Senescence of Broccoli Buds Is Related to Changes in Lipid Peroxidation

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Chlorophyll (Chl), total soluble protein, lipoxygenase (LOX) activity, C_{18} polyunsaturated fatty acid (PUFA), C_{18} PUFA hydroperoxides, and total fatty acid declined and thiobarbituric acid-reactive substances (TBA-RS) increased in senescing broccoli flower buds during postharvest storage of broccoli florets at 5 °C over 96 h. The nonpackaged storage treatment resulted in a significant decrease in moisture, Chl, soluble protein, PUFA, and LOX activity and an increase in TBA-RS. Modified atmosphere packaging (MAP) and automatic misting (AM) treatments maintained Chl and soluble protein. MAP and AM storage reduced losses in PUFA and LOX activity and also reduced increases in TBA-RS for up to 96 h of storage. Further analysis of the data showed that a correlation existed between deterioration indices and lipid peroxidation (LP) parameters and the changes in LP indices preceded Chl losses in senescing broccoli buds. These results indicate a relationship between changes in LP and broccoli bud senescence.

Keywords: *Packaging; broccoli buds; lipid peroxidation*

INTRODUCTION

Lipid peroxidation (LP) may contribute to postharvest deterioration of vegetables and fruits (Mazliak, 1987; Watada et al., 1990). Postharvest processing of vegetables and fruits can lead to lipid degradation and accumulation of free polyunsaturated fatty acids (PUFA) (Leshem, 1987; Mazliak, 1969; Yamauchi et al., 1986). PUFA are then oxidized enzymatically by lipoxygenase (LOX) or nonenzymatically by reactive oxygen species to form lipid hydroperoxides. Both LP and lipid hydroperoxides promote loss of membrane integrity, protein, and chlorophyll (Chl), resulting in deterioration.

Increased LP appears to be related to green tissue senescence during postharvest storage. When fresh spinach leaves were stored at 25 °C for 6 days, Yamauchi et al. (1986) and Yamauchi and Watada (1991) found that decreased linolenic acid (18:3) and increased thiobarbituric acid-reactive substances (TBA-RS) accompanied yellowing and Chl losses. Exposure to 10 ppm of ethylene resulted in further Chl loss and also enhanced malondialdehyde (MDA) formation in spinach leaves. Meir et al. (1992) reported that LP increased during senescence of detached parsley leaves, with a rise in Chl degradation and proteolysis. During cabbage leaf senescence, reduced relative levels of Chl and soluble proteins were observed with decreased ratios of PUFA/saturated fatty acid (SFA) of phospholipids (PL) and enhanced LOX activities (Cheour et al., 1992). *In vitro*, chlorophyll bleaching was accelerated by LP and inhibited by antioxidants (Orthoefer and Dugan, 1973; Imamura and Shimizu, 1974; Cohen et al., 1985; Klein et al., 1985).

Broccoli is a perishable green vegetable, and its deterioration is visibly manifested by Chl loss and yellowing of flower buds. Our previous study showed that deterioration (i.e., Chl and protein losses) of broccoli florets (weighing about 20 g with >1 in. long branchlets) was associated with decreased relative levels of PUFA. Modified atmosphere packaging (MAP) and automatic misting (AM) treatments prevented PUFA losses and maintained quality of broccoli florets during postharvest storage at 5 °C for 144 h (Zhuang et al., 1994). This observation led us to hypothesize that LP results in rapid broccoli deterioration after harvest. The objective of this study was to describe the relationship between LP and deterioration in broccoli flower buds under the same storage treatments. We chose broccoli flower buds (weighing about 3 mg/bud) rather than florets because the most obvious physiological features of broccoli postharvest senescence are found primarily in flower buds rather than stalks (Tian et al., 1994). Lebermann et al. (1968) observed that yellowing of the stalks was usually observable only in the samples with extremely yellowed flower heads (made up of the florets and less than 0.5 in. of stalk). Aharoni et al. (1985) reported that the broccoli inflorescence turns yellow due to opening of the flower buds and degradation of chlorophyll in the green sepal tissues. The inflorescence portions (weighing about 1 g) and florets (weighing about 5 g) produced more CO_2 and tended to produce more ethylene than branchlets or intact heads (Aharoni et al., 1985; King and Morris, 1994). In addition, homogeneous flower buds should have consistent physiological responses to postharvest treatment. Use of broccoli flower buds can reduce stalk-induced nonhomogeneous problems in analysis of small sample sizes (1 g).

In the current study, deterioration of broccoli buds was measured by moisture loss and total Chl and total soluble protein contents during simulated retail handling and storage (5 °C) over 96 h. LP was assessed by LP substrate (PUFA content), LP primary products (hydroperoxy derivatives of C_{18} PUFA), final LP breakdown products (short-chain n-aldehydes), and LP catalyst (soluble LOX activity), all at four different levels to obtain a better estimate of LP changes. It has been demonstrated that no single test can possibly measure all oxidative events at once and more reliability can be

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obtained when a combination of tests is employed for LP. In the previous studies, however, those parameters were only partially detected in senescing plant tissues (Thompson et al., 1987; Kumar and Knowles, 1993; Meir et al., 1992; Yamauchi et al., 1986; Yamauchi and Watada, 1991; Grossman and Leshem, 1978).

