

Response of Major Wild Leafy Vegetables to Controlled Atmosphere Storage

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Abstract

The effects of controlled atmosphere (CA) on quality maintenance and storage potential were estimated in four major, wild, leafy vegetables, specifically focusing on the incidence of CA-related disorders. After harvest, vegetables were pre-cooled to 4-5°C with a pilot-scale pressure pre-cooler. As a control treatment, perforated modified atmosphere packaging (MAP) was performed using 20 × 25 cm (W × L) polypropylene film bags with 8 punch holes. For CA treatments, two regimens were applied as 1% O₂ combined with 5% or 10% CO₂ atmospheric compositions. Discoloration was evaluated during storage and on the shelf. The resulting storage potential was determined by overall marketability. The vegetables used in this study could be sorted into two groups based on their sensitivity to CA or MA-related disorders. *Aster scaber* (edible aster) and *Cirsium setidens* (Korean thistle) were sensitive to carbon dioxide and/or low oxygen injuries. As a typical symptom of physiological disorder, blackening discoloration increased under high carbon dioxide conditions over 5%. Storage potential of vegetables at 0°C in the MAP condition was approximately 3 weeks for edible aster and 4 weeks for Korean thistle leaves. *Allium ochotense* (myeongyi) and *Heracleum moellendorffii* (East Asian hogweed) did not show low oxygen and high carbon dioxide injuries even under the 1% O₂ + 10% CO₂ regimen. Storability of these two commodities was longer than 4 weeks under both MAP and CA conditions. In myeongyi leaves, CA effects were clearly observed under 1% O₂ + 5-10% CO₂ conditions while in East Asian hogweed leaves, the beneficial effects were similar to those in MAP storage.

Additional key words: browning disorder, marketability, modified atmosphere packaging, shelf life, storage potential

Introduction

Wild, leafy vegetables are primarily consumed after a drying process due to difficulties in storing them as fresh produce. Nevertheless, the need for storage and distribution technology has increased recently as eating preferences are shifting from dried vegetables to fresh vegetables to gain their bioactive functions.

Refrigerated cold storage is a common technology to maintain fresh quality and extend storage potential. In some commodities, modified atmosphere packaging (MAP) or controlled atmosphere (CA)

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storage is a more effective storage method (Mattheis and Fellman, 2000; Kim et al., 2014). In the domestic market, however, CA storage can cause physiological disorders and has been applied in a limited scale. Carbon dioxide injury in 'Fuji' apples has been a continuous barrier preventing the expansion of the technology (Kim et al., 2014; Park and Lee, 2003; Park et al., 1997).

Another limiting factor for the application of CA storage, especially in wild leafy vegetables and herb plants, is the relatively short storability and weak benefit CA storage provides. For example, storage-life of chicory can be doubled under 3–4% O₂ + 4–5% CO₂ CA conditions as compared to air storage. However, acceptable marketability lasts only for 4–8 weeks (Rubatzky and Saltveit, 2016). In parsley, the benefit of CA storage was very limited when stored at 0°C (Heyes, 2016). In addition, a large investment for CA storage facilities is an economical risk for practical applications. From an economical point of view, MAP storage is widely applied for vegetables as an alternative technology to CA storage. In herb plants and wild leafy vegetables, MAP storage delayed the yellowing of chive (Aharoni et al., 1993) and parsley (Aharoni et al., 1989), maintained quality of basil, chervil, and coriander on the shelf (Wright, 2016) and extended storability of *Ligularia fischeri* (Mele et al., 2017). In contrast, MAP of leafy greens may stimulate decay and aggravate flavor, suggesting the need for appropriate perforation treatment on the film bags (Rushing, 2016).

Elucidating the optimum atmospheric composition is the basic approach for successfully applying CA and MAP storage while avoiding low-oxygen and/or carbon dioxide injuries. However, only little information is available on the optimized atmospheric composition for CA or MAP storage of fresh, wild, leafy vegetables. In fresh Fischer's ragwort (*Ligularia fischeri* Turcz.) leaves, high CO₂ seemed to induce or aggravate the incidence of browning symptoms (Park et al., 2015). As for MAP, Chung et al. (2010) reported beneficial effects of film packaging on the quality maintenance of *Aster glehni* and *Aruncus dioicus* during short-term storage. During 9-day MAP storage of *Aster glehni* at 4°C, the average concentrations of O₂ and CO₂ inside polypropylene (PP) packages were 6.3% and 13.5%, respectively.

