Pigment Changes in Parsley Leaves during Storage in Controlled or Ethylene Containing Atmosphere

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ABSTRACT

Pigments were monitored in parsley leaves stored in air, air + 10 ppm C_2H_4, or 10% O_2 + 10% CO_2 controlled atmosphere (CA). Chlorophylls a and b, as determined with HPLC, decreased sharply in leaves held in air or air + 10 ppm C_2H_4. The decrease was less in leaves held in 10% O_2 and 10% CO_2 CA. The oxidized product of chlorophyll a, 10-hydroxychlorophyll a, did not accumulate and chlorophyllide accumulated minimally. Xanthophylls decreased but new pigments, suspected to be esterified xanthophylls, formed with yellowing of leaves. Neither the pathway of Chl degradation or xanthophyll products were altered by C_2H_4 or CA.

Key Words: parsley, color, chlorophyll, controlled atmosphere, storage

INTRODUCTION

YELLOWING of leafy vegetables, such as parsley and spinach, occurs with degradation of chlorophyll (Chl). Temperature is the most influential factor in rate of degradation, but the atmosphere can also have an effect. Ethylene (C_2H_4) hastens the rate of Chl degradation (Watada, 1986), whereas, controlled atmosphere (CA) retards degradation (Kader, 1986).

In citrus fruit, C_2H_4 enhanced degradation of Chl resulted in increased chlorophyllase activity (Barmore, 1975; Shimokawa et al., 1978) and an accumulation of chlorophyllide (Amir-Shapira et al., 1987). However in spinach, a leafy tissue, increased degradation by C_2H_4 was not associated with increased chlorophyllide content (Yamauchi and Watada, 1991). This difference between citrus and spinach Chl degradation may be due to differences in the degradative pathway, which is not clearly understood.

Use of CA to retard color or Chl degradation has been shown with several vegetables including Brussels sprouts (Lyons and Rappaport, 1962), asparagus (Wang et al., 1971), and broccoli (Yang and Henze, 1988). Those studies showed that either green color or Chl content in the vegetable tissue was maintained under controlled atmosphere storage, but they did not describe changes in the products of Chl degradation. Knowledge of degradation products should be helpful in elucidating the mechanisms of Chl degradation.

Our objective was to describe the effects of C_2H_4 and controlled atmosphere storage on chlorophyll and its degraded product and also show formation of xanthophyll products, with yellowing of parsley leaves.

MATERIALS & METHODS

FRESH 'Forest Green' parsley (*Petroselium crisum* Nym.) was obtained from a local grower in Delaware and mature detached leaves free of defects or injury were used. About 150 g of leaves were placed in a lightly covered 3.8L glass jar, and triplicate lots were placed at 20°C under a stream of humidified air with or without 10 ppm C_2H_4 or a stream of humidified mixed gases (CA) of 10% O_2, 10% CO_2, and 80% N_2, as recommended by Apeland (1971). The gases were metered at a rate to maintain respiratory CO_2 levels at about 0.5%. Sublots of leaves were removed for analysis after 0, 1, 2, 3 and 5 days ethylene storage or after 0, 1, 2, 4 and 6 days CA storage.

Pigments were extracted by grinding 2.5g leaves in 20 mL cold acetone with 2.5 mL of 0.1% sodium carbonate (to adjust pH to about 7.0), with a mortar and pestle. The homogenate was filtered, the residue washed with 80% cold acetone until colorless, and the filtrate brought to a final volume of 50 mL. The entire extraction was done in low light and the combined extracts were kept in darkness. Aliquots

