HORTSCIENCE 33(1):114–117. 1998.

Chlorophyll and Xanthophyll Changes in Broccoli Florets Stored under Elevated CO_2 or Ethylene-containing Atmosphere

Naoki Yamauchi[1] and Alley E. Watada

Horticultural Crops Quality Laboratory, Beltsville Agriculture Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705-2350

Additional index words. Brassica oleracea, pigment, yellowing, quality

Abstract. **Chlorophylls and xanthophylls were monitored in broccoli (*Brassica oleracea* L. var. *italica* Plen.) florets stored in air, air + 10 ppm ethylene, or 10% CO_2 + 1% O_2 controlled atmosphere (CA) at 15 °C. Chlorophylls a and b, as measured with high-performance liquid chromatography, decreased in florets held in air. The decrease was accelerated by ethylene treatment and suppressed in CA. Chlorophyllide a and pheophorbide a were present in fresh broccoli florets, but the levels decreased significantly in all treatments during storage. The oxidized product of chlorophyll a, 13²-hydroxychlorophyll a, did not accumulate. Xanthophylls decreased, but new pigments, suggested to be esterified xanthophylls, formed with yellowing in stored florets. The chlorophyll degradative pathway in broccoli florets was not altered by ethylene or CA and differed from that reported for parsley (*Petroselium crisum* Nym.) and spinach (*Spinacia oleracea* L.) leaves.**

Yellowing of leafy vegetables occurs as chlorophyll (Chl) is degraded and xanthophyll pigments undergo changes. Degradation of Chl is a regulated process and can be hastened by ethylene or retarded by controlled atmosphere (CA) (Amir-Shapira et al., 1987; Yamauchi and Watada, 1991, 1993). In ethylene-treated citrus fruit, Chl decreases greatly with the increase of chlorophyllide (Chlide) (Amir-Shapira et al., 1987). We also reported that the decrease in Chl was effectively accelerated and Chlide was formed in ethylene-treated leafy vegetables such as spinach and parsley, and that CA inhibited Chl loss in parsley leaves (Yamauchi and Watada, 1991, 1993). CA had the same effects on Brussels sprouts (Lyons and Rappaport, 1962), asparagus (Wang et al., 1971), and broccoli (Yang and Henze, 1988).

Matile (1992) and Vicentini et al. (1995) indicated that Chl degradation in senescing leaves occurs in three steps. Initially, dephytylation by chlorophyllase results in formation of Chlide, which is converted to pheophorbide (Phb) by Mg-dechelatase. Oxygenolytic cleavage of the porphyrin ring of Phb results in colorless low-molecular-weight compounds. In vitro studies with broccoli extract also showed the same sequential steps in degradation of Chl to Chlide, Phb, and finally to the colorless low-molecular-weight compounds (Yamauchi and Watada, 1996). Another degradative product of Chl in the extract was 13²-hydroxychlorophyll a (Chl a-1). Maunders et al. (1983) reported an accumulation of Chl a-1 with a decrease of Chl a during senescence of excised barley and bean leaves. The in vivo pathway of Chl degradation in broccoli florets is not clear and how the pathway is affected by ethylene or CA is unknown.

With advanced yellowing following Chl degradation, xanthophylls undergo changes. Esterified xanthophylls are formed during advanced senescence of beech (*Fagus sylvatia* L.) leaves (Tevini and Steinmüller, 1985) and yellowing of parsley leaves (Yamauchi and Watada, 1993). The esterified xanthophylls in parsley leaves were identified as lutein, neoxanthin, and violaxanthin. Formation of these pigments probably is not linked to the Chl degradative pathway (Yamauchi and Watada, 1993); however, the pigment changes in yellowing broccoli florets are not known.

We report here the Chl degradative pathway in broccoli florets and the effects of ethylene and CA on the pathway. Additionally, we determined the changes in xanthophylls during yellowing of broccoli florets.

Materials and Methods

Fresh broccoli, grown in California, was purchased at the Jessup, Md., Distribution Center. Fresh broccoli heads (≈200 g per head) were placed in 30 closed containers at 15 °C. Streams of humidified gases were passed through the containers. Twelve containers received air alone, nine received air containing ethylene (10 $\mu L \cdot L^{-1}$), and nine received a mixture of 1% O_2, 10% CO_2, and 89% N_2 (CA) (Hardenburg et al., 1986). The gases were metered to maintain respiratory CO_2 levels at ≈0.5%. Three containers of each treatment were removed for analyses at scheduled intervals during the 7 d of storage.