In the present study, susceptibility to CA disorder was estimated to provide guidelines for atmospheric composition of practical MAP storage in major, wild, leafy vegetables. In addition, storage potential was determined for perforated MAP and CA storage.

Materials and Methods

Plant Sources and Pretreatment

Among four wild vegetables used in this experiment, Edible aster and East Asian hogweed were cultivated in Yeongyang county, while Korean thistle and myeongyi were cultivated in Hongcheon county and Yeongwol county, respectively. The wild vegetables were grown in open fields that were located at lower to mid mountainous areas. Young leaves of wild vegetables were harvested at the optimum maturity for consumption as fresh materials (Table 1). Sampling units were young shoots with several leaves in edible aster, Korean thistle, and East Asian hogweed, whereas the units were individual leaves in myeongyi. Immediately after harvest, leaves were precooled to 4°C by pressure cooling and then MAP or CA storage treatments began.

Table 1. Growing area and harvest date of wild leafy vegetables used in the present study

Commodity name		Growing region (county)	Cultivation	Harvest date, year
English name	Scientific name			
Edible aster	<i>Aster scaber</i>	Yeongyang	Open	May 19, 2014
Korean thistle	<i>Cirsium setidens</i>	Hongcheon	Open	May 8, 2015
Myeongyi	<i>Allium ochotense</i>	Yeongwol	Open	April 30, 2015
East Asian hogweed	<i>Heracleum moellendorffii</i>	Yeongyang	Open	July 27, 2015

Experimental Treatment

Perforated MAP treatment served as a control and two CA treatments were performed on four commodities in the same way. Perforated MAP was achieved by using 40 μm polypropylene (PP) bags ($W \times L$: 20×25 cm) on which four 8 mm-diameter punch holes were made on both sides (8 punch holes total). Each packaging unit was adjusted to 120 ± 5 g. Oxygen and carbon dioxide concentrations inside the package were maintained at the same levels as in the fresh air. In the two CA treatments, 1% $\text{O}_2 + 5\% \text{CO}_2$ and 1% $\text{O}_2 + 10\% \text{CO}_2$ regimens were established respectively, with nitrogen as a balance gas. CA was achieved by intermittent supply of premixed gases through gas tight glass jars. Storage temperature was 0°C and relative humidity (RH) was maintained above 95% in all the treatments during the 4- to 5-week storage period.

For shelf life evaluation, CA-stored leaves were packaged inside perforated PP bags of the same size as used in the MAP treatment. A cold-chain distribution system was simulated in the shelf-type refrigerated chambers at 7°C for 5 d.

Evaluation of Discoloration and Marketability

Investigation on the incidence of discoloration and ratings of marketability were performed by visual assessment twice, immediately after initial storage and after the 5 d shelf life.

Two discoloration symptoms were observed on the individual leaf or young stem basis. Incidence of black coloration (blackening) was estimated as an indicator of CA-related disorder in edible aster, Korean thistle, and East Asian hogweed, whereas yellowing was investigated to compare quality changes between air and CA storage in myeongyi leaves. All leaves with even slight discoloration were counted as injured or deteriorated in order to calculate percent incidence of discoloration. Percent incidence was calculated from the ratio of the number of injured leaves or young stems over the number of investigated leaves or stems.

The severity of the symptom was estimated in three categories: slight (negligible symptom, no problem for consumption), mild (visible spot discoloration, critical point of consumption), and severe (substantial discolored area, not acceptable). Overall marketability was assessed considering the incidence of discoloration, shriveling, and tissue breakdown. Ratings were made using a 5-point scale, where score 1 = not marketable, 3 = acceptable (critical level of consumers' acceptance), and 5 = excellent appearance, comparable to the quality at harvest. Three experienced panels assessed severity of discoloration and marketability ratings. The severity and rating scores were determined for each replicate through the consensus of assessment.