MATERIALS AND METHODS

Reagents. Palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), nonadecanoic acid (19:0), octadeca-9,11-*trans*-dienoic acid, and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. Linoleic acid hydroperoxide (HOO-18:2) and linolenic acid hydroperoxide (HOO-18:3) were prepared using soybean LOX-1 (Gardner, 1975).

Broccoli. Broccoli (cv. Iron Duke) heads (the entire inflorescence harvested from one plant, >200 g) were handled according to industry practices and shipped overnight on ice by a commercial vegetable grower/processor (Mann Packing, Salinas, CA) to the University of Kentucky Agricultural Engineering Pilot Plant or obtained through a local distributor. Heads were divided with a sharp knife into florets of about 20 g. Nonpackaged (NP) samples were immediately placed in storage at 5 °C and 60% relative humidity (RH). MAP samples were prepackaged in a commercial polymeric film, Cryovac PD-941 (Cryovac, Duncan, SC) and stored under the same conditions as the NP treatment. CO_2 and O_2 compositions in the headspace of the packages were monitored every 24 h using an O_2/CO_2 headspace analyzer (Zhuang et al., 1994). Levels inside packages equilibrated to about 4% CO_2 and 8.7% O_2 after 48 h. For AM treatment, NP samples were placed in the misting section of the display case and misting intervals were set at 4 s/4 min, providing a total of 43.5 mL of water/4 min at 5 °C. Samples of the broccoli florets were taken at 24 h intervals (0, 24, 48, 72, and 96 h). Broccoli flower buds (about 3 mg per bud) were trimmed on ice with scissors from floret pieces for analysis.

Moisture Content. Approximately 5 g of broccoli buds was used for each moisture content determination (Barth et al., 1992).

Chlorophyll. Total Chl (TChl) determinations were done using a Shimadzu Model UV160U spectrophotometer (Shimadzu Corp., Japan) as adapted by Barth et al. (1992). One gram of broccoli buds was homogenized in a precooled 6 mL of acetone/1 mL of 0.1 N NH_4OH solution using a Tekmar Tissumizer (Tekmar, Cincinnati, OH) for 1 min and then stored in the dark at −20 °C prior to centrifugation. The homogenate was spun at 2500*g* for 20 min at 5 °C and was decanted. Aliquots of the samples were diluted (1:4) prior to spectrophotometric readings of absorbance at 700, 663, 645, and 626 nm. Chl *a*, Chl *b*, and TChl were calculated using the following formula: Chl a (nmol/mL) = $14.18\ OD_{663} - 2.91\ OD_{645} - 0.22\ OD_{626}$; Chl b (nmol/mL) = $26.01\ OD_{645} - 4.66\ OD_{663} - 0.36\ OD_{626}$; TChl = Chl a + Chl b.

Protein Analysis. One gram of buds was homogenized with 1 mL of water on ice and then centrifuged at 13000*g* for 15 min at 4 °C. The protein solution (from the albumin bovine standard and 10-fold diluted aqueous supernatant of broccoli florets) was detected with the Bio-Rad method.

Fatty Acid Composition. Individual fatty acids (FA) were determined following procedures developed by Miquel and Browse (1992). One gram of broccoli buds was extracted with precooled chloroform/methanol/formic acid (10:10:1 v/v/v) in a mortar and pestle on −80 °C gel refrigerant (Cold Ice, Inc., Oakland, CA) after 100 μg of 19:0 internal standard was added. Total fatty acid (TFA) composition was determined by gas chromatography using a Hewlett-Packard FFAP capillary column (Hewlett-Packard, Little Falls, DE) after methylation (Dahmer et al., 1989).

Conjugated Dienes. C_{18} conjugated dienes were measured according to a modification of Iversen's method (Iversen et al., 1984) using HPLC. The lipid fraction was extracted as above for FA analysis, and the internal standard, octadeca-9,11-*trans*-dienoic acid, was added (14.7 μg/g of fresh weight of

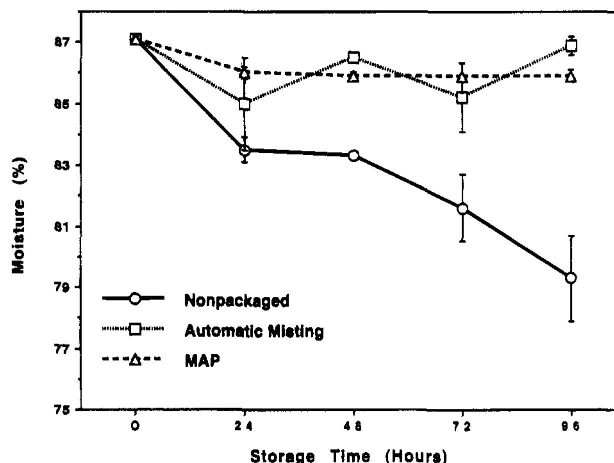

Figure 1. Moisture changes in broccoli buds during postharvest storage of broccoli florets under different treatments at 5 °C over 96 h. The moisture content was measured by water losses after broccoli buds were placed in a 100 °C oven for at least 48 h (means ± SEM, n = 6).

tissue). Lipid extracts (20 μL) were applied to a Sep-Pak octadecyl (C_{18}) cartridge which was preconditioned by washing twice with 2.5 mL of methanol/water/acetic acid (67:33:0.04) immediately before use. Samples were eluted with 1 mL of propan-2-ol/acetonitrile (2:1 v/v). The eluates from four cartridges were combined and dried using a rotary vacuum system at 30 °C. The residue was dissolved in 80 μL of acetonitrile/water/acetic acid (85:15:0.1 v/v/v) solution, and analyzed by HPLC using a Microsorb-MV C_{18} reversed-phase packing (250 × 4.6 mm) containing 5 μm fully capped particles, and the mobile phase of acetonitrile/water/acetic acid (85:15:0.1) was delivered at 1.5 mL/min. HOO-18:2 and HOO-18:3 and the internal standards were detected at 234 nm. HOO-18:2 and HOO-18:3 were identified by coelution with synthetic standards.

