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Phenolic metabolism and ethanolic fermentation of intact and cut lettuce exposed to CO_2-enriched atmospheres *

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ABSTRACT

Intact heads and cut leaf midrib tissue of crisphead lettuce (*Lactuca sativa*, L., cv. 'Salinas' and 'Vanguard') were kept in air or air + 5%, 10%, or 20% CO_2 for 10 or 20 days at 2.5°C and then transferred to air at 20°C for 12 hrs to study CO_2 effects on phenolic metabolism and fermentative products. Exposure of cut midribs to 20% CO_2 increased extractable activity of phenylalanine ammonia-lyase (PAL) when assayed at its optimum pH (8.5). However, under the 20% CO_2 treatment PAL activity was reduced as a result of a decrease in cytoplasmic pH. Total phenolic content of cut midribs was reduced under 20% CO_2-enriched atmosphere probably due to this decrease in PAL activity. The phenolic content rapidly increased after the tissue was transferred from 20% CO_2 at 2.5°C to air at 20°C when cytoplasmic pH returned to its normal value and the PAL activity was restored. Such effects of CO_2 on PAL and phenolics were of lower magnitude in intact heads, probably due to their lower sensitivity to phenolic metabolism. Off-flavor developed in the intact heads exposed to 20% CO_2, which was associated with increased concentrations of ethanol and acetaldehyde. When exposed to 20% CO_2, levels of ethanol and acetaldehyde in cut midribs were only about half of those in intact heads.

Key words: Lettuce; *Lactuca sativa*; Controlled atmosphere; Anaerobic volatile; Phenolic metabolism; Physiological disorder

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INTRODUCTION

Controlled atmospheres with reduced O_2 and/or elevated CO_2 concentrations are used to extend the storage life of some fruits and vegetables. In lettuce, however, elevated CO_2 atmospheres may cause a disorder called "brown stain"

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(BS). This disorder has been described as superficial oval to irregular necrotic areas with margins that are often darker than the slightly sunken centers of the lesions. Symptoms of BS usually occur in midribs and in the basal portion of the leaf when lettuce is exposed to more than 2% CO_2 atmospheres (Stewart and Uota, 1971; Brecht et al., 1973b,c; Lipton, 1977). Development of BS is highly dependent on storage temperature, with the highest incidence at 0°C and being negligible at 10°C (Stewart and Uota, 1971; Brecht et al., 1973a,b; Lipton, 1987). BS is not always evident when lettuce is under CO_2-enriched atmospheres and becomes more obvious after the tissue is transferred to air. Combination of high CO_2 with low O_2 increases BS severity (Brecht et al., 1973c; Kader et al., 1973). Another disorder of lettuce caused by CO_2 is "heart leaf injury" (HLI), which appears as a reddish brown discoloration in leaf margins or in the entire leaves of the center of intact head (Lipton, 1987; Kader and Lipton, 1990). Susceptibility to CO_2 injury varies among types of lettuce. Aharoni and Ben-Yehoshua (1973) observed that quality of 'romaine' lettuce was maintained better when held in 2% to 10% O_2 combined with 5% CO_2 than in air, and no injury was found at CO_2 levels up to 12% at 1°C for about 2 weeks. Lipton (1987) showed that sensitivity of 'romaine' lettuce to CO_2 was not consistently enhanced when low O_2 was combined with 7.5% CO_2 and BS was induced at 0°C only. However 'butterhead' and 'crisphead' lettuce present a higher incidence of BS than that reported for 'romaine' lettuce (Stewart and Uota, 1971; Brecht et al., 1973b; Tartaru and Weichmann, 1974). These differences may be due to the greater resistance to gas diffusion in the head lettuce.

Minimally processed (cut) lettuce, unlike whole heads, is less sensitive to CO_2 injury (McDonald et al., 1990). When cells are ruptured by cutting during minimal processing, wound-induced biochemical reactions are initiated that shorten storage life. To minimize wilting, discoloration, and microbial contamination, modified atmospheres, either generated by respiration of minimally processed lettuce or attained by gas flushing, are currently used (Bolin and Huxsoll, 1991; King et al, 1991). Ballantyne et al. (1988) found that polymer films containing shredded lettuce in an equilibrated atmosphere of 1% to 3% O_2 and 5% to 6% CO_2 maintained a better quality during 14 days at 5°C than that held in air. However, discoloration and fermentation odors have been reported when CO_2 levels were higher than 20% in chopped lettuce packaged in plastic bags (McDonald et al., 1990).