Measurement of Respiration Rate

Respiration rates were measured by using a 2.0 L respirometer (Kim et al., 2014) immediately after storage and 5 d on the

shelf. All the samples were equilibrated to 20°C before putting into the closed system in which 40-50 g leaf samples were held for 4 h. The rates were calculated based on the changes in concentrations of CO₂. Gas concentrations were analyzed by using a gas chromatograph (model 600D, Young Lin Instrument Co., Ltd., Anyang, Korea) equipped with a Porapak Q packed column and a thermal conductivity detector.

Experimental Design and Data Analysis

Experiments were carried out in a completely randomized design with 4 replicates. Each replicate consisted of a 120±5 g leaf sample package. Data were analyzed by analysis of variance (ANOVA) and mean separation was determined by Duncan's multiple range test (DMRT) at the 5% level (SAS, 1990).

Results

Incidence of Discoloration and Marketability of Edible Aster and Korean Thistle

In edible aster, discoloration was not observed and marketability was maintained at the excellent level until 3-week storage (Table 2). After 4-week storage, blackening developed up to 30% in perforated MAP, 67.6% in 1% O₂ + 5% CO₂, and even reached 100% in 1% O₂ + 10% CO₂ treatment. The incidence of the symptom significantly increased on the shelf especially in MAP-treated edible aster. Marketability decreased below the acceptable level in all the treatments by the end of 4-week

Table 2. Incidence of discoloration and marketability ratings of edible aster leaves during 4-week storage and 5-day shelf life as influenced by CA treatment

Storage atmosphere ^e	Incidence of discoloration (%/ severity) ^z			Marketability rating (score 1 to 5) ^y		
	3 weeks	4 weeks	4 weeks + 5 days	3 weeks	4 weeks	4 weeks + 5 days
Control: MAP, perforated	0.0	30.0 c ^x (mild)	58.1 b (mild)	5.0	2.0 a	2.0 a
CA: O ₂ 1% + CO ₂ 5%	0.0	67.6 b (severe)	69.8 b (severe)	5.0	2.0 a	1.0 a
CA: O ₂ 1% + CO ₂ 10%	0.0	100.0 a (severe)	100.0 a (severe)	5.0	1.0 a	1.0 a

^z% incidence = No. of suffered leaves/No. of leaves investigated.

Severity: slight = negligible symptom, mild = critical point of consumption with visible spot discoloration, severe = substantial discolored area (not acceptable).

^yMarketability rating score 1 = not marketable, 3 = acceptable (critical level of consumers' acceptance), 5 = excellent.

^xMean separation within columns by Duncan's multiple range test at $p < 0.05$.

Table 3. Incidence of discoloration and marketability ratings of Korean thistle leaves during 4-week storage and 5-day shelf life as influenced by CA treatment

Storage atmosphere	Incidence of discoloration (%/ severity) ^z			Marketability rating (score 1 to 5) ^y		
	3 weeks	4 weeks	4 weeks + 5 days	3 weeks	4 weeks	4 weeks + 5 days
Control: MAP, perforated	16.3 b ^x	36.1 b (slight)	40.1 c (slight)	4.0 a	3.3 a	3.0 a
CA: O ₂ 1%+CO ₂ 5%	25.7 b	42.3 b (slight)	74.2 b (mild)	3.3 b	3.3 a	2.0 b
CA: O ₂ 1%+CO ₂ 10%	74.4 a	90.2 a (severe)	97.5 a (severe)	1.9 c	1.6 b	1.0 c

^z% incidence = No. of suffered leaves/No. of leaves investigated.

Severity: slight = negligible symptom, mild = critical point of consumption with visible spot discoloration, severe = substantial discolored area (not acceptable).

^yMarketability rating score 1 = not marketable, 3 = acceptable (critical level of consumers' acceptance), 5 = excellent.

^xMean separation within columns by Duncan's multiple range test at $p < 0.05$.

storage (Table 2).

