Leaf color. Surface color of spinach leaves was objectively measured using a Minolta CR-400 chromameter (Minolta, Osaka, Japan), calibrated with a white tile. Five leaves per atmosphere condition were measured at 0, 3, 6, 9, and 12 d of storage. The change in total color that occurred during the storage (ΔE) was determined using the formula:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where ΔL^* , Δa^* , and Δb^* are differences in spinach color between day 0 and the sampling day (Pandurangi and LaBorde, 2004).

Mineral and trace elements. All mineral and trace elements were analyzed using a method described by van der Walt et al. (2009).

Determination of antioxidant activity. Antioxidant activity (by free radical scavenging) of the extracts was determined using Trolox equivalent antioxidant capacity assay as described by Awika and Rooney (2004).

Evaluation of total flavonoid content. The total flavonoids were measured using a modified calorimetric method described by Yoo et al. (2008) and slightly modified by Mudau et al. (2015).

Statistical analysis. Data were subjected to analysis of variance using IBM SPSS statistics version 23.0. Means were separated using Duncan's multiple range test. The Wilcoxon Mann-Whitney test was used for sensory analysis to compare treatments applied during postharvest. The significant differences were considered at $P < 0.05$.

Results

Weight loss (MAP). Table 1 showed that baby spinach leaves stored under MA from 3 to 12 d significantly reduce % weight loss. The difference between the highest and lowest weight loss was 0.94% when stored at 4 °C, whereas at 10 and 20 °C, the highest weight loss was 5.03% and 5.79%. All treatments significantly maintained percentage weight loss with the highest achieved at 12 d of storage.

Untreated (normal) atmosphere. Percentage weight loss (%) in spinach leaves stored under normal air showed demonstrated similar trends in all storage temperature for 12 d (Table 1). After 12 d, the highest weight loss (7.56%) was observed in samples stored at 20 °C. The least weight loss (2.24%) was observed in the samples stored at 4 °C. This storage temperature showed great potential in maintaining weight than at 10 and 20 °C. However, the CA samples maintained more weight greater than those that were exposed to untreated atmosphere.

Gas composition. Figure 1A and B demonstrated the changes in the headspace gas composition of baby spinach in MA and normal air conditions at different temperatures over the storage period. There was a rapid decrease of O₂ ranging from 4.9% to 2.5% on the 3 d of storage at 20 °C. A faster CO₂ production was observed at 20 °C on the 3 d of storage under baby spinach leaves stored at normal air condition, ranging from 15.5% to 19.2%. Irrespective of gas conditions, samples stored at 20 °C had the lowest O₂ and the highest CO₂ followed by those stored at 10 and 4 °C.

Sensory analysis. Significant differences ($P < 0.05$) in the overall visual appearance and off-odor were observed among baby spinach samples stored in MA and normal air condition at different temperatures over the storage duration (Figs. 2 and 3). Samples from all treatments scored high with similar values for day 0 and no significant difference was noted ($P > 0.05$), whereas on the day 3, samples stored in normal air at 20 °C showed signs of a faster deterioration in quality from all other treatments (Fig. 2). Samples in MA at 10 °C had better quality than those in

normal air condition stored at 4 °C on the 6 and 9 d of storage. In comparison between samples in MA and normal air conditions, significantly faster quality deterioration was observed in samples exposed to normal air. This was evident in that after 6 d of storage; all samples in normal air irrespective of the storage temperature were found to fall short of acceptable marketability. On the 12 d, samples in CA stored at 4 °C still had overall quality for acceptable marketability.

Moderate off-odor was observed after 12 d of storage in spinach samples under MA when stored at 4 °C (Fig. 3). However, samples exposed to normal air stored at similar temperature after 12 d had strong off-odor. Furthermore, no significant differences ($P > 0.05$) were observed among samples under MA and normal air at 4 °C

during the entire storage period. Similar trend was observed in samples stored at the storage temperature of 10 and 20 °C for modified and normal air conditions, respectively. Samples exposed to normal air at 20 °C revealed a defect which occurred rapidly because a strong off odor was evident on day 6 of storage.

