

## DIFFERENTIAL RESPONSES OF INTACT AND MINIMALLY PROCESSED LETTUCE TO HIGH CARBON DIOXIDE ATMOSPHERES

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### ABSTRACT

Intact heads and minimally processed midrib tissue and green tissue of Vanguard lettuce (*Lactuca sativa*, L.) were kept in air or in air + 5%, 10%, or 20% CO<sub>2</sub> for 10 or 20 days at 2.5C and then transferred to air at 20C for 12 hours. All the CO<sub>2</sub> treatments caused severe injury in intact heads, with "heart leaf injury" as the major symptom. For minimally processed lettuce, senescent browning was severe in air-stored tissue and elevated CO<sub>2</sub> atmospheres prevented or reduced this disorder. No brown stain was observed in processed green leaf tissue treated with 5% or 10% CO<sub>2</sub>. However, brown stain occurred in the processed green tissue exposed to 20% CO<sub>2</sub> and in intact heads and processed midrib tissue exposed to 5%, 10%, or 20% CO<sub>2</sub>, with the processed midrib tissue affected the most. Off-flavor developed in the intact heads exposed to 20% CO<sub>2</sub>. The physiological bases that caused these differential responses are discussed.

### INTRODUCTION

Exposure to CO<sub>2</sub> concentrations above 2% may cause a disorder named "brown stain" (BS) in lettuce. This disorder is characterized by the occurrence of superficial oval to irregular necrotic areas with margins that are often darker than the slightly sunken centers of the lesions. Another disorder of lettuce caused by CO<sub>2</sub> is "heart leaf injury" (HLI), which appears as a reddish brown discoloration in the leaf margins or in the entire leaves of the center of intact head (Kader and Lipton, 1990).

The popularity of salad bars and fast-food outlets has increased the market for chopped and shredded lettuce. Minimally processed lettuce, unlike whole heads, is less sensitive to CO<sub>2</sub> injury (McDonald et al., 1990). When cells are ruptured by cutting, wound-induced biochemical reactions are initiated. This may decrease visual quality and shorten storage life. Senescent browning (SB) is a common feature of product aging, which can be enhanced by wounding during minimal processing. Modified atmosphere packaging is currently used to avoid wilting, discoloration, and contamination by microorganisms (Ballantyne et al., 1988).

Both SB and BS can be caused by oxidation of phenolic compounds in the presence of polyphenol oxidase. In this paper, we report on the differential responses of intact heads and minimally processed lettuce to CO<sub>2</sub> injury and SB and discuss the physiological bases for these differential responses.

### MATERIALS AND METHODS

**Materials and treatments** - Heads of Vanguard lettuce were obtained from a commercial shipper in San Joaquin Valley, California. Wrapper leaves were discarded and intact heads and minimally processed leaf midrib tissue and leaf green tissue were used separately. For each treatment, 8 to 9 heads were used.

To obtain the minimally processed tissues, leaves were torn off and then excised. For midrib tissue, segments were excised about 2 cm wide and extending 4 cm up the leaf, starting 1 cm from the base of each leaf, as previously described by Ke and Saltveit (1989). Green tissue was obtained by excising segments from the green part of leaf blade. The processed tissues were then rinsed with 50 ul-liter<sup>-1</sup> chlorine solution and centrifuged in a salad spinner to remove surface water. About 200 g green tissue and midrib tissue were placed in 2-liter and 1-liter glass jars, respectively. The jars were covered with 4 layers of cheesecloth and then placed together with the intact heads in a 25-liter container at 2.5C. A continuous flow of air or air + 5%, 10%, or 20% CO<sub>2</sub> gas mixture was passed through the containers. The whole heads and processed lettuce tissues were kept in the stated conditions for 10 or 20 days and then transferred to air at 20C for 12 hr.

