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Effect of ethylene and 1-methylcyclopropene on chlorophyll catabolism of broccoli florets

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Abstract

Branchlets of broccoli (*Brassica oleracea* L.) were used to examine ethylene-stimulated chlorophyll catabolism. Branchlets treated with: 1) air (CK); 2) 1 $\mu L \cdot L^{-1}$ 1-methylcyclopropene (1-MCP) for 14 hr at 20 °C; 3) 1000 $\mu L \cdot L^{-1}$ ethylene (C_2H_4) for 5 hr at 20 °C; or 4) 1-MCP then C_2H_4, were stored in the dark at 20 °C for up to 3 d. Chlorophyll (Chl) content and branchlet hue angle decreased during the storage period and 1-MCP treatment delayed this change. Chl degradation in broccoli was accelerated by exposure to C_2H_4, especially for Chl a. Prior treatment with 1-MCP prevented degreening stimulated by C_2H_4. Lipoxygenase activity was not altered by any of the treatments, however, 1-MCP with or without ethylene resulted in reduced activity of chlorophyllase (Chlase) and peroxidase (POD). Exposure to C_2H_4 stimulated Chlase activity and extended the duration of high POD activity. Treatment with 1-MCP followed by C_2H_4 resulted in reduced POD activity and delayed the increase in Chlase activity. The results suggest chlorophyll in broccoli can be degraded via the POD – hydrogen peroxide system. Exposure to C_2H_4 enhances activity of Chlase and extends the duration of high POD activity, and these responses may accelerate degreening. Treatment with 1-MCP delays yellowing of broccoli, an effect that may be due to the 1-MCP-induced reduction in POD and Chlase activities.

Abbreviations: 1-MCP – 1-methylcyclopropene, C_2H_4 – ethylene, Chl – chlorophyll, Chlase – chlorophyllase, h° – hue angle, LOX – lipoxygenase, POD – peroxidase

Introduction

Degreening of plant organs during ripening and senescence is the result of the breakdown of chlorophyll (Chl) (Amir-Shapira et al. 1987; Wang 1977). Chl degradation is regulated through several pathways. Chlorophyllase (Chlase) catalyzes the release of the phytol chain from Chl to form chlorophyllide, the first step in Chl catabolism (Sabater and Rodrisol 1978); and Chlase activity is stimulated by ethylene (Amir-Shapira et al. 1987; Trebitsh et al. 1993). Peroxidation of polyunsaturated fatty acids in plant tissues can be catalyzed by lipoxygenase (LOX) to form various primary and secondary oxidation products (Hildebrand 1989). Yamauchi et al. (1986, 1987) reported that chloroplast lipids decrease in spinach leaves during senescence, and suggested that free radicals produced by unstable hydroperoxides could degrade Chl. A peroxidase-hydrogen peroxide system functioning in the presence of phenolic compounds degrades Chl in parsley (Yamauchi and Minamide 1985) and spinach leaves (Yamauchi and Watada 1991).

The green color of broccoli (*Brassica oleracea* L. var. italica Plen) is an important commercial quality index. Degreening of broccoli occurs rapidly after harvest during storage at 20 °C (Wang 1977). Ethylene plays an important role in regulating Chl loss (Tain et al. 1994). High CO_2, exogenous ethanol or acetaldehyde inhibits broccoli ethylene production and delays degreening (Abe et al. 1995; Gong 1993). Treatment of broccoli with the ethylene action inhibitor 1-methylcyclopropene (1-MCP) results in lower

respiration and ethylene production, delayed degreening and an extension of broccoli storage life (Fan and Mattheis 2000; Ku and Wills 1999). The objective of this study was to further examine the roles of ethylene and 1-MCP in stimulating and preventing Chl degradation in harvested broccoli.

Materials and methods

Materials and treatments

'Windsor' broccoli was harvested at a commercial farm near Quincy, WA. The branchlets were separated from the whole head and divided into four groups of similar weight. Branchlets were treated with 1) air (control); 2) 1 $\mu L \cdot L^{-1}$ 1-MCP for 14 hr at 20 °C (1-MCP); 3) 1000 $\mu L \cdot L^{-1}$ ethylene for 5 hr at 20 °C (C_2H_4); or 4) 1-MCP then C_2H_4 (1-MCP + C_2H_4). There were 12 branchlets × 3 replicates per treatment. Treated branchlets were held at 20 °C in the dark for up to three days. 1-MCP was generated from EthylBloc® provided by Rohm and Haas, Co., Philadelphia, PA.

Determination of color and chlorophyll concentration

Broccoli color was determined on the top of the branchlet florets (20 branchlets) with a colorimeter (Minolta CR-200, Osaka, Japan) with an 8 mm measuring aperture measuring tristimulus values L* (lightness), a* (red/green), and b* (yellow/blue). The data were converted to hue angle (h° = arctangent [b*/a*]), ranging from green – (180°) to yellow – (90°) (McGuire 1992). Broccoli florets were cut with a knife and stored at −80 °C until assayed. Analyses for Chl a, Chl b and total Chl were conducted using the method of MacKinney (MacKinney 1941). There were four or five replicates for each treatment.