Leaf color. Statistical analysis of color changes in spinach leaves during storage showed slight differences in MA at all three storage temperatures. In general, the nature of samples stored under normal air showed significantly lower a*, higher b*, and higher chroma than samples stored in MA. The color differences (ΔE) increased ($P < 0.05$) in both MA and normal air samples and most rapid changes were observed at 20 °C (Fig. 4). Whereas, samples stored at 4 and 10 °C

Fig. 3. Influence of modified atmosphere and normal air conditions on the off-odor in baby spinach leaves stored at 4, 10, and 20 °C for 12 d.

Fig. 4. Change in total color (ΔE) of spinach packaged with modified atmosphere (5% O₂; 15% CO₂; balance N₂) and control (78% N₂; 21% O₂) at 4, 10, and 20 °C for 12 d. Each value represents the mean of five determinations at each temperature $\pm$ SE. Data points indicate color change SE.

Fig. 5. Effect of modified atmosphere on the Mg (A), Fe (B), and Zn (C) in the spinach leaves stored at 4, 10, and 20 °C for 12 d. Data points indicate mean SE.

Fig. 6. Total antioxidant activity (mg·kg⁻¹ DW) of baby spinach leaves stored under modified atmosphere (5% O₂; 15% CO₂; balance N₂) and control (78% N₂; 21% O₂), at 4 °C for 12 d. Data points indicate mean total antioxidant activity SE.

remained constant during the entire storage duration.

Mineral and trace elements. Modified atmospheric pressure revealed a similar effect of normal air on Mg, Fe, and Zn. Magnesium concentration was highly retained in the lowest storage temperature

regardless of the surrounding gas composition on the spinach leaves (Fig. 5A). After 12 d of storage, spinach leaves stored at 4 °C under MA and normal air conditions had Mg concentration of 11 and 9.2 mg·kg⁻¹ dry weight (DW). The least concentration of Mg was pronounced in spinach leaves stored

at 20 °C under MA (7.5 mg·kg⁻¹ DW) and normal air (7 mg·kg⁻¹ DW). The rate in which Fe was declining was very slow compared with Mg and Zn concentration. Iron concentration ranged between 0.4 and 0.67 mg·kg⁻¹ DW in both gas conditions (Fig. 5B). Iron concentration was not significantly different ($P > 0.05$) between MA and normal air conditions. From 3 to 9 d of storage, Zn concentrations were similar at 4 and 10 °C (Fig. 5C).

Total antioxidant activity. Total antioxidant activity decreased in all treatments; however, the baby spinach stored under MA showed better activity when compared with those stored in a normal air. During storage period at 4 °C, total antioxidant activities were closer in both MA and normal air conditions. Nevertheless, at the end of the experiment baby spinach leaves stored under MA demonstrated a higher maintenance of total antioxidant activity when compared with those stored in the normal air environment (Fig. 6).

On the other hand, it was found that the total antioxidant activity in baby spinach leaves stored under normal atmosphere drastically decreased, after day 3 in particular, from 6.45 to 5.58 mg·kg⁻¹ DW, whereas those stored under MA slightly decreased from 6.45 to 5.91 mg·kg⁻¹ DW (Fig. 7). Thus,

the total antioxidant activity was significantly affected as the difference was statistically significant. It can be concluded that the MA technique is ideal as it delays degradation of leaf pigment. Immediately, after day 3 of storage period, it was clear that the highest level of total antioxidant activity was observed in the baby spinach leaves stored at 4 °C under MA when compared with the other samples stored at 10 and 20 °C under both MA and normal air atmosphere (Figs. 5–7).

In fact, the total antioxidant activity in the baby spinach leaves stored under MA at 4 °C after 3 and 12 d of storage period was 6.39 and 4.41 mg·kg⁻¹ DW, respectively, as shown in Fig. 8. Whereas, the lowest total antioxidant activity after 3 d (3.65 mg·kg⁻¹ DW) and 12 d (2 mg·kg⁻¹ DW) was observed at 20 °C in baby spinach leaves stored under untreated atmosphere. Although the baby spinach

leaves stored under normal atmosphere had shown very poor total antioxidant sustainability, baby spinach leaves stored at 4 °C resembled the steadiest of total antioxidant over those stored at 10 and 20 °C under normal air.