Discoloration occurred earlier during storage of Korean thistle, starting at 3 weeks of storage and eventually reached 36% in perforated MAP, 42% in 1% O₂ + 5% CO₂ CA and 90% in 1% O₂ + 10% CO₂ CA treatment after 4 weeks of storage (Table 3). The symptom intensity during the 5-d shelf life was slight in the perforated MAP but substantial in CA treatments, showing significant differences among treatments.

The severity of the discoloration of Korean thistle leaves was slight to mild until the incidence increased approximately to 40% (Table 3). Thus, leaf quality was acceptable and marketability rating scores over 3.0 were shown after 4-week storage under MAP and 1% O₂ + 5% CO₂ CA. The marketability after 5-d shelf life, however, was only acceptable in MAP-treated leaves. The incidence of the injury increased over 70% in the treatment of 1% O₂ + 10% CO₂ CA. The symptom was mild immediately after storage, while severe after the following 5-d shelf life.

Incidence of Discoloration and Marketability of Myeongyi and East Asian Hogweed Leaves

Myeongyi leaves did not show any blackening discoloration until 5-week storage plus 5-d shelf life in all the treatments. Instead, yellowing discoloration occurred, especially in the perforated MAP treatment (Table 4). The incidence of yellowing symptom after 5-week perforated MAP storage and the following 5 d on the shelf were 6.7% and 25.6%, respectively with statistical significance at $p < 0.05$. In comparison, the incidence was slight in CA treatments showing less than 5% in 1% O₂ + 5% CO₂ and 1% level in 1% O₂ + 10% CO₂ treatment even on the shelf. As a result, marketability on the shelf declined below the acceptable level in MAP-stored leaves after 5-week storage, whereas CA-stored leaves maintained excellent quality even

Table 4. Incidence of discoloration and marketability ratings of myeongyi leaves during 5-week storage and 5-day shelf life as influenced by CA storage

Storage atmosphere	Incidence of yellowing (%) ^z			Marketability rating (score 1 to 5) ^y		
	3 weeks	5 weeks	5 weeks + 5 days	3 weeks	5 weeks	5 weeks + 5 days
Control: MAP, perforated	0.0 a ^x	6.7 a	25.6 a	5.0 a	3.9 a	2.5 b
CA: O ₂ 1% + CO ₂ 5%	0.0 a	0.0 b	4.5 b	5.0 a	4.3 a	3.9 a
CA: O ₂ 1% + CO ₂ 10%	0.0 a	1.1 b	1.0 b	5.0 a	4.4 a	4.0 a

^z% incidence = No. of suffered leaves/No. of leaves investigated.

^yMarketability rating score 1 = not marketable, 3 = acceptable (critical level of consumers' acceptance), 5 = excellent.

^xMean separation within columns by Duncan's multiple range test at $p < 0.05$.

Table 5. Incidence of discoloration and marketability ratings of East Asian hogweed leaves during 4-week storage and 5-day shelf life as influenced by storage method

Storage atmosphere	Incidence of discoloration (%/ severity) ^z			Marketability rating (score 1 to 5) ^y		
	3 weeks	4 weeks	4 weeks + 5 days	3 weeks	4 weeks	4 weeks + 5 days
Control: MAP, perforated	26.8 a ^x	45.5 ab (slight)	53.8 b (slight)	5.0 a	4.0 a	3.5 a
CA: O ₂ 1% + CO ₂ 5%	30.8 a	30.7 b (slight)	54.8 b (slight)	5.0 a	4.3 a	3.2 b
CA: O ₂ 1% + CO ₂ 10%	23.6 a	58.4 a (slight)	63.8 b (slight)	5.0 a	3.6 b	3.2 b

^z% incidence = No. of suffered leaves/No. of leaves investigated.

Severity: slight = negligible symptom, mild = critical point of consumption with visible spot discoloration, severe = substantial discolored area (not acceptable).

^yMarketability rating score 1 = not marketable, 3 = acceptable (critical level of consumers' acceptance), 5 = excellent.

^xMean separation within columns by Duncan's multiple range test at $p < 0.05$.

after the 5-week storage period and 5-d shelf life.