Surface color of broccoli florets was monitored with a chromameter (Minolta CR-300, Osaka, Japan) and reported as hue angle (McGuire, 1992).

Pigments were extracted by grinding 3.0-g florets in 20 mL cold acetone and 2.0 mL distilled water with a mortar and pestle, filtering the homogenate, rewashing the residue with 80% cold acetone until the residue was colorless, and bringing the final volume to 30 mL. Aliquots of the combined extracts were used for photometric analysis of Chls or passed through a nylon filter (0.2 mm pore size; PGC Scientific, Gathersburg, Md.) for high-performance liquid chromatography (HPLC) analysis of pigments. Chl content was determined by the method of Arnon (1949).

Pigments were separated and quantified with HPLC using a photodiode array detector and modified method of Yamauchi and Watada (1991). The absorption spectra of the pigments were recorded between 200 and 600 nm at the rate of 12 spectra per min. Pigments were separated on a Nova-Pak C18 column (Waters, Milford, Mass.), 3.9 × 150 mm, using two solvents: "A", 80 methanol : 20 water, and "B", ethyl acetate. "A" was added to "B" at a linear rate for a 30-min period until a 25:75 mixture was attained, and the 25:75 mixture was then kept isocratic for an additional 5 min. The flow rate was 1 $mL \cdot min^{-1}$ and the injection volume was 50 µL. Data from the photodiode array detector were stored and processed by means of a Hewlett-Packard model 9153 disk drive, color display monitor 35741 (Hewlett-Packard, Avondale, Pa.). Identification of Chls and their derivatives were based on retention time and by the visible absorption spectra.

Individual pigments from acetone extracts were identified by the method described previously (Yamauchi and Watada, 1991). The relative contents of Chl a-1 and Chlide a were reported as peak areas because prepared standards were useful for identification, but not for quantification.

Results

The hue angle of broccoli florets decreased during storage due to yellowing (Fig. 1). The decrease started after day 4 with samples in CA and was gradual relative to those in air. The hue angle of samples in air and ethylene dropped sharply after day 3 and 2, respectively.

Pigments present in the broccoli florets were eluted in the following sequence: Chlide a, Phb a, neoxanthin, violaxanthin, lutein, Chl b, Chl a-1, Chl a, pheophytin (Phy) a, and b-carotene (Fig. 2).

Chl a content of all samples decreased during storage (Fig. 3). The Chl a content in CA samples decreased gradually and ≈21% was lost by day 4. The decrease occurred

Received for publication 23 Dec. 1996. Accepted for publication 20 June 1997. Use of company or product name by the U.S. Dept. of Agriculture does not imply approval or recommendation of the product to the exclusion of others which also may be suitable. We gratefully acknowledge Mr. Willard Douglas for excellent technical assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Current address: Faculty of Agriculture, Yamaguchi Univ., 1677-1 Yoshida, Yamaguchi 753, Japan.