Total flavonoid content. Total flavonoids in samples stored in MA ranged from 6.12 to 25.14 mg·kg⁻¹ DW, whereas total flavonoids of untreated (normal) samples ranged from 5 to 25.14 mg·kg⁻¹ DW in all three different temperatures (Figs. 9–11). Total flavonoid contents decreased as the storage duration increased in all treatments. However, baby spinach samples under MA when stored at 4 °C showed higher capacity to retain the high levels of total flavonoids from the beginning of storage until day 12 (Fig. 9). There was a significant difference between modified samples stored at 4 and 20 °C after day 6 of storage.

Discussion

The headspace gas composition completely relies on the fresh produce O₂ consumption and CO₂ production rate when packaged and stored (Mastromatteo et al., 2012). The increase in O₂ consumption and CO₂ production with relative increase in temperature over storage time could be attributed to the respiration activity of baby spinach. Samples in CA had slow O₂ consumption and CO₂ production rate compared with those stored in normal air condition. Thus, the findings in this study suggest that CA coupled by low temperature over storage period could reduce respiration rate, which leads to extended shelf life of the fresh produce. Similar findings were obtained in previous studies for baby spinach (Kaur et al., 2011; Kou et al., 2014; Tudela et al., 2013). Furthermore, similar results were found by other authors for different vegetables (Leceta et al., 2015).

Weight loss during storage period varies depending on the vegetable, packaging conditions (include modified atmospheric pressure), and also, temperature levels. Modified atmosphere has proved to reduce weight loss compared with normal air. Similar findings were confirmed in the study conducted by Antmann et al. (2008), who observed a weight loss of 72% after 6 d of storage at 5 °C in unpackaged mushrooms, whereas mushroom in packaged conditions reduced weight loss. Reduced weight loss in plants stored under MA could be caused by the fact that the respiration rate is passive, and also carbohydrate resources were consumed slowly in the atmospheric condition. In this current study, weight loss increased with storage duration gradually. However, at higher temperature it has been observed that there is a rapid increase. This could be attributed to the fact that high temperature may increase the respiration rate which may result in dehydrated product. Mudau et al. (2015) and Watada et al. (1987) reported similar findings as greater loss of weight was observed in baby spinach samples stored at 20 °C. Zenoozian (2011) reported the maximum weight loss of spinach during storage at 10 and 25 °C when stored in normal air; however, active MA packaging had minimum loss of weight.

Spinach samples under MA were considered not acceptable for marketing after 9 and 12 d at 20 and 10 °C, respectively. Similar findings were reported by Pandrangi and LaBorde (2004), who found that spinach under normal air stored at 4, 10, and 20 °C were commercially unacceptable after 8, 6, and 4 d, respectively. Although less pronounced in their studies, we assume that the extended of commercial acceptability in our findings was effected by an introduction of modified atmospheric pressure.

The shelf life of the stored produce is pronounced to be influenced largely by sensory quality parameters, among others, odor, which may either invigorate or discourage consumers' consumption. Off-odor became notably more perceptible in spinach leaves

Fig. 7. Total antioxidant activity (mg·kg⁻¹ DW) of baby spinach leaves stored under modified atmosphere (5% O₂; 15% CO₂; balance N₂) and control (78% N₂; 21% O₂), at 10 °C for 12 d. Data points indicate mean total antioxidant activity SE.

Fig. 8. Total antioxidant activity (mg·kg⁻¹ DW) of baby spinach leaves stored under modified atmosphere (5% O₂; 15% CO₂; balance N₂) and control (78% N₂; 21% O₂), at 20 °C for 12 d. Data points indicate mean total antioxidant activity SE.