Thiobarbituric Acid-Reactive Substances. One gram of broccoli buds was homogenized in 1.0 mL of ice-cold 0.2 M phosphate buffer (pH 6.5, 1% Triton X-100) using a precooled mortar and pestle on ice. The homogenate was centrifuged at 10000*g* (5 °C, 15 min), and the supernatants were immediately frozen in liquid nitrogen and stored at −80 °C before use. A 67 μL aliquot of bud extract was mixed with 933 μL of 20% TCA plus 0.5% TBA in 1.5 mL polypropylene centrifuge tubes and incubated at 95 °C for 30 min. The tubes were then cooled immediately in an ice bath and centrifuged for 10 min (10000*g*, 5 °C). Following centrifugation, absorbance readings were made at 532 and 600 nm for MDA (Peever and Higgins, 1989) and at 455 nm for aldehydes (Meir et al., 1992). For calculations (Meir et al., 1992), an extinction coefficient (E) of 1.56×10^5 M/cm was used for MDA at 532 nm, and an E of 0.457×10^5 M/cm was used at 455 nm as an average of the E obtained for five aldehydes (propanal, butanal, hexanal, heptanal, and propanal−dimethylacetal).

Lipoxygenase Assay. Aliquots of broccoli buds were prepared as described for the protein assay. LOX activity was measured by spectrophotometry at 234 nm in the presence of 18:2 (Zhuang et al., 1994).

Statistical Analysis. The entire experiment was conducted two times with three replicates per experiment. All six replicates from two replicated experiments were used for statistical analysis. Analysis of variance was performed on the data over time and storage treatments. Estimates of mean square values were generated using the General Linear Model (GLM) procedure on the SAS statistical software package (SAS, 1988). Significance was determined at 0.05 level by least-squares difference (lsd).

RESULTS AND DISCUSSION

Moisture. The initial moisture level of broccoli buds was 87% of total fresh weight (Figure 1). Compared to

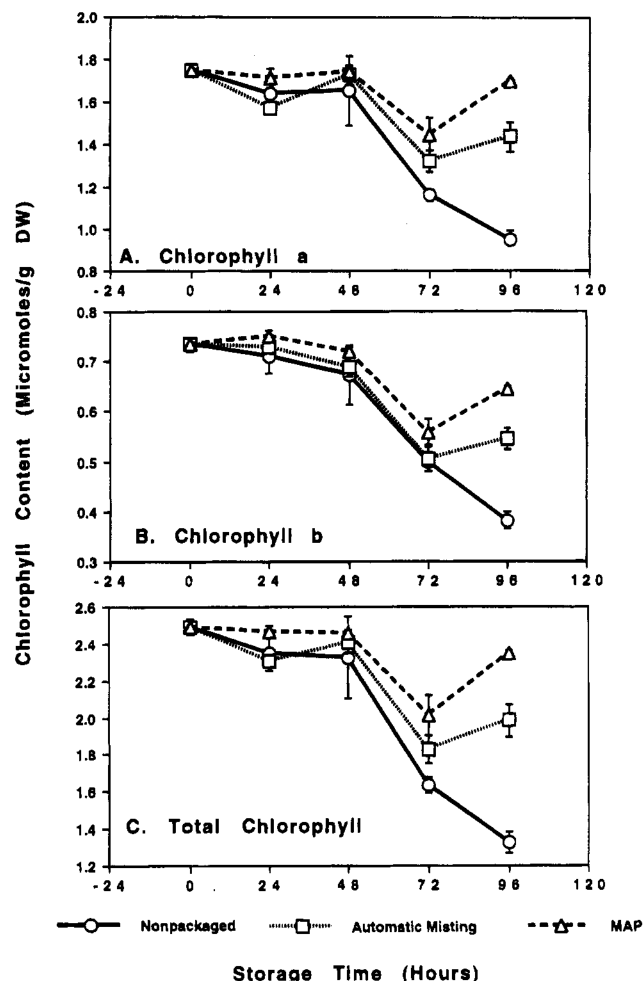

Figure 2. Effect of postharvest treatment on chlorophyll (Chl) levels (dry weight basis) in broccoli buds during postharvest storage (5 °C) over 96 h: (A) Chl *a*; (B) Chl *b*; (C) total Chl. Chl was extracted using 80% acetone in the dark and was determined spectrophotometrically (means ± SEM, $n = 6$).