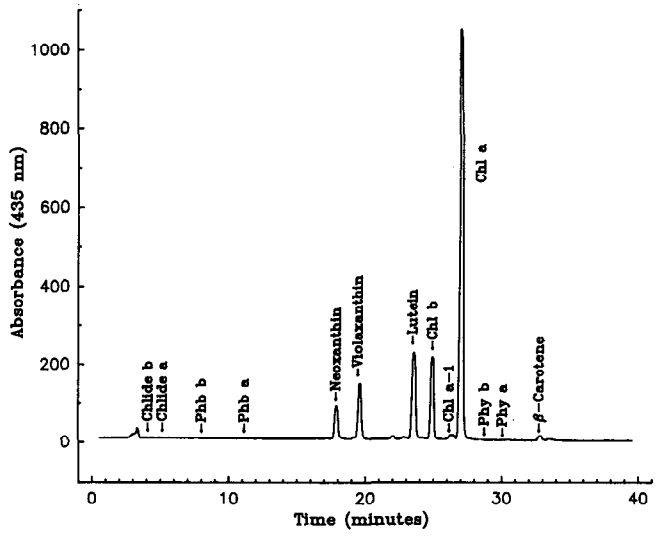

Fig. 1.—HPLC chromatogram of pigments extracted from fresh parsley leaves. Columns and solvent gradient of HPLC system described in text. Chl–chlorophyll, Chlide–chlorophyllide, Phy–pheophytin, Phb–pheophorbide.

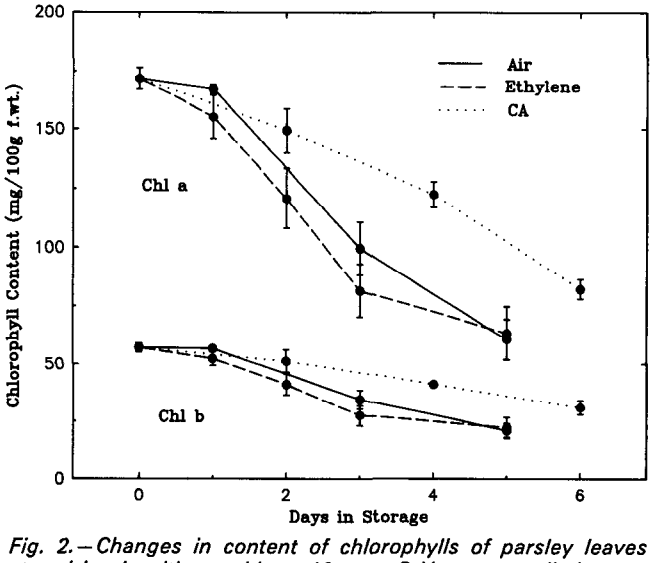

Fig. 2.—Changes in content of chlorophylls of parsley leaves stored in air with or without 10 ppm C_2H_4 or controlled atmosphere of 10% O_2, 10% CO_2.

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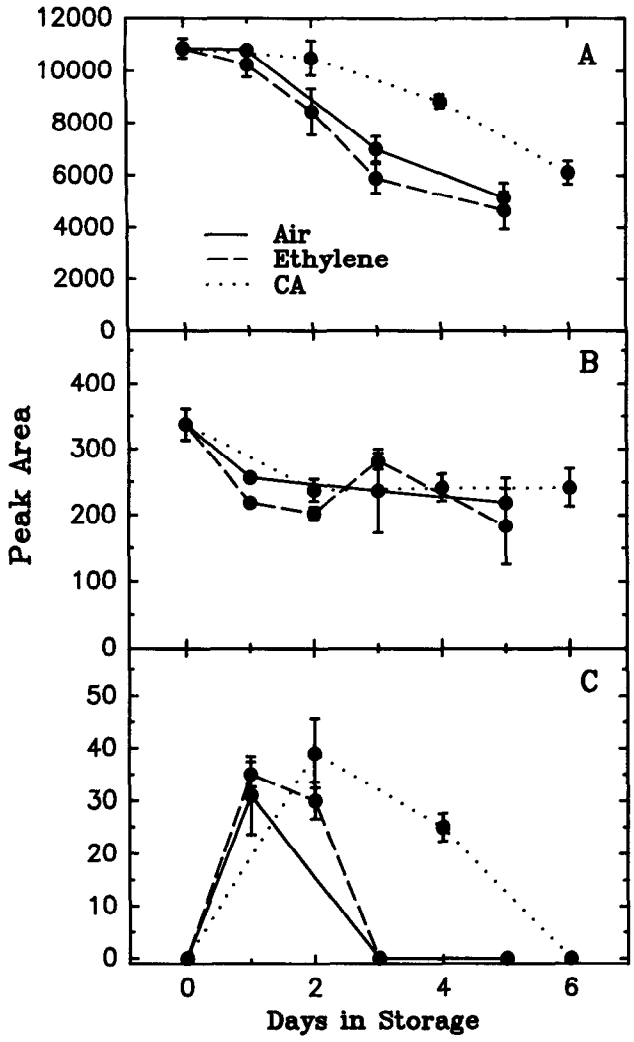