Senescent browning (SB), a common feature of product deterioration, can be prevented or reduced by using CO_2-enriched atmospheres. Both SB and BS can be caused by brown pigments generated by the oxidation of phenolic compounds in the presence of the enzyme polyphenol oxidase. These phenolic compounds come from the hydroxylation of cinnamic acid which is formed by the deamination of phenylalanine catalyzed by PAL. Siriphanich and Kader (1985) and Ke and Saltveit (1989a) observed that under high CO_2 atmospheres PAL activity was induced whereas phenolic production and browning were inhibited until lettuce tissue was transferred from CO_2 to air. After the transfer, tissue browning occurred, which was associated with a rapid increase in soluble phenolic content.

Elevated CO_2 concentration may decrease intracellular pH in plant tissue (Bown, 1985). This may in turn change activities of many enzymes and distribution of auxin, gibberellins, and some organic acids. Siriphanich and Kader (1986) found that cytoplasmic pH of lettuce tissue held in air was 6.7 whereas in tissue exposed to 20% CO_2 the pH dropped to 6.3. When the 20% CO_2-treated tissue was transferred to air, cytoplasmic pH returned to its original value. PAL is located in cytoplasm (Shaw et al., 1990) and the optimum pH for PAL activity in lettuce tissue is 8.5 (Siriphanich and Kader, 1985). Therefore, reduction in PAL activity may occur if cytoplasmic pH moves from its optimum.

Reduced O_2 and/or elevated CO_2 atmospheres may induce fermentative metabolism in fruits and vegetables. Ethanol and acetaldehyde are usually the major products of plant fermentation and their accumulation correlates with off-flavor development (Ke et al., 1991a,b).

In this paper, we report on the differential responses of intact heads and cut lettuce tissues to elevated CO_2 atmospheres on PAL, soluble phenolic content, and fermentative products and discuss the relationships between these responses and incidence and severity of HLI, BS, SB, and off-flavor.

MATERIALS AND METHODS

Materials and treatments

Heads of 'Salinas' and 'Vanguard' cultivars of crisphead lettuce were obtained from a commercial shipper in Salinas, California, and transported in an air-conditioned car to the University of California at Davis. After arrival the heads were stored at 0°C for less than 48 hrs until experiments were initiated. Lettuce heads were sorted for uniform overall visual appearance. Wrapper leaves were discarded and intact heads and cut leaf midrib tissue were used separately. For each treatment, 8 to 9 heads were used. To obtain the cut 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 (1989a). The cut tissues were then rinsed with water containing 50 $\mu l \cdot l^{-1}$ NaOCl (chlorine) solution and centrifuged in a salad spinner to remove surface water. About 200 g of midrib tissue were placed in 1-l glass jars. The jars were covered with 4 layers of cheesecloth and then placed together with the intact heads at 2.5°C in a 25-l container ventilated with a continuous flow of air or air + 5%, 10%, or 20% CO_2. Light was excluded by covering the containers with opaque paper. The CO_2 concentrations were monitored periodically with an infrared gas analyzer. The whole heads and cut lettuce tissues were kept in the stated conditions for 10 or 20 days and then transferred to air at 20°C for 12 h.

After each period, symptoms of HLI, BS, and SB were evaluated. Midribs from intact heads were excised. Cut edges (about 2 mm from each side) of minimally processed tissues were removed and discarded. The midribs obtained from intact heads and processed tissues were used immediately for analyses of PAL activity, phenolic content, and concentrations of anaerobic volatiles. Three replicates per treatment were used in all the analyses.

Assay of PAL activity

PAL activity was assayed as previously described by Ke and Saltveit (1986). To measure the maximum extractable activity of PAL, 4 g fresh midrib tissue was homogenized at 0°C in 16 ml of 50 mM borate buffer (pH 8.5) containing 5 mM 2-mercaptoethanol and 0.4 g insoluble polyvinylpyrrolidone. In a similar way, 4 g midrib tissue was homogenized at 0°C in 16 ml of 50 mM NaH_2PO_4 buffer (containing 5 mM 2-mercaptoethanol and 0.4 g insoluble polyvinylpyrrolidone) at pH 6.7 and pH 6.3 to mimic the cytoplasmic conditions of the tissue held under air and 20% CO_2, respectively. The increase in absorbance at 290 nm over 1 h in sample with 0.55 ml of 100 mM L-phenylalanine added minus that with 0.55 ml water added was used to determine PAL activity. One unit of enzyme activity was defined as the amount of PAL that produced 1 μmol of cinnamic acid in 1 h under the specified conditions.