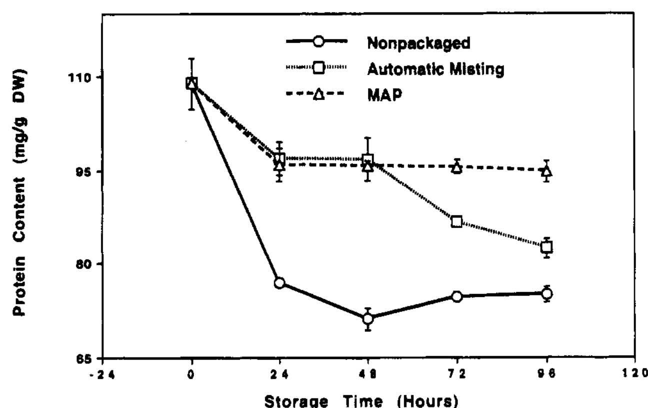

Figure 3. Changes in total water-soluble protein levels in broccoli buds during postharvest storage (5 °C) over 96 h. Broccoli bud protein was extracted with cold water on ice. The content was determined by a Bio-Rad method (means ± SEM, $n = 6$).

the 90% moisture levels of broccoli florets (Zhuang et al., 1994), broccoli buds contained lower levels of water. A nearly linear loss ($r^2 = 0.90$) in moisture of broccoli buds was observed in NP samples over 96 h of storage, significantly lower than that for either MAP or AM treatment. At the end of the storage period, the moisture content decreased to 80% ($P < 0.01$). No significant difference was observed between MAP and AM storage through the entire experimental period. Similar effects of storage treatments on moisture losses in broccoli florets were also reported under the same storage conditions, with a larger reduction from total fresh weight in NP samples (Zhuang et al., 1994).

Total Chlorophyll Retention. The initial TChl content of broccoli buds in our experiments was about 2.5 μmol/g of dw (Figure 2C). This level is 4–5 times higher than Chl levels in broccoli florets. Of the TChl content of broccoli buds, more than two-thirds was Chl a (1.8 μmol/g of dry weight). During storage, TChl, Chl *a*, and Chl *b* showed similar trends under each storage condition. By 96 h, no significant changes were observed in Chl *a*, Chl *b*, and TChl of MAP-treated broccoli buds ($P < 0.06$), whereas in the NP samples about 50% of Chl a (1.0 μmol/g of dw), Chl *b* (0.4 μmol/g of dw) and TChl (1.4 μmol/g of dw) were lost ($P < 0.01$). Levels of all forms of Chl in AM samples (20% less than the initial level) were between those of MAP and NP samples by 96 h ($P < 0.01$). No significant changes in Chl levels among these storage treatments were found by 48 h of storage ($P < 0.60$). After 48 h, Chl significantly decreased in NP samples ($P < 0.05$). There was no significant difference between MAP and AM treatments. These results are consistent with previous observations that MAP and AM maintained Chl in broccoli florets under low-temperature storage (Makhlouf et al., 1990; Barth et al., 1993; Zhuang et al., 1994). Also, the significant changes in TChl of the differently treated broccoli florets occurred after 48 h of storage (Zhuang et al., 1994).

Soluble Protein Content. Changes in total soluble protein contents of broccoli buds during storage based on dry weight are shown in Figure 3. Initial protein levels of broccoli buds were about 110 mg/g of dw, higher than the protein levels of broccoli florets by 20% as reported earlier (Zhuang et al., 1994). Over 96 h of storage, protein content in broccoli buds significantly declined ($P < 0.01$). NP treatment resulted in a 40% decrease in total protein (76 mg/g of dw) at the end of storage. In AM-treated samples, the protein level was 85 mg/g of dw by 96 h, about 20% less than the initial level ($P < 0.01$). No significant loss was observed in MAP-treated samples by 96 h ($P = 0.10$). During storage, significant changes in soluble protein occurred in the first 24 h for NP treatments ($P < 0.01$). In the first 48 h, the protein levels did not significantly change in MAP- and AM-treated broccoli buds. However, a significant difference was detected at the end of 96 h of storage.

Fatty Acid Composition. Table 1 shows the effect of different storage conditions on FA contents in broccoli buds during postharvest storage at 5 °C for 96 h. TFA content of broccoli buds was about 50 mg/g of dw initially, about 2 times higher than that of green plant leaves. Seven major FA were identified by comparison with commercial standard FA for 16:0, 18:0, oleic acid 18:1, 18:2, and 18:3 and with FA mutant lines *Arabidopsis* for hexadecadienoic acid (16:2) and hexadecatrienoic acid (16:3) (Browse and Somerville, 1991).

We found that postharvest storage at low temperature resulted in significant decreases in TFA and 16:0, 16:2, 18:2, and 18:3 FA fractions of broccoli buds. During storage, a significant reduction occurred in the first 24 h. TFA levels dropped to 36.18 mg in NP samples, to 35.71 mg in AM samples, and to 42.40 mg in MAP samples from an initial 50.76 mg/g of dw. This rapid decrease was also observed in FA, 16:0, 18:2, and 18:3 fractions. These FA as well as TFA were reduced by 50% in NP samples and by 30% in AM- and MAP-