The typical discoloration symptom on East Asian hogweed leaves was the development of black spots without yellowing. Black spots appeared after 3-week storage in all the treatments (Table 5). Unlike edible aster and Korean thistle leaves, however, there was not a significant increase in the incidence of injury in East Asian hogweed leaves by CA treatments. Another difference was the severity of the symptom. Despite the incidence of black spots being over 50% during the 5-d shelf life, marketability was evaluated above the acceptable level with a score higher than 3.0 since the symptom was very slight and negligible.

Changes in Respiration Rates after Storage according to the Susceptibility to CA-related Disorder

In edible aster, respiration rates were higher in MAP-stored leaves than those in CA-stored leaves immediately after storage (Table 6). In contrast, after the 5-d shelf life, the rates were higher in CA-stored leaves resulting in a remarkable increase on the shelf compared to relatively less increases in MAP-stored leaves.

In Korean thistle leaves, respiration rates were higher in 1% O₂ + 5% CO₂ CA-stored leaves immediately after 4 weeks of storage (Table 6). The increase on the shelf was also noticeable in the same treatment.

An increasing pattern of respiration rates during storage and on the shelf in myeongyi leaves was similar to that in edible aster (Table 7). However, the increases in CA-stored leaves were not as distinguishable as in CA-stored edible aster leaves.

Changes in respiration rates of East Asian hogweed leaves were completely different from the other three commodities. Respiration continuously reduced during storage and the following shelf life (Table 7).

Table 6. Respiration rates after 4-week storage and 5-day shelf life as influenced by CA treatment in the wild, leafy vegetables susceptible to CA-disorder

Storage atmosphere	Respiration rate, 20°C (CO ₂ mL·kg ⁻¹ ·h ⁻¹)					
	Edible aster			Korean thistle		
	At harvest	After storage + shelf life		At harvest	After storage + shelf life	
	4 weeks	4 weeks + 5 days		4 weeks	4 weeks + 5 days	
Control: MAP, perforated		106.7 a ^z	185.0 b		90.4 a	106.8 b
CA: O ₂ 1% + CO ₂ 5%	63.7 ± 4.3	81.2 b	309.1 a	134.3 ± 9.2	100.1 b	104.7 b
CA: O ₂ 1% + CO ₂ 10%		12.9 c	312.1 a		135.1 a	236.5 a

^zMean separation within columns by Duncan's multiple range test at $p < 0.05$.

Table 7. Respiration rates after 4- to 5-week storage and 5-day shelf life as influenced by CA treatment in the wild, leafy vegetables resistant to CA-disorder

Storage atmosphere	Respiration rate, 20°C (CO ₂ mL·kg ⁻¹ ·h ⁻¹)					
	Myeongyi			East Asian hogweed		
	At harvest	After storage + shelf life		At harvest	After storage + shelf life	
	5 weeks	5 weeks + 5 days		4 weeks	4 weeks + 5 days	
Control: MAP, perforated		129.3 a ^z	193.1 ab		46.4 a	37.0 a
CA: O ₂ 1% + CO ₂ 5%	68.4 ± 2.1	115.1 a	181.5 b	79.0 ± 2.3	54.3 a	35.6 a
CA: O ₂ 1% + CO ₂ 10%		123.4 a	226.4 a		51.6 a	39.2 a

^zMean separation within columns by Duncan's multiple range test at $p < 0.05$.

Discussion

In edible aster and Korean thistle leaves, blackening discoloration was observed even inside perforated MAP suggesting that the disorder may occur under air condition when the storage period exceeds 3 weeks. Remarkable increases in the incidence of the blackening symptom, however, indicated that CA with high CO₂ concentrations might aggravate the disorder. CA may not be a direct inducing factor of the disorder, but seems to be an aggravating factor. The idea that high CO₂ rather than low O₂ primarily influences the incidence of the disorder could be supported by a previous MAP study. Oh et al. (1997) observed faster quality deterioration inside MAP storage under 15% O₂ + 5% CO₂ condition compared to air- or nitrogen-package conditions. Supplementary studies of specified CA regimens are needed to elucidate the critical level that induces or aggravates the discoloration in edible aster and Korean thistle leaves. Although the symptom was very similar, discoloration of East Asia hogweed leaves does not seem to be directly related to the CA environment since the incidence of the disorder was not significantly different between perforated MAP (air) and CA treatments.