Fig. 9. Total flavonoid contents (mg·kg⁻¹ DW) in baby spinach leaves stored under modified atmosphere (5% O₂; 15% CO₂; balance N₂) and control atmosphere (78% N₂; 21% O₂), stored at 4 °C for 12 d. Data points indicate mean total antioxidant activity SE.

Fig. 10. Total flavonoid contents (mg·kg⁻¹ DW) in spinach leaves stored under modified atmosphere (5% O₂; 15% CO₂; balance N₂) and control atmosphere (78% N₂; 21% O₂), stored at 10 °C for 12 d. Data points indicate mean total flavonoids SE.

Fig. 11. Total flavonoid contents (mg·kg⁻¹ DW) in spinach leaves stored under modified atmosphere (5% O₂; 15% CO₂; balance N₂) and control atmosphere (78% N₂; 21% O₂), stored at 20 °C for 12 d. Data points indicate mean total flavonoids SE.

exposed to normal air stored at 10 and 20 °C as the storage duration increased. When considering O₂ evolution, oxygen uptake will rise with an increase in storage temperature. Most importantly, low O₂ and high CO₂ concentrations retarded the respiration rate. Off-odor was less pronounced in MA samples stored at 4 and 10 °C, whereas similar trend was observed with those samples exposed to normal air at 4 °C. It was only MA samples stored at 4 °C which were found to be commercially acceptable after 12 d of storage. These findings were in agreement with results found by Tudela et al. (2013), who reported that baby spinach exposed to low O₂ with CO₂ was rejected after 10 d at 7 °C.

According to Rizzo and Muratore (2009), color remains to be the core parameter that customers apply when judging the quality of vegetables. Our findings were similar to those reported by Pandrangi and LaBorde (2004). In their study, they found that there was no significant difference after 8, 6, and 4 d at 4, 10, and 20 °C. Rizzo and Muratore (2009) reported that unacceptable color change from green to yellow was more noticeable in controls, whereas MAs of celery leaves showed no signs of color change.

The postharvest storage retained high level of antioxidant under low temperature. This findings concur with the study by Mudau et al. (2017), who reported that the antioxidant content were mostly retained at 4 °C when compared with 10 and 22 °C. This could be attributed to metabolic reactions that are greatly reduced, although are not shut down completely. Pereira et al. (2014) experimented the effect of CA on the total antioxidant activity in the radicchio (*Cichorium intybus* L.) when stored at 1 °C for 10 d and observed that there was a slight increase in the total antioxidant activity for first 3 d of storage, and thereafter declined drastically. Chu et al. (2002) stressed that there is no clear indication on the causes of antioxidant activity variation in the vegetables during storage. However, it is commonly understood that the antioxidant activity of vegetables totally depends on several compounds, such as flavonoids, phenolic acids, amino acids, and ascorbic acid.

Total flavonoids were well maintained at 4 and 10 °C, whereas there was a drastic decline at 20 °C. Gil et al. (1999) reported an increase of total flavonoids in Swiss chard during storage at 6 °C under CA, whereas this increase was not significant in the air-stored Swiss chard. The present study findings concurred with the study by Gil et al. (1999) in that there was no significant difference between the total flavonoids in plants stored in CA and those stored in normal air, except at high storage temperature. Elsewhere, Martínez-Sánchez et al. (2006) reported that the flavonoids content in the wild rocket leaves remained constant or even increased during storage when stored in CAs at temperature of 1.5 to 4 °C for 14 d, whereas flavonoids in leaves stored under normal air kept on declining even when stored at low

temperature. This could indicate that MAP can have pronounced effects of metabolism of phenolics depending on the variety or cultivar. Modified atmosphere packaging also plays a crucial role in reducing the oxygen level around the produce during storage, which inhibits the existence of aerobic microorganisms.

When considering all conditions under which this study was carried out, it has been found that baby spinach leaves stored under CA (5% O₂; 15% CO₂; balance N₂) at 4 °C for 9 d maintained a high level of antioxidant activity and flavonoids. Common observation was that substantial losses of nutrients occurred at each storage temperature. Thus, it is ideal to apply a combination of control atmosphere and low temperature to promote longer shelf life without compromising the produce quality during storage.

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