Table 1. Effect of Different Storage Conditions on Fatty Acid Composition of Broccoli Buds during Postharvest Storage at 5 °C for 96 h[a]

fatty acids[b]	storage time 0 h	24 h	48 h	72 h	96 h
		Nonpackaged (NP)			
16:0	10.4^{a,x}	7.4^{bc,x}	8.0^{b,x}	6.8^{c,x}	4.7^{d,y}
16:2	1.3^{a,x}	0.9^{bc,x}	1.0^{b,x}	0.9^{bc,x}	0.8^{c,x}
16:3	2.2^{a,x}	1.6^{a,y}	1.9^{a,x}	1.6^{a,xy}	1.8^{a,x}
18:0	1.0^{a,x}	0.7^{b,x}	0.8^{ab,x}	0.7^{b,x}	0.6^{c,y}
18:1	0.5^{a,x}	0.5^{a,x}	0.3^{a,y}	0.3^{a,x}	0.2^{a,y}
18:2	8.6^{a,x}	6.7^{b,x}	6.7^{b,y}	5.6^{bc,x}	4.5^{c,y}
18:3	26.8^{a,x}	18.4^{c,y}	20.6^{b,y}	17.2^{c,x}	14.2^{d,y}
total	50.8^{a,x}	36.2^{bc,y}	39.3^{b,y}	32.9^{c,x}	26.8^{d,y}
		Automatic Misting (AM)			
16:0	10.4^{a,x}	7.3^{bc,x}	8.6^{b,x}	7.1^{c,x}	6.2^{c,x}
16:2	1.3^{a,x}	0.8^{c,x}	1.0^{b,x}	0.9^{bc,x}	0.8^{c,x}
16:3	2.2^{a,x}	1.8^{a,x}	2.1^{a,x}	1.8^{a,x}	2.2^{a,x}
18:0	1.0^{a,x}	0.8^{bc,x}	0.9^{b,x}	0.8^{bc,x}	0.7^{c,xy}
18:1	0.5^{a,x}	0.5^{a,x}	0.5^{a,x}	0.3^{a,x}	0.4^{a,x}
18:2	8.6^{a,x}	6.4^{c,x}	7.4^{b,x}	6.1^{c,x}	6.0^{c,x}
18:3	26.8^{a,x}	18.2^{c,y}	21.7^{b,x}	18.8^{c,x}	17.9^{c,x}
total	50.8^{a,x}	35.7^{c,y}	42.1^{b,x}	35.7^{c,x}	34.1^{c,x}
		Modified Atmosphere Storage (MAP)			
16:0	10.4^{a,x}	8.6^{b,x}	8.4^{b,x}	7.6^{bc,x}	6.3^{c,x}
16:2	1.3^{a,x}	1.1^{ab,x}	1.1^{ab,x}	1.0^{bc,x}	0.8^{c,x}
16:3	2.2^{a,x}	1.9^{a,x}	2.1^{a,x}	1.5^{a,y}	2.0^{a,x}
18:0	1.0^{a,x}	0.9^{ab,x}	0.9^{a,x}	0.7^{a,xy}	0.8^{a,x}
18:1	0.5^{a,x}	0.3^{a,x}	0.4^{a,xy}	0.3^{a,x}	0.3^{a,x}
18:2	8.6^{a,x}	7.2^{ab,x}	7.4^{ab,xy}	6.0^{b,x}	6.0^{b,x}
18:3	26.8^{a,x}	22.3^{bc,x}	23.4^{ab,x}	19.4^{c,x}	19.5^{c,x}
total	50.8^{a,x}	42.4^{bc,x}	44.2^{ab,x}	36.5^{c,x}	35.8^{c,x}

[a] Total fatty acids were measured by a direct transmethylation method (Dahmer et al., 1989) after lipids were extracted following Browse's procedure (Miquel and Browse, 1992). Values represent mg/g of dw. Means in the same row with the same letter (a–d) do not differ significantly ($P < 0.05$). Statistical analysis was made by SAS program (SAS, 1988). Means in the same column with a common superscript (x–z) are not different ($P < 0.05$). [b] 16:0, palmitic acid; 16:2, hexadecadienoic acid; 16:3, hexadecatrienoic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3 linolenic acid.

treated samples by 96 h. About 70% of total losses in TFA reduction resulted from C_{18} PUFA losses, with 50% from 18:3.

Storage methods also impacted the FA metabolism in broccoli buds during storage. During the first 24 h of storage, AM and NP treatments resulted in significantly lower levels of 18:3 and TFA. NP treatments also caused significant loss in 16:3 (by 20%). At 48 h, significant losses in 18:1, 18:2 18:3, and TFA were detected in only NP samples. At the end of 96 h of storage, NP treatment resulted in significant decreases in 16:0, 18:0, 18:1, and C_{18} PUFA. No significant difference was detected in C_{16} PUFA among treatments or in C_{18} PUFA between AM and MAP samples after 24 h of storage.

Further analysis of the relative composition of FA showed only small changes in PUFA over the entire experiment period and among the treatments (data are not shown), suggesting that most of the losses in FA throughout storage might be due to β-oxidation rather than LP. However, it is worth mentioning that only a small portion of FA may be involved in LP in the tissue. For example, less than 3% of total FA and less than 0.3% triene PUFA in this study were used for other TBA-reactive aldehydes and for MDA, respectively, on the basis of our calculation. So, a small, nonsignificant change in FA is also enough to cause significant increases in LP products. Additionally, no significant changes in relative levels of total PUFA do not mean no changes in PUFA of a specific lipid fraction, such as triacylglyceral (TG), free fatty acids (FFA), glycolipids (GL), or phospholipids (PL). In fact, our preliminary results in the same experiment showed that there is a significant decrease in relative levels of PUFA in GL and TG fractions at the later stage of storage. However, no significant differences were observed in PL and FFA fractions. A similar result has been found in previous research. In tea leaves, PUFA of neutral lipids were specifically used for LP metabolism (Hatanaka et al., 1976). In potato tubers, GL was utilized for LP (Galliard, 1970). This result implied that changes in total PUFA or total individual C_{18} PUFA in broccoli buds may not be a good indicator for LP changes during postharvest storage.