Fig. 3.—Relative changes of chlorophyll a (A), chlorophyll a-1 (B), and chlorophyllide (C) of parsley leaves held in air with or without 10 ppm C_2H_4 or controlled atmosphere of 10% O_2, 10% CO_2.

were used for spectrophotometric (Shimadzu, Model UV-260) analysis or passed through a Millipore filter (0.45 μm pore size) for HPLC analysis.

The HPLC with photodiode array detector system reported previously (Yamauchi and Watada, 1991) was modified. The absorption spectra of the pigments were recorded between 200 and 600 nm at the rate of 12 spectra/min. Pigments were separated by a Vydac C, ultrasphere column, 4.6 × 250 mm, using two solvents: A, 80% methanol, and B, ethyl acetate. Ethyl acetate was added to 80% methanol at a linear rate until a 50:50 mixture was attained at the end of 20 min. The 50:50 mixture then was run for an additional 20 min. Flow rate was 1 mL/min and injection volume was 50 μL.

Identification of individual pigments from the acetone extract were carried out by methods described previously (Yamauchi and Watada, 1991). Chl content was determined by the method of Arnon (1949). The relative contents of 10-hydroxychlorophyll (Chl a-1) and chlorophyllide were reported as peak area because prepared standards were useful for peak identification, but not for quantification.

RESULTS

HPLC of fresh parsley leaves showed sequential elution of neoxanthin, violaxanthin, lutein, Chl b, Chl a-1, Chl a and β-carotene during a 40 min run (Fig. 1). The elution time of chlorophyllide (Chlide) a and b, pheophorbide a and b, and pheophytin a and b are shown as reference points. Identity of each pigment was based on retention time, and in some instances were confirmed by the absorption spectra of the eluting peak.

Chl a content in parsley leaves stored in air with or without C_2H_4 decreased at the same rate and were about 36% of the original level after 5 days storage (Fig. 2). Chl a of leaves exposed to air with or without C_2H_4 decreased at the same rate. Chl a of leaves held in CA decreased, but the rate was slower and the content was maintained 20% longer than those in leaves held in air with or without C_2H_4. Chl b decreased in all leaves and like Chl a, the decrease in samples held in air with or without C_2H_4 was greater than in those held in CA.

The relative level of Chl a-1, the oxidized form of Chl a, was about 3% that of Chl a, based on absorbance units (Fig. 3). Chl a-1 of all treatments decreased by about 30% after one day of storage and then leveled off during the remainder of storage.

The relative level of chlorophyllide was 10% of Chl a-1. With storage, a small accumulation was noted initially after storage in leaves from all treatments, but the accumulation did not continue nor was it retained. The accumulation was retained longer in CA stored leaves where rate of Chl degradation was slower than that of air or C_2H_4 treated samples. Pheophytin a content was low and decreased in leaves of all treatments (data not shown).

The contents of xanthophylls, which included lutein, violaxanthin, and neoxanthin, decreased with yellowing of the leaves. However, at the same time, several new peaks occurred on the HPLC chromatograms with the yellowing (Fig. 4). The wavelengths of maximum absorbance of the new peaks were similar to that of the xanthophylls, such as neoxanthin, violaxanthin and lutein (Table 1). Spectral properties of these peaks were also similar to that of parent xanthophyll, as shown for peaks 6, 7 and 9 compared with parent xanthophyll in Fig. 5. Elution times of these new peaks (except peak 1) were considerably longer than those of the xanthophylls.

DISCUSSION

Chl CONTENT of parsley leaves decreased during storage at 20°C and was not hastened by 10 ppm C_2H_4 which was unexpected. The effect of C_2H_4 may be apparent at a lower holding temperature where the degradation of the control sample would not be so rapid. CA of 10% O_2 and 10% CO_2 was effective in reducing the rate of Chl degradation to the extent that the shelf life would be extended about 20% longer than that of air-held samples. Wang (1979) postulated that CO_2 inhibition of Chl degradation in broccoli may be due to inhibitory effect of CO_2 on C_2H_4 production or action.