Determination of phenolic content

The procedure for extraction and measurement of total soluble phenolic content from midrib tissue was that described by Hyodo et al. (1978) using *p*-coumaric acid as the standard.

Determination of fermentative volatiles

Ten g fresh midrib tissue was homogenized at 0°C with an Ultra-Turrox Tissue Homogenizer in 15 ml water. The homogenate was filtered with 4 layers of cheesecloth and frozen until analyzed. Five ml sample was placed in a 10-ml screw-cap test tube and covered with a plastic cap. After 1-h incubation at 60°C, a 1-ml headspace gas sample was taken to measure concentrations of acetaldehyde and ethanol by a HP5890A gas chromatograph (Hewlett Packard, Palo Alto, CA) with a flame ionization detector (at 250°C) and a glass column (2 mm × 1.8 m) containing 5% Carbowax on 60/80 Carbopack (at 85°C).

RESULTS AND DISCUSSION

As reported earlier (Mateos et al., 1993), HLI occurred in intact heads of both 'Vanguard' and 'Salinas' cutivars of crisphead lettuce when exposed to 5%, 10%, or 20% CO_2. No HLI was observed in cut lettuce tissues. On the other hand, there was more BS development in the cut tissues exposed to CO_2. The elevated CO_2 atmospheres inhibited SB and maintained better visual quality in the minimally processed lettuce.

The effects of elevated CO_2 atmospheres on extractable PAL activity (Table 1) and phenolic content (Fig. 1) were more pronounced with cut midribs than with intact heads of lettuce. For intact heads, only the 20% CO_2 treatment for 20 days significantly increased extractable PAL activity. For cut midribs, however, 5%, 10%, and 20% CO_2 all resulted in higher extractable PAL activity and the effect increased with CO_2 concentration (Table 1). These differential responses were probably due to the wounding effects on the cut midrib tissue since wounding

TABLE 1

Effect of exposure to air or air + 5%, 10% or 20% CO_2 at 2.5°C for 20 days on extractable activity of phenylalanine ammonia-lyase (PAL), assayed at pH 8.5, in intact heads and cut 'Vanguard' lettuce

Treatment	PAL activity ($\mu mol \cdot g^{-1} \cdot h^{-1}$)	
	Intact head	Cut lettuce
Air	0.045	0.035
5% CO_2	0.049	0.085
10% CO_2	0.056	0.102
20% CO_2	0.083	0.158
LSD at $P = 0.01$	0.037	

could induce phenolic metabolism and make lettuce tissue become more sensitive to environmental stress (Ke and Saltveit, 1989b).

Although exposure to 20% CO_2 increased the extractable activity of PAL in cut midribs when the enzyme was extracted and assayed at pH 8.5 (Fig. 1D), phenolic