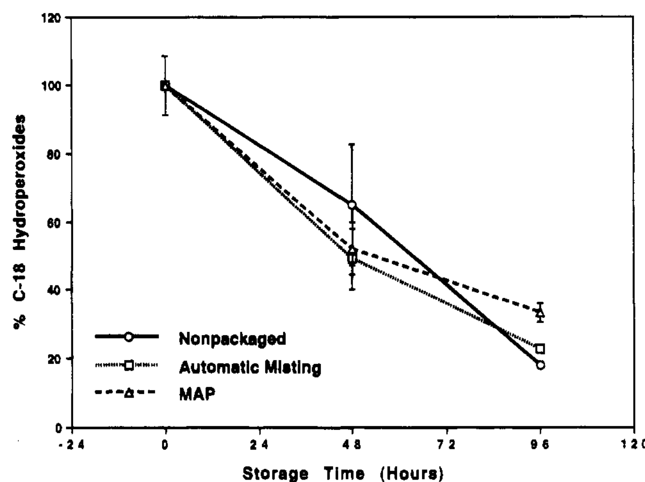

Figure 4. Effects of modified atmosphere packaging (MAP) and automatic misting (AM) on C_{18} hydroperoxide contents in broccoli buds during postharvest storage (5 °C) over 96 h. C_{18} hydroperoxides were extracted using chloroform/methanol/formic acid (10:10:1) containing 0.01% BHT at −20 °C, and their contents were identified and quantitatively determined using an HPLC C_{18} column.

A significant decrease in relative levels of total PUFA through 144 h of storage was reported in our previous work (Zhuang et al., 1994). The different observations between this experiment and our previous one may result from various metabolic responses of different organs to storage treatments. In the earlier study, more than 50% of the tissues that were used for FA analysis were broccoli stems. However, in the current investigation, only buds were employed.

Hydroperoxides of Linoleic Acid and Linolenic Acid. Our FA analysis showed that broccoli buds predominantly lost C_{18} PUFA rather than C_{16} PUFA during senescence. Figure 4 shows relative changes in hydroperoxides of C_{18} PUFA of broccoli buds during postharvest storage. Initial levels of C_{18} hydroperoxides were 1.69 μmol/g of TFA and 2.41 μmol/g of C_{18} PUFA. The hydroperoxides decreased in broccoli buds over 96 h of storage. Hydroperoxide levels declined by 40% at 48 h and by 70% over 96 h. The reduction trend was not altered by different storage conditions. However, by 96 h, hydroperoxide levels in MAP samples were slightly higher than those in AM and NP samples, and a difference between AM and NP samples was also observed. A reduction in lipid hydroperoxides (by measuring 234 nm absorbance) was also reported by Thompson et al. (1987) in senescing cut carnation flowers.

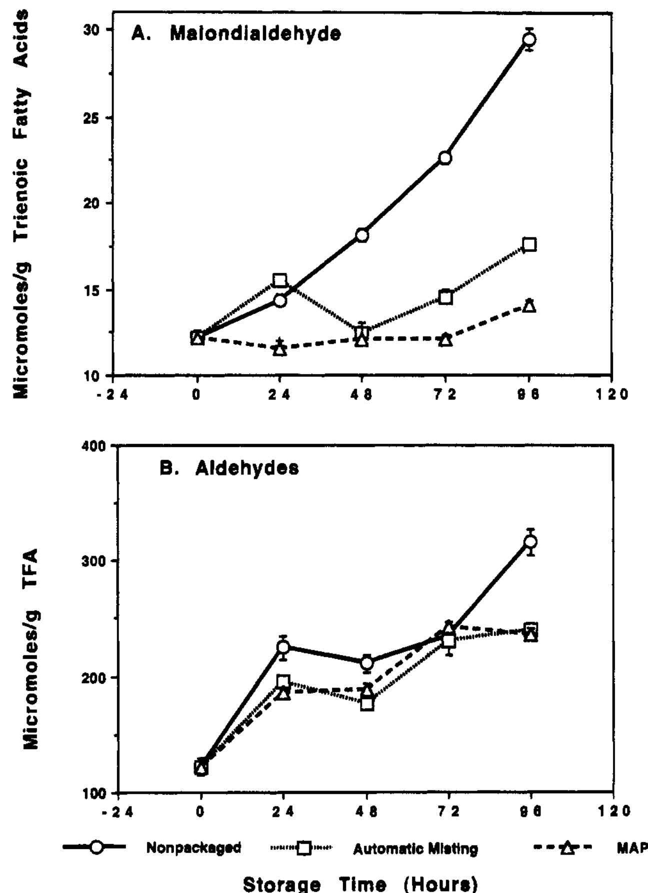

Figure 5. Changes in lipid peroxidation (LP) derivatives (TBA-RS) of broccoli buds during postharvest storage (5 °C) over 96 h: (A) malondialdehyde (MDA); (B) TBA-reactive aldehydes. The samples were ground with phosphate buffer on ice. The products of LP, MDA and short-chain aldehydes, were measured spectrophotometrically after aliquots were incubated with thiobarbituric acid (TBA) (means ± SEM, n = 6).