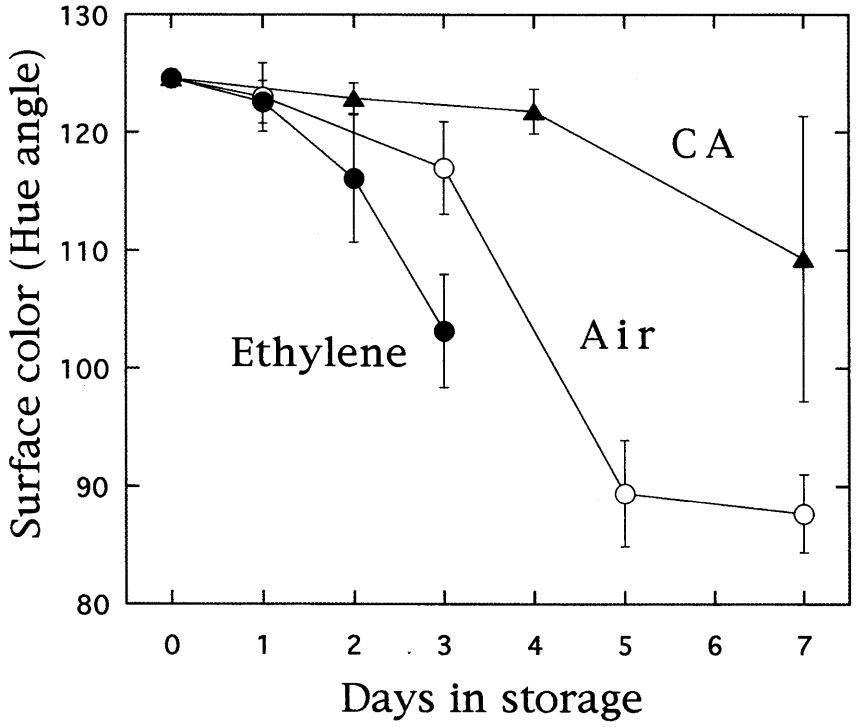

Fig. 1. Changes in surface color (hue angle) of broccoli florets held in air with or without 10 $\mu L \cdot L^{-1}$ ethylene or CA containing 10% CO_2, 1% O_2 at 15 °C. The vertical bars represent the average values with SD (n = 3).

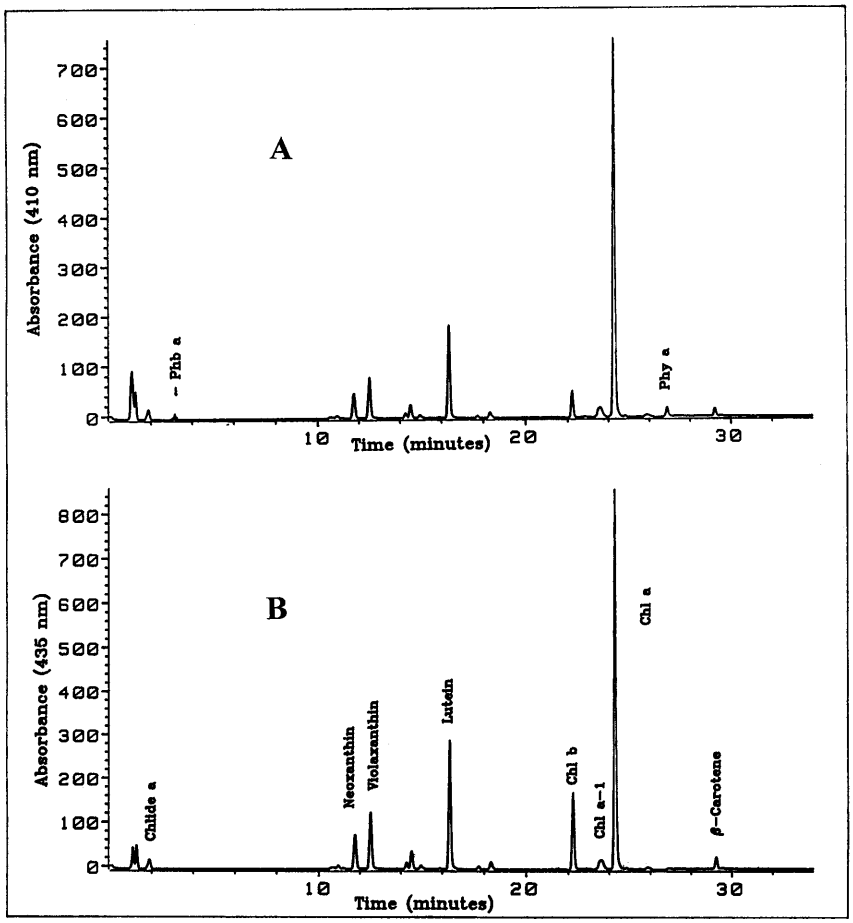

Fig. 2. HPLC chromatogram of pigments extracted from fresh broccoli florets. Column and solvent gradient of HPLC system are described in text. Pigments were measured at (**A**) 410 nm for pheophorbide a and pheophytin a and at (**B**) 435 nm for chlorophylls and carotenoids. Chlide = chlorophyllide, Phb = pheophorbide, Chl = chlorophyll, Phy = pheophytin.

earlier and was greater with samples in the air plus ethylene atmosphere than with those in air, with the loss being 44% and 77% on day 3, respectively. Chl b also decreased during storage and the pattern of decrease among the different treatments was similar to that of Chl a.