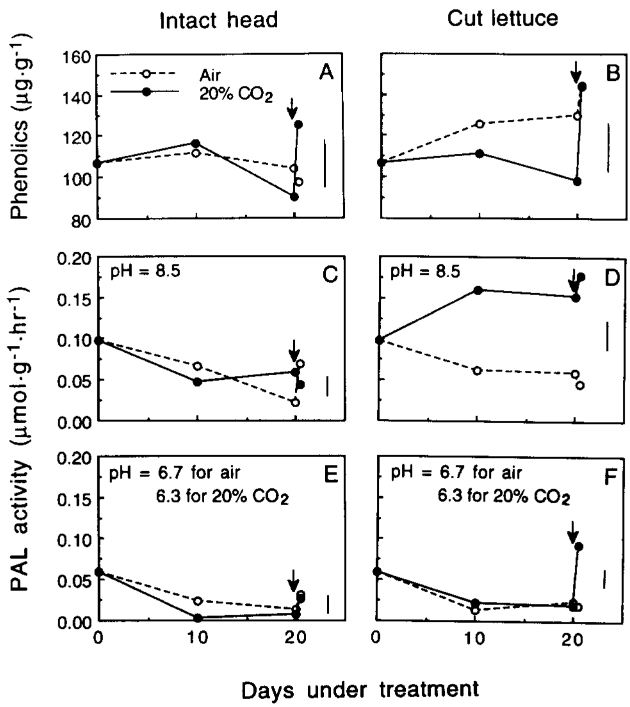

Fig. 1. Effects of exposure to air or air + 20% CO_2 at 2.5°C for 10 or 20 days followed by transfer (indicated by arrow) to air at 20°C for 12 h on total soluble phenolic content (A and B) and phenylalanine ammonia-lyase (PAL) activity (C, D, E, and F) of intact heads and cut 'Salinas' lettuce. PAL was extracted and assayed at its optimum pH 8.5 (C and D) and at mimicked cytoplasmic pH of 6.7 and 6.3 (E and F) for air control tissue and the tissue exposed to 20% CO_2, respectively. Vertical bars represent LSD at $P = 0.01$.

content of the midribs kept under CO_2 was actually lower than that in air control tissue (Fig. 1B). The increase in phenolic content in cut midribs exposed to air (Fig. 1B) might have been caused by wounding (Ke and Saltveit, 1989b) and it was associated with the development of SB symptom. Exposure to 20% CO_2 prevented such an increase in phenolic content (Fig. 1B) and probably also inhibited polyphenol oxidase and, therefore, reduced SB in the cut midrib tissue.

Comparison of data shown in Fig. 1B and 1D indicated that phenolic content and extractable PAL activity did not correlate when the cut midribs were kept under 20% CO_2. Although more PAL proteins might have been synthesized during the 20% CO_2 treatment as suggested by an increase in its extractable activity measured at pH 8.5, this enzyme might not be functioning to its full potential under the elevated CO_2 atmosphere. PAL is located in cytoplasm (Shaw et al., 1990) and the optimum pH for lettuce PAL is 8.5 (Siriphanich and Kader, 1985). Exposure to 20% CO_2 reduced cytoplasmic pH from 6.7 to 6.3 (Siriphanich and Kader, 1986). Therefore, the extractable PAL activity assayed at pH 8.5 may not reflect the real in vivo PAL activity in lettuce cytoplasm. When we mimicked the in vivo cytoplasmic pH changes and measured PAL at pH 6.7 for air control tissue and at pH 6.3 for the tissue exposed to 20% CO_2, there was no significant difference in PAL activity between the two treatments (Fig. 1E and F). These results suggested that although PAL was induced under 20% CO_2, this enzyme had a decreased activity through a lowering of the cytoplasmic pH due to the elevated CO_2 concentration. When lettuce tissue was transferred from 20% CO_2 to air, however, cytoplasmic pH returned to its normal value of 6.7 (Siriphanich and Kader, 1986) and the activity of PAL was restored. The induced PAL resulted in higher activity in vivo (Fig. 1F) and phenolic content rapidly increased (Fig. 1B). Due to the lower sensitivity of intact heads to phenolic metabolism, their responses to CO_2 as related to PAL and phenolic content were not as obvious as those of cut midribs.

For both intact heads and cut midribs, 5% or 10% CO_2 did not significantly influence acetaldehyde and ethanol concentrations (Table 2). The 20% CO_2 treatment greatly enhanced ethanol content and moderately increased acetaldehyde concentration in both intact heads and cut midribs. However, ethanol and acetaldehyde contents in cut midribs were only about half of those in intact heads.

TABLE 2

Effects of exposure to air or air+5%, 10% or 20% CO_2 at 2.5°C for 20 days on acetaldehyde and ethanol contents of intact heads and cut 'Vanguard' lettuce

Treatment	Acetaldehyde ($\mu l \cdot kg^{-1}$)		Ethanol ($\mu l \cdot kg^{-1}$)	
	Intact head	Cut lettuce	Intact head	Cut lettuce
Air	9.2	7.8	120	48
5% CO_2	12.5	8.2	142	52
10% CO_2	11.3	9.0	109	49
20% CO_2	17.3	11.1	604	304
LSD at $P=0.01$	2.5		50	