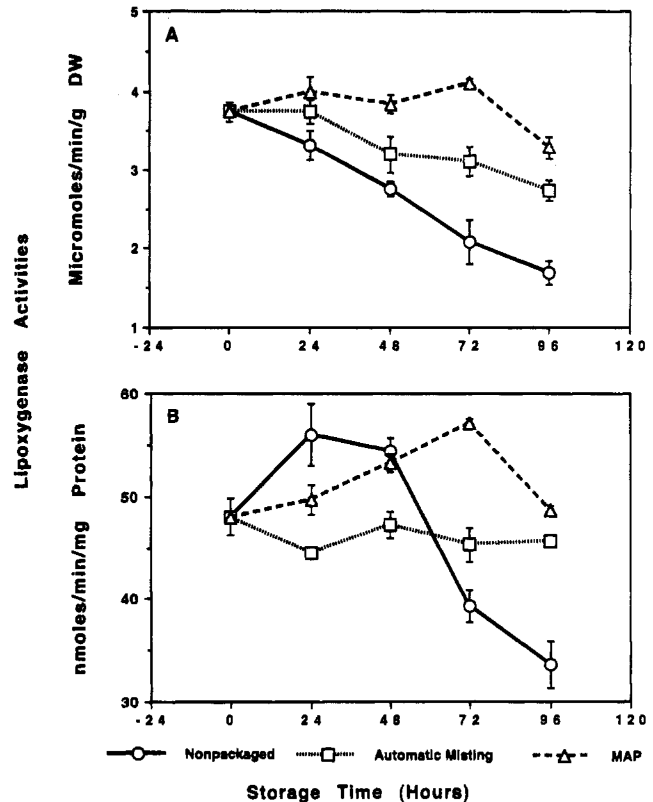

Figure 6. Effect of postharvest treatments on lipoxygenase (LOX) activity in broccoli buds at 5 °C over 96 h of storage: (A) LOX activity expressed on dw basis; (B) LOX activity expressed on protein basis. LOX was extracted with cold water on ice, and its activity was determined spectrophotometrically at 234 nm in the presence of linoleic acid (18:2) (means ± SEM, n = 6).

Thiobarbituric Acid-Reactive Substances. Of the total TBA-RS, less than 10% consisted of MDA in stored broccoli buds; the other 90% was predominantly composed of other TBA-reactive aldehydes (Figure 5). These levels were also much higher than C_{18} hydroperoxides. A similar result was also reported by Meir et al. (1992) during parsley leaf senescence. In contrast to the changes in hydroperoxy derivatives of C_{18} PUFA, TBA-RS in broccoli buds significantly increased during storage ($P < 0.01$). By the end of storage, MDA rose 2-fold. The other TBA-reactive aldehydes were enhanced 2-fold in MAP- and AM-treated samples and 3-fold in NP-treated samples. Different storage methods showed different effects on the changes in those aldehydes ($P < 0.01$). By 24 h, NP and AM treatments resulted in significant increases in MDA formation ($P < 0.01$), and NP significantly enhanced the production of other aldehydes. At the end of 96 h of storage, TBA-RS levels in NP-treated samples were significantly higher than those in either AM- or MAP-treated samples ($P < 0.01$). No significant difference was observed between AM and MAP treatments. The significantly increased TBA-RS with decreased C_{18} hydroperoxides suggests that lipid hydroperoxide synthesis may be a rate-limiting step in the *in vivo* LP pathway. In fact, several lines of evidence have shown that hydroperoxide synthesis by LOX is a limiting step during C_6 aldehyde formation via a LOX-hydroperoxide lyase pathway (Zhuang et al., 1992; Matoba et al., 1985; Sekiya et al., 1984).

Lipoxygenase Activity. A linear decline ($r^2 = 0.86$) in LOX activity on a dry weight basis was observed in NP samples, with 50% of losses at the end of storage (Figure 6A). In AM samples LOX activity also decreased over 96 h ($P < 0.02$). However, LOX activity in MAP samples was maintained almost at its initial activity level up to 96 h ($P = 0.22$).

The same effects of storage treatments were also found when LOX activity was expressed on a protein basis (Figure 6B). No significant difference was found among the three different treatments at 24 h ($P < 0.10$). By 48 h, a significant difference was observed in LOX activities based on dry weight ($P < 0.01$). However, no changes were found on a protein basis. At the end of storage, NP treatment resulted in a 30% loss in LOX activity. No significant difference was detected between MAP and AM treatments. Although increased LOX activity has been related to plant senescence during storage (Mazliak, 1969; Feys et al., 1980; Zamora et al., 1985; Pauls and Thompson, 1984; Thompson et al., 1987; Cheour et al., 1992), contradictory results were reported recently. Kar and Feierabend (1984) observed a marked decline in LOX activity in senescing wheat and rye leaves. With advancing age of soybean leaves (Zhuang et al., 1992) and potato seed tubers (Kumar and Knowles, 1993), there was a loss rather than increase in LOX activities. On the other hand, LOX *in vitro* has been demonstrated to be a self-destructive enzyme; that is, LOX catalyzes its own destruction during oxygenation of FA substrates (Smith and Lands,