The relative level of Chl a-1, the oxidized form of Chl a, was ≈7% of that of Chl a, based on absorbance units (Fig. 4). The content in CA samples remained unchanged until day 4 and then decreased to less than one-half of the original level by day 7. With samples held in air plus ethylene, the content decreased beginning after day 1 down to 35% and 22% of the original level by days 3 and 5, respectively (Fig. 4).

The relative level of Chlide a was ≈4% of that of Chl a, based on absorbance units. The contents of these pigments in all samples decreased considerably during storage (Fig. 4). The level of Phy a was low (Fig. 2) and decreased with yellowing of broccoli florets under air or ethylene treatment (data not shown); that of Phb a was very low in green broccoli florets (Fig. 2) and was not detected with advanced yellowing (data not shown).

Several new peaks appeared with yellowing (Fig. 5). The spectral properties and wavelengths of maximum absorbance of the new peaks were similar to those of the xanthophylls, neoxanthin, violaxanthin, and lutein (Fig. 6).

Discussion

In our in vitro study with broccoli extract (Yamauchi and Watada, 1996), we suggested that Chl was degraded by chlorophyllase to form Chlide, which subsequently was degraded to Phb. We proposed that Phb was then oxidized to colorless low-molecular-weight compounds. The results here with broccoli florets confirm this pathway.

Chlide a was present on day 0, but decreased as it was degraded to Phb. Phb was present initially only in small quantity and probably was oxidized to colorless compounds as soon as it was formed. Chl degradation was enhanced significantly by ethylene and suppressed by CA; however, neither altered the pathway. This Chl degradation pathway in broccoli florets is apparently different from that noted with spinach and parsley leaves. In the leaves, Chlide a accumulated with yellowing and storage (Yamauchi and Watada, 1991, 1993), which was not noted in the broccoli florets. More study is needed to support this speculation.

In broccoli florets, Chl a-1 decreased during storage, whereas a slight increase was noted in the broccoli extract and the accumulation in the extract was enhanced by linoleic acid (Yamauchi and Watada, 1996). Chl a-1 is formed when Chl oxidase bleaches Chl in the presence of unsaturated or saturated fatty acids such as stearic acid (Lüthy et al., 1984; Schoch et al., 1984). This enzyme is clearly different from lipoxygenase (Lüthy et al., 1984). We have shown that Chl a-1 is formed when lipoxygenase bleaches Chl in the presence of linoleic acid (Yamauchi and Watada,

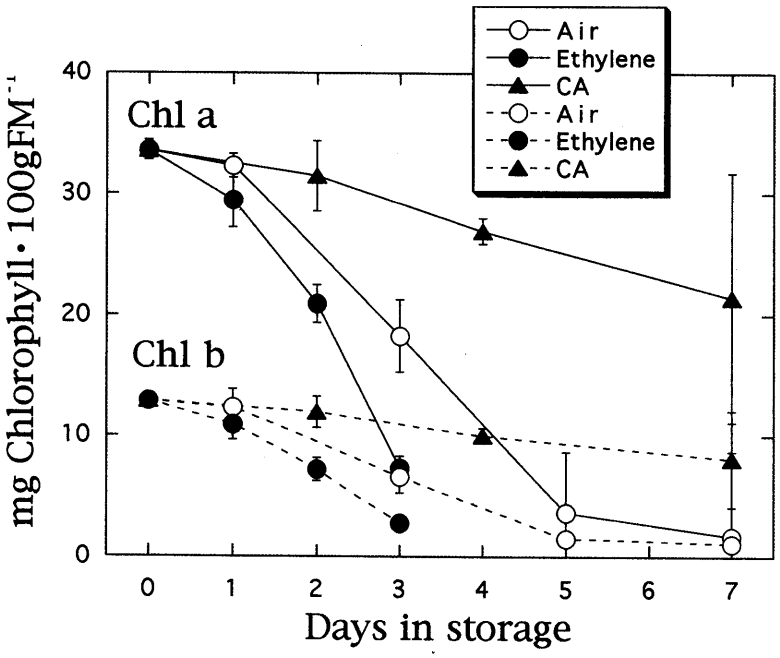

Fig. 3. Changes in content of chlorophylls of broccoli florets stored in air with or without 10 μL·L^{-1} ethylene or CA containing 10% CO_2, 1% O_2 at 15 °C. The vertical bars represent the average values with SD (n = 3). Chl = chlorophyll.