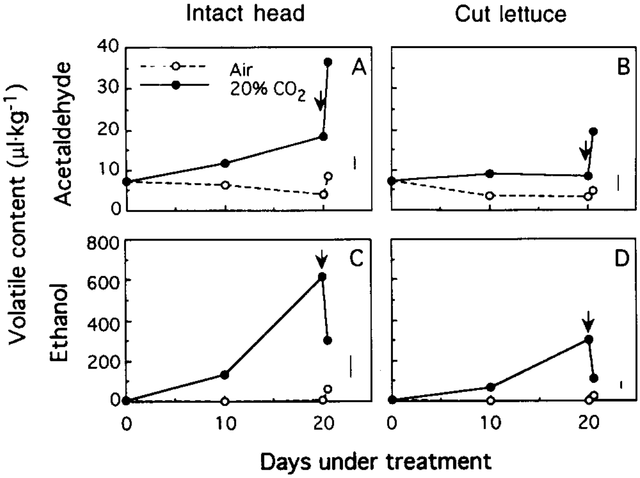

Fig. 2. Effects of exposure to air or air + 20% CO_2 at 2.5°C for 10 or 20 days followed by transfer (indicated by arrow) to air at 20°C for 12 h on acetaldehyde (A and B) and ethanol (C and D) concentrations in intact heads and cut 'Salinas' lettuce. Vertical bars represent LSD at $P = 0.01$.

Acetaldehyde and ethanol contents increased as duration of exposure to 20% CO_2 was extended (Fig. 2). After intact heads or cut midribs were transferred from 20% CO_2 at 2.5°C to air at 20°C for 12 h, acetaldehyde content increased while ethanol concentration decreased (Fig. 2), suggesting that some ethanol might have been converted into acetaldehyde during this period.

Ke et al. (1991a,b) observed a good correlation between accumulation of ethanol and acetaldehyde and development of off-flavor in several fruits stored in low O_2 and/or high CO_2. Intact heads have greater resistance to gas diffusion compared to cut lettuce, which might have resulted in a higher CO_2 and lower O_2 concentrations in the center of the heads and limited diffusion of anaerobic volatiles out of the commodity. This may explain the higher ethanol and acetaldehyde concentrations in the intact heads. An informal tasting indicated occurrence of off-flavor in the intact heads but not in the cut lettuce midribs exposed to 20% CO_2 at 2.5°C for 20 days.

The results from this study indicate differential responses to elevated CO_2 atmospheres between intact heads and minimally processed lettuce tissues, as indicated by differences in phenolic metabolism and accumulation of anaerobic volatiles. HLI and off-flavor development in intact heads kept under 20% CO_2 appear to be related to their higher resistance to gas diffusion and enhanced concentrations of anaerobic volatiles. Minimal processing (cutting) reduces lettuce resistance to gas diffusion and increases its tolerance to CO_2-enriched atmospheres. On the other hand, cutting causes wounding responses, especially the wound-induced phenolic metabolism accelerates SB incidence and increases sensitivity to BS. Intact lettuce heads should not be kept under elevated CO_2 atmospheres to avoid BS, HLI, and off-flavor. For minimally processed lettuce, an optimum CO_2 concentration, temperature, and storage duration can be selected to maximize the beneficial effect of CO_2 on inhibiting SB while minimize its detrimental effect due to BS and potential development of off-flavor.

REFERENCES

Aharoni, N. and Ben-Yehoshua, S., 1973. Delaying deterioration of romaine lettuce by vacuum cooling and modified atmosphere produced in polyethylene bags. J. Am. Soc. Hort. Sci., 98: 464–468.

Ballantyne, A., Stark, R., and Selman, J.D., 1988. Modified atmosphere packaging of shredded lettuce. Int. J. Food Sci. Technol., 23: 267–274.

Bolin, H.R. and Huxsoll, C.C., 1991. Effect of preparation procedures and storage parameters on quality retention of salad-cut lettuce. J. Food Sci., 56: 60–67.

Bown, A.W., 1985. CO_2 and intracellular pH. Plant Cell and Environ., 8: 459–465.

Brecht, P.E., Kader, A.A., and Morris, L.L., 1973a. Influence of the postharvest temperature on brown stain of lettuce. J. Am. Soc. Hort. Sci., 98: 399–402.