From a biochemical point of view, the blackening symptom might result from an enzymatic browning reaction. Most wild vegetables contain high phenolic compounds (Jeon et al., 2012; Lee et al., 2005; Lee et al., 2014) and postharvest oxidation of the compound may occur as the leaf tissues undergo senescence. In wild leafy vegetables that are susceptible to CA-related disorders, the tissue breakdown seemed to be aggravated in low oxygen and high carbon dioxide atmospheres. The biochemical mechanism of CA- or MA-related disorder was proposed in persimmons (Choi, 1998). Under inadequate atmospheric conditions, CA or MA may disturb normal energy metabolism and damages cellular integrity, thus leading to cellular compartments breaking down between organelles and accelerating enzymatic browning of phenolic compounds. In contrast, the blackening discoloration observed in perforated MAP-treated leaves under air condition might be due to cell senescence along with the degradation of chlorophylls and abnormal cellular metabolism (Toivonen and Brummell, 2008).

The changing pattern of respiration and its relationship with the incidence of discoloration symptoms were different according to commodities (Tables 6 and 7). Higher respiration rates in edible aster and Korean thistle leaves after CA storage or on the shelf indicate changes in energy metabolism. The need of energy metabolism seems to be a temporary event to maintain homeostasis, as was proposed in CA-stored apples during the induction of flesh browning disorder (Kim and Park, 2008) and in peppers during the expression of chilling injury symptoms (Lim, 2008; Lim and Cho, 2009). Relatively small increases in respiration rates of Korean thistle leaves compared to those of edible aster leaves could be explained by slight to mild discoloration symptoms, despite the high incidence of the disorder (Table 3). Compared to high respiration in the two CA-disorder susceptible commodities, moderate increases or decreases in respiration rates in non-susceptible myeongyi and East Asian hogweed leaves may be a consequence of normal senescence. In order to clarify the relationship between the incidence of storage disorders and energy metabolism, more detailed investigations should supplement the time course changes in respiration.

In edible aster, Korean thistle, and East Asian hogweed leaves, the beneficial effects of virtual MA or CA seemed to be minimal. In the present study, when a 5-d shelf life at 7°C was counted as a prerequisite, the storage potential of perforated MA packaged fresh leaves at 0°C was approximately 3 weeks in edible aster and 4 weeks in Korean thistle as well as East Asian hogweed. A similar estimation was reported in the study of MA-stored edible aster at 1°C, where appearance quality was maintained for 16 d under air condition (Oh et al., 1997). As for East Asian hogweed, Kim (2012) reported beneficial effects of heat-sealed PP film packaging in which 1% O₂ + > 7% CO₂ atmosphere was established. In contrast, our study showed no significant benefit from 1% O₂ + 5% CO₂ and 1% O₂ + 10% CO₂ CA storage. Unlike the three commodities that

showed blackening symptoms under perforated MA (air) and CA conditions, the storage potential of myeongyi leaves was extended by CA. Storage potential of myeongyi leaves was shorter than 5 weeks in perforated MAP storage, while the potential was longer than 5 weeks both under 1% O₂ + 5% CO₂ and 1% O₂ + 10% CO₂ CA conditions. Short-term high CO₂ treatment may be helpful in maintaining quality as was observed in cherry tomatoes (Sangwanangkul et al., 2017).

The present study indicates that long-term storage of most wild leafy vegetables is not an easy approach, even when using CA or MAP technology. Alternatively, wild, leafy vegetables have been stored by freezing or undergoing a freeze-drying process (You et al., 2009). From this point of view, storage technology should focus on estimating the precise storage potential in order to supply high quality commodities rather than extending the storage period for fresh, wild, leafy vegetables. Even for a specific commodity such as myeongyi that can be stored for longer periods under the CA condition, MAP is a more practical approach since a CA facility is not economically viable for small-sized storage. In most cases, the benefits of MAP storage occur from simple barrier effects on water vapor movement. As quality maintenance is dependent on actual changes in O₂ and CO₂ concentrations, virtual MAP technology could be achieved by using a micro-perforated film that controls the permeability of O₂ and CO₂.

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