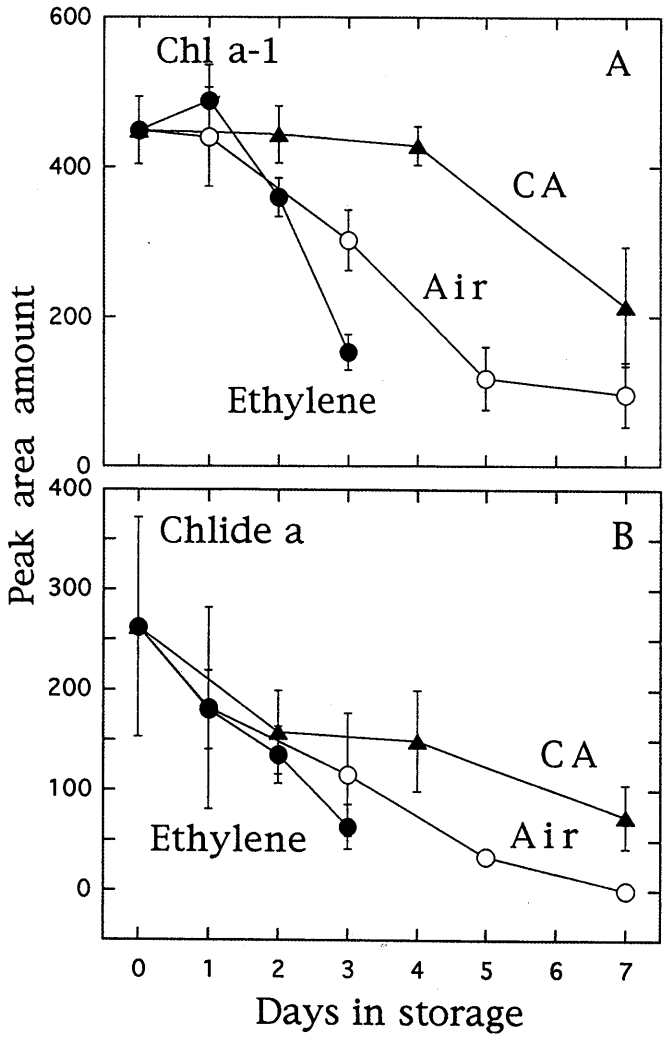

Fig. 4. Relative changes of chlorophyll a-1 (**A**) and chlorophyllide a (**B**) of broccoli florets held in air with or without 10 uL·L^{-1} ethylene or CA containing 10% CO_2, 1% O_2 at 15 °C. The vertical bars represent the average values with SD (n = 3). Chl = chlorophyll, Chlide = chlorophyllide.

1989). We also noted that a small amount of Chl a-1 was found as an intermediate when Chl a was degraded by peroxidase (Yamauchi and Watada, 1994). Thus, Chl a-1 is formed when Chl oxidase, lipoxygenase, and/or peroxidase are present and active. In broccoli florets, the lack of accumulation of Chl a-1 with yellowing implies that some Chl was bleached directly to colorless, low-molecular-weight compounds and/or that Chl a-1 was formed as an intermediate and did not accumulate.

New peaks appeared with yellowing of broccoli florets, which are suspected to be esterified xanthophylls of lutein, violaxanthin, and neoxanthin. These same compounds also formed during the yellowing of parsley leaves (Yamauchi and Watada, 1993). These xanthophylls are known to be esterified with fatty acids, such as palmitic and linolenic acids (Egger and Schwenker, 1966). Tevini and Steinmüller (1985) noted that the esterified xanthophylls were detected with senescence of beech leaves and deposited in the plastoglobuli of chloroplasts. We suspect that the new xanthophylls in the yellowing broccoli were also esterified with fatty acids and had accumulated in the plastoglobuli.

In conclusion, most of the Chl in the broccoli florets is degraded by the pathway through Chlide and Phb to form colorless, low-molecular-weight compounds. The pathway seems not to be altered by ethylene or CA treatment.