Assay of chlorophyllase activity

Chlase activity was measured by the modified procedure of Holden (Holden 1961). Acetone powder (100 mg) prepared from broccoli floret tissue was extracted with 10 ml of 5 mM phosphate buffer (pH 7.0; including 50 mM KCl, 0.24% Triton X-100, and 2.5% PVPP) on ice. After 60 min, the extract was centrifuged at 10,000 g for 10 min. The supernatant (0.5 ml) was added to 0.6 ml reaction mixture containing 50 μg Chl and 0.24% Triton X-100 in 50 mM MES buffer in a test tube. The tubes were incubated for 30 min at 25 °C, then the reaction was stopped by addition of 4 ml acetone, 4 ml hexane, and 1 ml of 10 mM KOH. The acetone phase was removed, then absorbance at 667 nm was measured using a HP8451A diode array spectrophotometer (Hewlett Packard, Palo Alto, CA).

Assays of lipoxygenase and peroxidase activity

Acetone powder (100 mg) prepared from broccoli floret tissue was extracted with 10 ml of 0.1 M Tris-HCl buffer (pH 7.2) including 2.5% PVPP. This mixture was centrifuged for 10 min at 10,000 g, then the supernatant was collected and used for LOX and POD assays. LOX activity was determined by monitoring bleaching of methylene blue at 660 nm (Romero and Barrett 1997). POD activity was measured using the method of Vetter et al. (1958) with modification. The supernatant was added to MES buffer (pH 4.5) containing 0.1% p-phenylenediamine and 0.05% H_2O_2. After addition of supernatant, absorbance at 485 nm was monitored for three min.

Protein determination

Protein content in the supernatant was determined by the method of Bradford (Bradford 1976) using a dye reagent from Bio-Rad (Bio-Rad Laboratories, Hercules, CA).

Experimental design and statistical analysis

The experiment was conducted using a completely random design with four treatments and three replications per treatment. Means were analyzed using the SAS ANOVA procedure (SAS Institute, Cary, NC) and comparison of treatment means was performed using Duncan's multiple range test ($p \leq 0.05$).

Results

Degreening of broccoli florets

Chl in broccoli florets decreased during storage at 20 °C (Figure 1) and exposure to 1000 $\mu L \cdot L^{-1}$ C_2H_4 for 5 hr accelerated Chl degradation. Chl degradation was reduced following treatments with 1-MCP with or without subsequent exposure to C_2H_4.

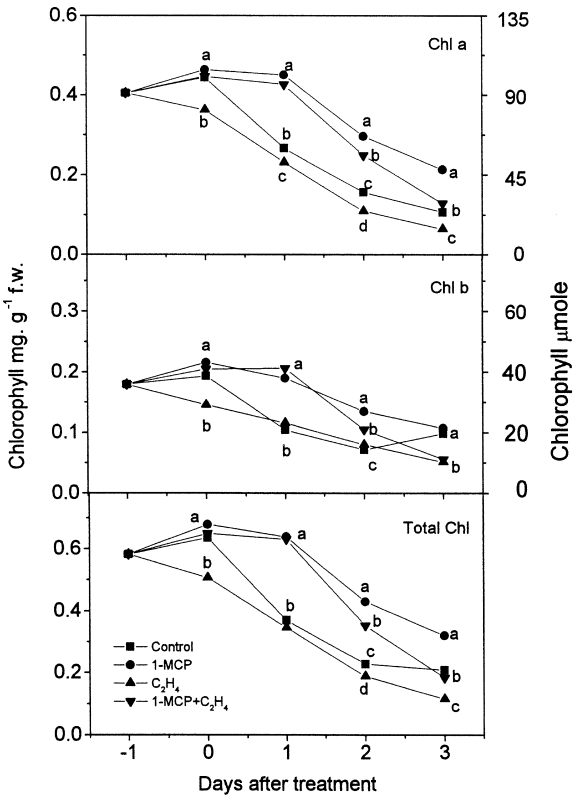

Figure 1. Chlorophyll content in broccoli branchlets. Branchlets were treated with air (control); 1 $\mu L \cdot L^{-1}$ 1-MCP for 14 hr at 20 °C (1-MCP); 1000 $\mu L \cdot L^{-1}$ ethylene for 5 hr at 20 °C (C_2H_4); 1-MCP then C_2H_4 (1-MCP + C_2H_4). Branchlets were held at 20 °C in the dark for up to three days after treatment. Means (n = 4) followed by different letters are significantly different ($p < 0.05$) within the same analysis date.