In examining the degraded products of Chl associated with yellowing of parsley leaves, Chl a-1 had not accumulated whereas chlorophyllide a accumulated slightly, but not to the extent of the amount of Chl degraded. In the orange flavedo, the peroxidase reaction has been shown to bleach chlorophyll (Huff, 1982). Thus the lack of chlorophyllide accumulation in the parsley may be due to the presence of peroxidase reaction, which converted chlorophyllide to a colorless compound. The changes in the chlorophyllide were similar to those noted with spinach (Yamauchi and Watada, 1991), but were different from those of ethylene treated citrus. There chlorophyllase activity (Barmore, 1975; Shimokawa et al., 1978; Amir-Shapira et al., 1987) and chlorophyllide (Amir-Shapira et al., 1987) increased with yellowing. Neither the ethylene treatment nor CA had a significant effect on formation of Chl a-1 or chlorophyllide.

With the decrease in the xanthophyll content, new pigments (peaks) were noted with the yellowing of the leaves. The new peaks were suspected to be esterified xanthophylls based on similar spectral characteristics and elution at a considerably later time than the xanthophylls. Esterified xanthophylls have been reported to appear with ripening of citrus flavedo (Eilati et al., 1972) and senescence of beech leaves (Tevini and Steinmaller, 1985). Others have reported that xanthophylls were esterified with fatty acids, such as palmitic and linolenic acid (Egger and Schwenker, 1966). The esterified xanthophylls are

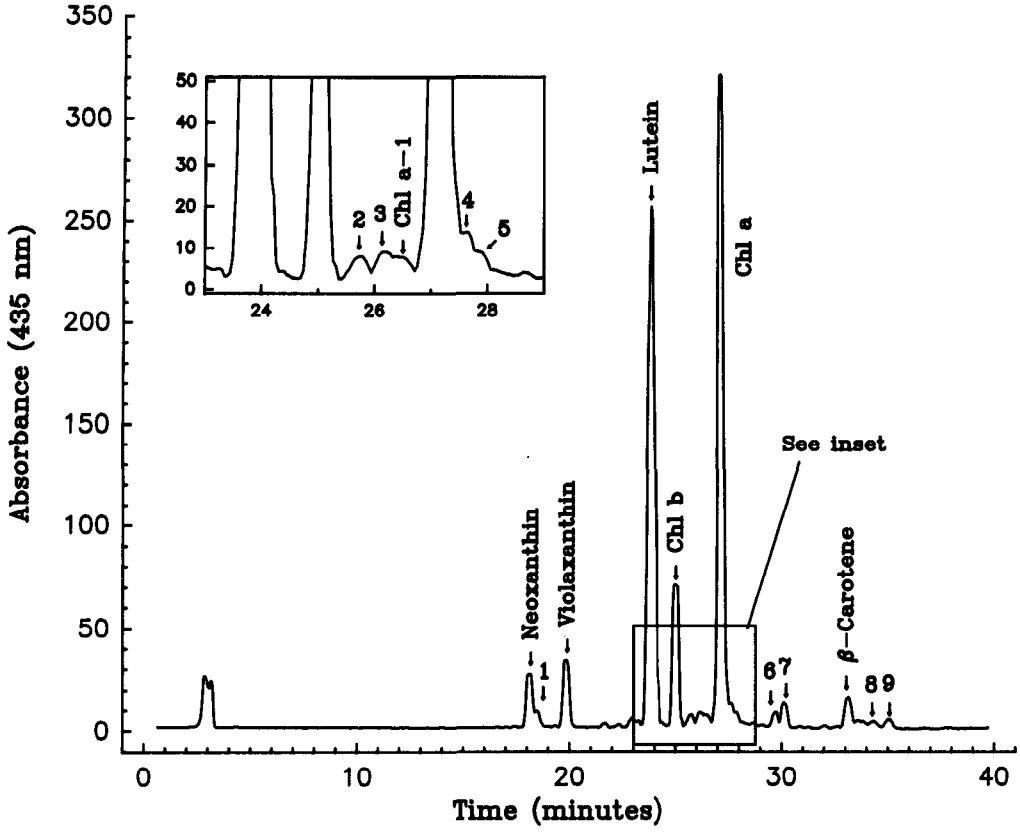

Fig. 4.—HPLC chromatogram of pigments extracted from parsley leaves held in air with 10 ppm C_2H_4 for 5 days at 20°C. Columns and solvent gradient of HPLC system described in text.