Literature Cited

Amir-Shapira, D., E.E. Goldschmidt, and A. Altman. 1987. Chlorophyll catabolism in senescing plant tissue: In vivo breakdown intermediates suggest different degradative pathways for citrus fruit and parsley leaves. Proc. Natl. Acad. Sci. 84:1901–1905.

Arnon, D.I. 1949. Copper enzymes in chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24:1–15.

Egger, K. and U. Schwenker. 1966. The esters of lutein in autumn leaves. Z. Pflanzenphysiol. 54:407–416.

Hardenburg, R.E., A.E. Watada, and C.Y. Wang. 1986. The commercial storage of fruits, vegetables, and florist and nursery stocks. USDA Agr. Handbook No. 66. Washington, D.C.

Lüthy, B., E. Martinoia, P. Matile, and H. Thomas. 1984. Thylakoid-associated chlorophyll oxidase: Distinction from lipoxygenase. Z. Pflanzenphysiol. 113:423–434.

Lyons, J.M. and L. Rappaport. 1962. Effect of controlled atmospheres on storage quality of Brussels sprouts. Proc Amer. Soc. Hort. Sci. 81:324–331.

Matile, P. 1992. Chloroplast senescence, p. 413–440. In: N. Baker and H. Thomas (eds.). Crop photosynthesis: Spatial and temporal determinants. Elsevier, Amsterdam.

Maunders, M.J., S.B. Brown, and H.W. Woolhouse. 1983. The appearance of chlorophyll derivatives in senescing tissue. Phytochemistry 22:2443–2446.

McGuire, R.G. 1992. Reporting of objective color measurements. HortScience 27:1254–1255.

Schoch, S., W. Rudiger, B. Lüthy, and P. Matile. 1984. 13^2-Hydroxychlorophyll a, the first product of the reaction of chlorophyll oxidase. J. Plant Physiol. 115:85–89.

Tevini, M. and D. Steinmüller. 1985. Composition

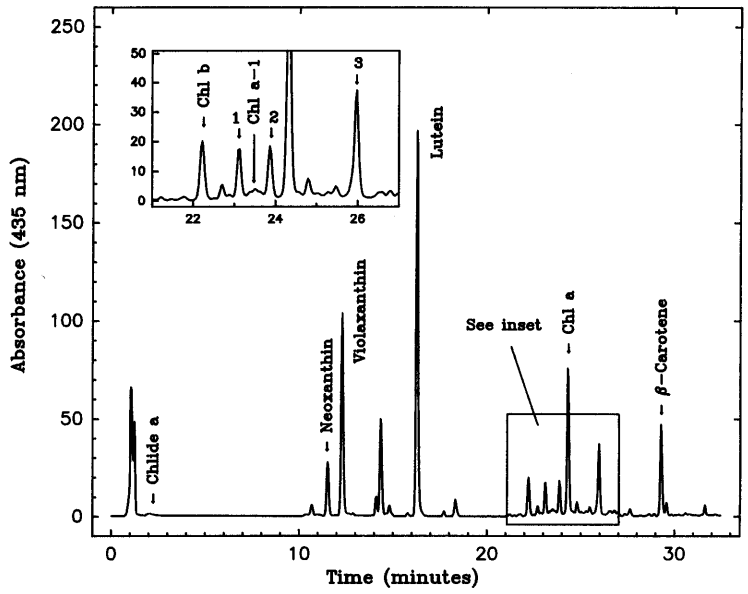

Fig. 5. HPLC chromatogram of broccoli florets after 5 d storage in air at 15 °C, showing three new peaks (**1, 2, and 3**) that appeared as the florets became yellow. Column and solvent gradient of HPLC system are described in text. Chlide = chlorophyllide, Chl = chlorophyll.