Brecht, P.E., Morris, L.L., Cheyney, C., and Janecke, D., 1973b. Brown stain susceptibility of selected lettuce cultivars under controlled atmospheres and temperatures. J. Am. Soc. Hort. Sci., 98: 261–264.

Brecht, P.E., Kader, A.A., and Morris, L.L., 1973c. The effect of composition of the atmosphere and duration of exposure on brown stain of lettuce. J. Am. Soc. Hort. Sci., 98: 536–538.

Hyodo, H., Kuroda, H., and Yang, S.F., 1978. Induction of phenylalanine ammonia-lyase and increase in phenolics in lettuce leaves in relation to the development of russet spotting caused by ethylene. Plant Physiol., 62: 31–35.

Kader, A.A., Brecht, P.E., Woodruff, R., and Morris, L.L., 1973. Influence of carbon monoxide, carbon dioxide and oxygen levels on brown stain, respiration rate and visual quality of lettuce. J. Am. Soc. Hort. Sci., 98: 485–488.

Kader, A.A. and Lipton, W.J., 1990. Lettuce. In: Lidster, P.D. et al. (Editors), Controlled atmosphere disorders of commercial fruits and vegetables. Agriculture Canada Publication No., 1847/E, pp. 45–46, 48.

Ke, D. and Saltveit, M.E. Jr., 1986. Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in iceberg lettuce. HortScience, 21: 1169–1171.

Ke, D. and Saltveit, M.E. Jr., 1989a. Carbon dioxide induced brown stain development as related to phenolic metabolism in iceberg lettuce. J. Am. Soc. Hort. Sci., 114: 789–794.

Ke, D. and Saltveit, M.E. Jr., 1989b. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. Physiol. Plant., 76: 412–418.

Ke, D., Goldstein, L., O'Mahony, M., and Kader, A.A., 1991. Effects of short-term exposure to low O_2 and high CO_2 atmospheres on quality attributes of strawberries. J. Food Sci., 56: 50–54.

Ke, D., Rodriguez-Sinobas, L., and Kader, A.A., 1991b. Physiology and prediction of fruit tolerance to low-oxygen atmospheres. J. Am. Soc. Hort. Sci., 116: 233–260.

King Jr., A.D., Magnuson, J.A., Torok, T., and Goodman, N., 1991. Microbial flora and storage quality of partially processed lettuce. J. Food Sci., 56: 459–461.

Lipton, W.J., 1977. Toward an explanation of physiological disorders of vegetables induced by high CO_2 or low O_2. In: Dewey, D.H. (Editor), Controlled atmospheres for the storage and transport of perishable agricultural commodities. Hort Rept. 28. Michigan State Univ., East Lansing, pp., 137–141.

Lipton, W.J., 1987. Carbon dioxide-induced injury in romaine lettuce stored in controlled atmospheres. HortScience, 22: 461–463.
Mateos, M., Ke, D., Kader, A., and Cantwell, M., 1993. Differential responses of intact and minimally processed lettuce to high carbon dioxide atmospheres. Acta Hort. (In press).
McDonald, R.E., Risse, L.A., and Barmore, C.R., 1990. Bagging chopped lettuce in selected permeability films. HortScience, 25: 671–673.
Shaw, M., Bolwell, G.P., and Smith, C., 1990. Wound-induced phenylalanine ammonia-lyase in potato (Solanum tuberosum) tuber discs: significance of glycosylation and immunolocalization of enzyme subunits. Biochem. J., 267: 163–170.
Siriphanich, J. and Kader, A.A., 1985. Effects of CO_2 on total phenolics, phenylalanine ammonia-lyase and polyphenol oxidase in lettuce tissue. J. Am. Soc. Hort. Sci., 110: 249–253.
Siriphanich, J. and Kader, A.A., 1986. Changes in cytoplasmic and vacuolar pH in harvested lettuce tissue as influenced by CO_2. J. Am. Soc. Hort. Sci., 111: 73–77.
Steward, J.K. and Uota, M., 1971. Carbon dioxide injury and market quality of lettuce held in controlled atmospheres. J. Am. Soc. Hort. Sci., 96: 27–30.
Tartaru, D.P. and Weichmann, J., 1974. Storage of butter type head lettuce in controlled atmospheres. Acta Hort., 38: 75–78.