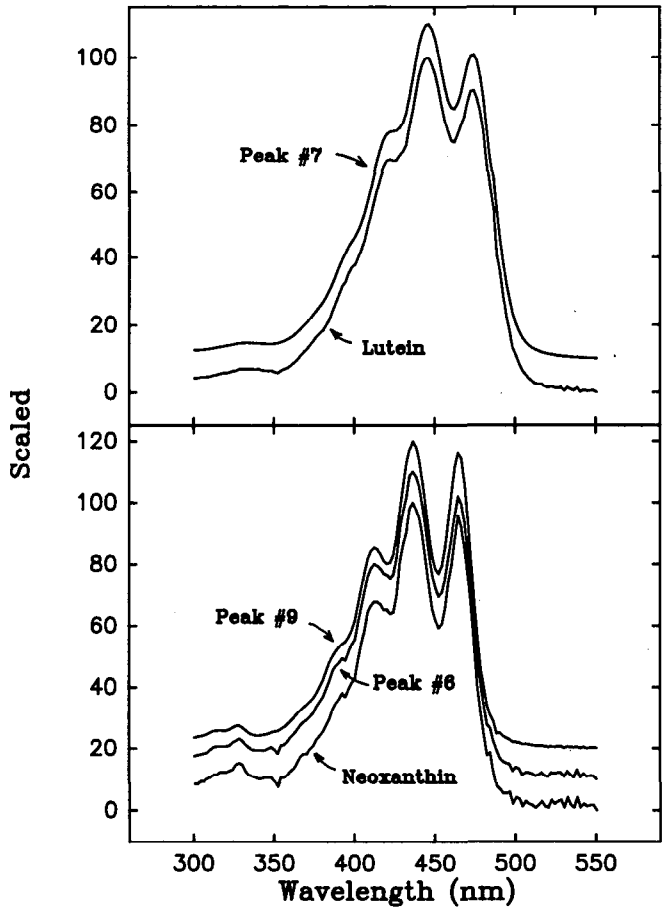

Fig. 5.—Absorption spectra of xanthophylls and new compounds that developed with yellowing of parsley leaves during storage.

Table 1—Spectral maxima of xanthophylls and new components that formed during storage of parsley leaves

Xanthophylls and new components	Wavelength (max)[a]		
Neoxanthin	413,	436,	465
Violaxanthin	417,	439,	469
Lutein	420,	447,	475
Peak #1	417,	441,	471
Peak #2	421,	441,	471
Peak #3	413,	437,	465
Peak #4	415,	435,	465
Peak #5	415,	435,	465
Peak #6	413,	437,	465
Peak #7	423,	445,	473
Peak #8	421,	441,	473
Peak #9	413,	437,	465

[a] Wavelength (max) of the pigments was measured by photodiode array detector.

deposited in the plastoglobuli during senescence (Tevini and Steinmaller, 1985). In parsley leaves, the xanthophylls may be esterified with fatty acids and accumulated in the plastoglobuli of chloroplasts.

These results imply that the pathway by which Chl is degraded in parsley leaves was not altered by C_2H_4 or CA treatments. The lack of effect by C_2H_4 on the rate of degradation needs further study, in that others have reported it to hasten color and Chl degradation. Further study is also needed to determine if formation of xanthophyll pigments is interrelated with Chl degradation. With a better understanding of the effect of C_2H_4 or CA individually (or in combination) on these pigment changes, the knowledge would be beneficial in developing improved handling and storage conditions to maintain color quality of parsley and other leafy vegetables. Additionally, as pathways are understood and enzymes that regulate the pathways are defined, the information would be useful in manipulating genes to develop cultivars that retain color and do not senesce rapidly.

REFERENCES

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