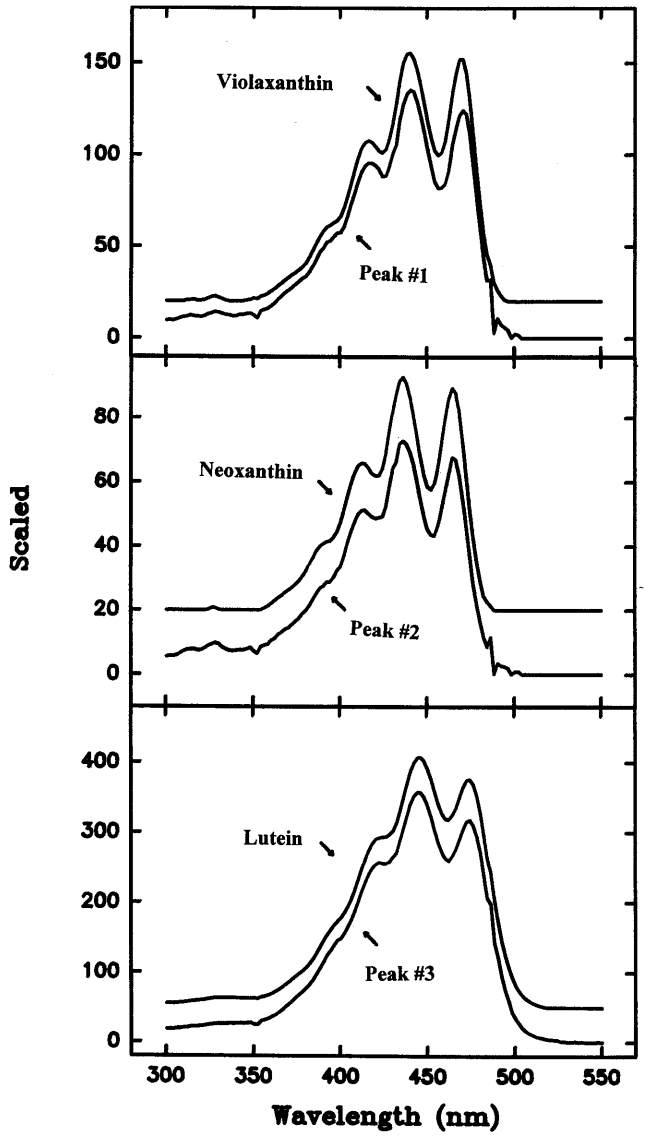

Fig. 6. Comparison of absorption spectra of HPLC chromatograms of peaks #1, #2, and #3 of yellow broccoli florets with those of the xanthophylls, violaxanthin, neoxanthin, and lutein, respectively.

and function of plastoglobuli. II. Lipid composition of leaves and plastoglobuli during beech leaf senescence. Planta 163:91–96.

Vicentini, F., S. Hörtensteiner, M. Schellenberg, H. Thomas, and P. Matile. 1995. Chlorophyll breakdown in senescent leaves: Identification of the biochemical lesion in a *stay-green* genotype of *Festuca pratensis* Huds. New Phytol. 129:247–252.

Wang, S.S., N.F. Haard, and G.R. Di Marco. 1971. Chlorophyll degradation during controlled-atmosphere storage of asparagus. J. Food Sci. 36:657–661.

Yamauchi, N. and A.E. Watada. 1989. Studies on the yellowing phenomenon of harvested leafy vegetables. 6. Chlorophyll degradation by lipoxygenase. J. Jpn. Soc. Hort. Sci. 58 (Suppl. 1):532–533.

Yamauchi, N. and A.E. Watada. 1991. Regulated chlorophyll degradation in spinach leaves during storage. J. Amer. Soc. Hort. Sci. 116:58–62.

Yamauchi, N. and A.E. Watada. 1993. Pigment changes in parsley leaves during storage in controlled or ethylene containing atmosphere. J. Food Sci. 58:616–618.

Yamauchi, N. and A.E. Watada. 1994. Effectiveness of various phenolic compounds in degradation of chlorophyll by in vitro peroxidase-hydrogen peroxide system. J. Japan. Soc. Hort. Sci. 63:439–444.

Yamauchi, N. and A.E. Watada. 1996. Mechanism of chlorophyll degradation in broccoli flower buds. J. Jpn. Soc. Hort. Sci. 65 (Suppl. 1):544–545.

Yang, Y.J. and J. Henze. 1988. Influence of CA-storage on external and internal quality features in broccoli (*Brassica oleracea* var. *italica*). II. Changes in chlorophyll and carotenoid contents. Gartenbauwenschaft 53:41–43.