**Estimation of disorders** - Severity of disorders was estimated using two parameters: extent of affected area and intensity of discoloration. Extent for BS and SB was rated using a scale of 0 to 9 according to the percentage of surface area affected where 0 = no injury or browning and 9 = more than 90% of the surface affected. Intact heads were dissected and incidence of HLI was estimated according to the percentage of heads affected. Discoloration intensity was divided into three categories: low = colorless damage or yellow lesions, moderate = well defined brown lesions, and high = dark brown lesions.

## RESULTS AND DISCUSSION

**Heart leaf injury** - The major symptom of CO<sub>2</sub> injury in intact heads was HLI, which was present after exposure to 5%, 10%, or 20% CO<sub>2</sub> for 20 days at 2.5C (Fig. 1). An additional 12 hr in air at 20C following CO<sub>2</sub> treatment increased the intensity of the injury. While 100% of the heads were injured in 10% or 20% CO<sub>2</sub> treatment, only about 50% of the heads were injured in 5% CO<sub>2</sub>-treated lettuce. An informal tasting indicated off-flavor in the heads exposed to 20% CO<sub>2</sub>. The high incidence of HLI and development of off-flavor would make the CO<sub>2</sub>-treated intact heads objectionable to consumers.

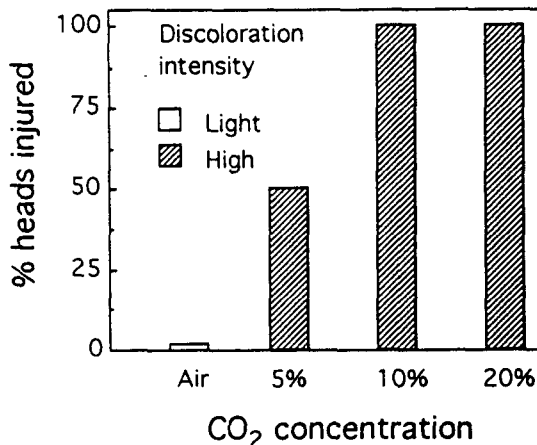


Fig. 1. Development of heart leaf injury in intact heads of Vanguard lettuce kept in air or in air + 5, 10 or 20% CO<sub>2</sub> at 2.5C for 20 days followed by transfer to air at 20C for 12 hr.

**Brown stain** - BS was observed in all the CO<sub>2</sub>-treated intact heads and minimally processed midribs after 20 days at 2.5C followed by transfer to air at 20C for 12 hr (Table 1). However, intact heads had less BS than processed midribs. Severity of BS increased as duration of CO<sub>2</sub> treatment was extended and the subsequent 12 hour exposure to air at 20C enhanced symptom development. No visual BS symptom was observed in cut midribs exposed to 20% CO<sub>2</sub> at 2.5C for 20 days, but colorless lesions appeared after additional 12 hr in air at 20C. After exposure to 5% or 10% CO<sub>2</sub> at 2.5C for 20 days, BS appeared and/or increased within 6 to 7 hr following the transfer of the midribs to air at 20C. For processed green tissue, no BS was observed if exposed to 5% or 10% CO<sub>2</sub>. However, BS occurred when the cut green tissue was exposed to 20% CO<sub>2</sub>.

Table 1. Development of brown stain and senescent browning in intact heads and minimally processed midrib and green tissues of Vanguard lettuce kept in air or in air + 5%, 10% or 20% CO<sub>2</sub> at 2.5C for 10 or 20 days followed by transfer to air at 20C for 12 hr. Numbers represent rating score for extent of affected area using a scale of 0 to 9 (0 = no injury or browning and 9 = more than 90% of the surface affected) and letters represent intensity of discoloration (L = low, M = moderate, and H = high).