1970, 1972; Downing et al., 1970). The velocity of the LOX-catalyzed reaction decreased as a linear function of substrate utilization. The mechanism of LOX self-destruction is believed to be via modification of protein by lipid hydroperoxide-derivative free radicals and MDA. If this is also the case, *in vivo* LOX activity should decline rather than increase as LP increases during senescence. The expectation is consistent with our observation of LOX changes during broccoli bud senescence. Furthermore, LP can be a self-propagating process and therefore should not require high levels of LOX activity once it is initiated. A significant increase in LOX activity based on water-soluble protein levels in NP samples over the first 48 h of the storage period and a steady increase in the LOX activity in MAP samples through the first 72 h of storage may result from the loss in water of broccoli buds over storage (Figures 6 and 1). Bell and Mullet (1991) and Mason and Mullet (1990) showed that water deficit induced expression of LOX genes in soybean and pea tissues. The following decreases in LOX activity at the later storage times in both treatments could be caused by greater inactivation of LOX by increased LP and/or bud deterioration.

Our investigation (Figures 1−3 and Table 1) showed that broccoli buds deteriorated during 96 h of low-temperature storage, expressed as losses in total soluble protein and Chl levels. Rates and extents of deterioration of broccoli buds appeared to depend on the storage treatments. Under NP and AM storage, total soluble protein loss in broccoli buds was >20% by the end of storage (Figure 3). A decrease in Chl was also observed in AM- and NP-treated samples (Figure 2). Comparison of Chl loss with protein loss over storage showed that soluble protein levels changed in a trend similar to that of Chl. However, an apparent drop in protein content occurred earlier (in the first 24 h) than the Chl loss (after 48 h). A difference was detected in protein content between MAP and AM samples by 72 h (Figure 3) rather than by 96 h for Chl (Figure 2). This result suggests that in broccoli buds the soluble proteins are more sensitive to postharvest deterioration than Chl. Proteolysis preceding Chl degradation was also observed in parsley leaves (Meir et al., 1992) and oat leaves (Gepstein and Thimann, 1981) during their senescence.

LP indices appeared to change in broccoli buds among the treatments during storage at 5 °C for 96 h. NP treatment resulted in significant decline in LOX activity and increased levels of MDA. However, these parameters in AM- and MAP-treated broccoli buds were maintained. During storage, the early reduction in LOX activity (based on dw) and increase in TBA-RS were also observed in NP-treated samples (Table 1; Figures 5 and 6). By 96 h, TBA-RS increased 2-fold (Figure 5), with a reduction in early LP products, C_{18} PUFA hydroperoxides, and LOX activities on a dry weight basis (Figure 4).

A correlation was found between broccoli bud deterioration and lipid metabolism during postharvest storage. NP treatment, which resulted in the most deterioration of broccoli buds (Figures 1−3), also caused the greatest changes in PUFA (Table 1), C_{18} PUFA hydroperoxides (Figure 4), and LOX activity losses (Figure 6) and TBA-RS accumulation (Figure 5) by 96 h of storage. Little difference between AM and MAP samples in soluble protein (Figure 3) and Chl contents (Figure 2) was consistent with little significant differences observed between those two treatments in LP indices, C_{18} PUFA (Table 1), TBA-RS (Figure 5), and LOX activities per protein level (Figure 6) by 96 h. Over the storage period, a linear correlation was found between either Chl or moisture losses and each of all four LP indices in NP samples ($r^2 > 0.740$ or $r^2 > 0.900$) in NP-treated samples. In AM-treated samples, a similar correlation existed between either Chl or protein and any one of C_{18} hydroperoxides, LOX activity, and TBA-RS parameters ($r^2 > 0.700$). A correlation between C_{18} hydroperoxides or TBA-RS with each of TChl, protein, and moisture ($r^2 > 0.700$) was also observed in MAP-treated samples (data are not shown). These results showed that changes in TBA-RS and C_{18} hydroperoxides are better related to the senescence of broccoli buds during storage. On the other hand, the decreases in LOX activity and increase in TBA-RS of NP samples preceded Chl losses, suggesting that LP may be responsible for Chl color losses during broccoli bud senescence. *In vitro*, LP has been shown to be able to accelerate bleaching of chlorophyll (Orthoefer and Dugan, 1973; Imamura and Shimizu, 1974; Cohen et al., 1985; Klein et al., 1985). The correlation between senescence parameters and LP parameters among the treatments and the kinetic pattern of these parameters over storage indicate that LP could be involved in broccoli bud senescence under low-temperature storage.

ABBREVIATIONS USED

16:0, palmitic acid; 16:2, hexadecadienoic acid; 16:3, hexadecatrienoic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 19:0, nonadecanoic acid; AM, automatic misting; BHT, butylated hydroxytoluene (2,6-di-*tert*-butyl-*p*-cresol); Chl, chlorophyll; FA, fatty acid; dw, dry weight; FFA, free fatty acids; HOO-18:2, linoleic acid hydroperoxide; GL, glycolipids; HOO-18:3, linolenic acid hydroperoxide; LOX, lipoxygenase; LP, lipid peroxidation; MAP, modified atmosphere packaging; MDA, malondialdehyde; NP, nonpackaged; PL, phospholipids; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TBA, thiobarbituric acid; TBA-RS, thiobarbituric acid-reactive substances; TFA, total fatty acid; TG, triacylglycerol.

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