Treatment	Brown stain			Senescent browning		
	10 d	20 d	20 d + 12 hr	10 d	20 d	20 d + 12 hr
<u>Intact head</u>						
Air	0	0	0	0	1 L	1 L
5% CO <sub>2</sub>	0	1 L	1 M	0	0	0
10% CO <sub>2</sub>	1 L	3 M	3 M	0	0	0
20% CO <sub>2</sub>	0	0	2 L	0	0	0
<u>Midrib tissue</u>						
Air	0	0	0	3 M	4 M	9 H
5% CO <sub>2</sub>	0	0	2 M	1 L	4 M	5 M
10% CO <sub>2</sub>	1 L	2 M	4 M	1 L	2 M	7 M
20% CO <sub>2</sub>	0	0	8 L	0	0	0
<u>Green tissue</u>						
Air	0	0	0	2 L	2 L	4 M
5% CO <sub>2</sub>	0	0	0	1 L	1 L	1 L
10% CO <sub>2</sub>	0	0	0	0	0	1 L
20% CO <sub>2</sub>	0	1 H	3 H	3 L	1 H	3 H

**Senescent browning** - While almost no SB was observed in intact heads, processed midribs kept in air showed the highest incidence and severity of SB (Table 1). Extent and intensity of the symptoms increased with time and became more severe after 12 hr in air at 20C. High CO<sub>2</sub> atmospheres reduced SB although browning was significant in 5% or 10% CO<sub>2</sub>-treated midribs after 20 days. When processed midribs were kept under 20% CO<sub>2</sub>, no SB was observed. For processed green tissue, some browning was observed when held under 20% CO<sub>2</sub> and, it was difficult to tell if this browning was caused by BS or

by SB since the two symptoms were mixed. For the 5% or 10% CO<sub>2</sub>-treated green tissue, SB was very slight.

From the above observations, it can be seen that intact heads had a higher susceptibility to CO<sub>2</sub> injury than cut lettuce tissues. The severe symptoms of HLI and/or off-flavor development would make the CO<sub>2</sub>-treated intact heads unsalable. Therefore, intact heads should be stored in air to prevent CO<sub>2</sub> injury. For minimally processed lettuce, SB was a major problem. Since CO<sub>2</sub> generally inhibited SB, it should be taken into account in selecting the appropriate CO<sub>2</sub> concentration for the storage of minimally processed lettuce. The 20% CO<sub>2</sub> treatment prevented SB and delayed appearance of browning symptom of the BS disorder when the processed midribs were held in CO<sub>2</sub> and after transfer to air at 20C for up to 12 hr. Therefore, processed midribs can be kept in 20% CO<sub>2</sub> at 2.5C for 20 days with better visual quality than those held in air. The processed midribs exposed to 5% or 10% CO<sub>2</sub> also had better overall visual quality than those kept in air. Processed green tissue, however, had the best quality when treated with 5% or 10% CO<sub>2</sub>. The 20% CO<sub>2</sub> treatment caused browning in the processed green tissue after transfer to air. The minimally processed lettuce should be used immediately following removal from the CO<sub>2</sub> treatment to avoid enhanced browning during subsequent exposure to air.

The results from this study indicate differential responses to elevated CO<sub>2</sub> atmospheres between intact heads and minimally processed lettuce tissues, as shown by their varied sensitivities to CO<sub>2</sub> injury and SB. These differential responses are physically initiated by cutting lettuce heads into pieces. This cutting, on one hand, reduces lettuce resistance to gas diffusion and, on the other hand, causes wounding responses. HLI and off-flavor development in intact heads kept under 20% CO<sub>2</sub> may be related to their higher resistance to gas diffusion and enhanced concentrations of anaerobic volatiles. The wounding responses, especially the wound-induced phenolic metabolism, in minimally processed lettuce tissues may accelerate SB and make the tissues become more sensitive to BS. Elevated CO<sub>2</sub> atmospheres may, on one hand, inhibit SB and, on the other hand, induce BS. The criteria in selecting the optimum CO<sub>2</sub> concentration, temperature, and treatment duration for postharvest handling of minimally processed lettuce are to maximize the beneficial effect of CO<sub>2</sub> on inhibiting SB and to minimize its detrimental effect due to BS. The effects of elevated CO<sub>2</sub> atmospheres on phenolic metabolism and ethanolic fermentation will be presented in a separate paper elsewhere.

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