Floret color, expressed as h° (Figure 2), decreased similarly for controls and broccoli exposed to ethylene. Treatment with 1-MCP or 1-MCP + C_2H_4 delayed initiation of the decrease in h° by one day. Treatment differences in h° were greatest three days after treatment. Florets treated with 1-MCP and C_2H_4 had the highest and lowest °h values, respectively. After 3 d at 20 °C, the h° value for broccoli treated with 1-MCP then C_2H_4 was higher than h° of controls.

Lipoxygenase activity

Extractable LOX activity in broccoli florets decreased during the post-treatment period at 20 °C (Figure 3). At days 0 and 1, LOX activity was highest in florets treated with 1-MCP then exposed to C_2H_4. At 3 d after the treatments, 1-MCP alone resulted in the highest LOX activity. Exposure to C_2H_4 did not increase LOX activity; and there was no apparent correlation between LOX activity and degreening of florets.

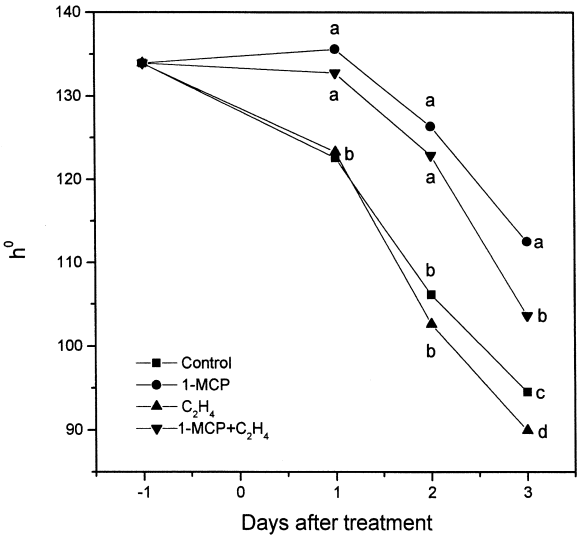

Figure 2. Color (hue angle) on broccoli branchlet surface. Branchlets were treated with air (control); 1 $\mu L \cdot L^{-1}$ 1-MCP for 14 hr at 20 °C (1-MCP): 1000 $\mu L \cdot L^{-1}$ ethylene for 5 hr at 20 °C (C_2H_4); 1-MCP then C_2H_4 (1-MCP + C_2H_4). Branchlets were held at 20 °C in the dark for up to three days after treatment. Means (n = 4) followed by different letters are significantly different ($p < 0.05$) within the same analysis date.

Chlorophyllase activity

Chlase activity decreased during the treatments and through day 1 after the treatments for controls and broccoli treated with 1-MCP with or without subsequent exposure to C_2H_4 (Figure 4). Chlase activity increased between day 0 and 2 for florets exposed to C_2H_4. Chlase activity increased more than 4 fold between days 2 and 3 in florets treated with 1-MCP + C_2H_4.

Peroxidase activity

POD activity was highest in control florets at the end of the treatment period (Figure 5). Exposure to C_2H_4 resulted in increased POD activity in broccoli florets one day after the treatment. Changes in POD activity were delayed by 1-MCP and 1-MCP + C_2H_4 treatments where the increase in POD activity was delayed until the third day after treatment. Regardless of treatments, POD activity increased by the end of experiment.

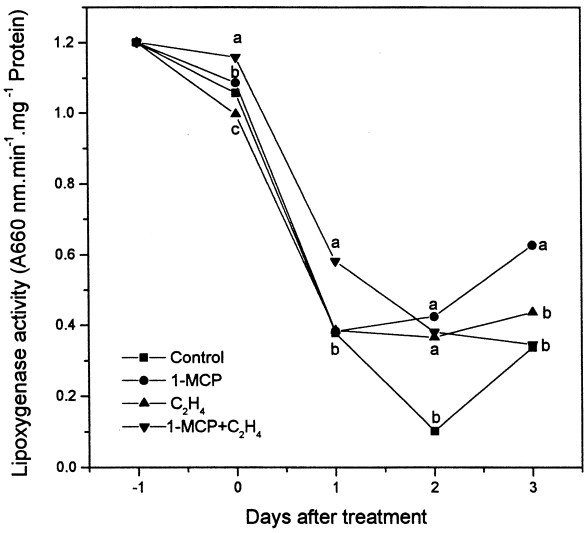

Figure 3. Lipoxygenase activity (A660 nm·min^{-1}·mg^{-1} Protein) in broccoli branchlets. Branchlets were treated with air (control); 1 μL·L^{-1} 1-MCP for 14 hr at 20 °C (1-MCP): 1000 μL·L^{-1} ethylene for 5 hr at 20 °C (C_2H_4); 1-MCP then C_2H_4 (1-MCP + C_2H_4). Branchlets were held at 20 °C in the dark for up to three days after treatment. Means (n = 3) followed by different letters are significantly different ($p < 0.05$) within the same analysis date.

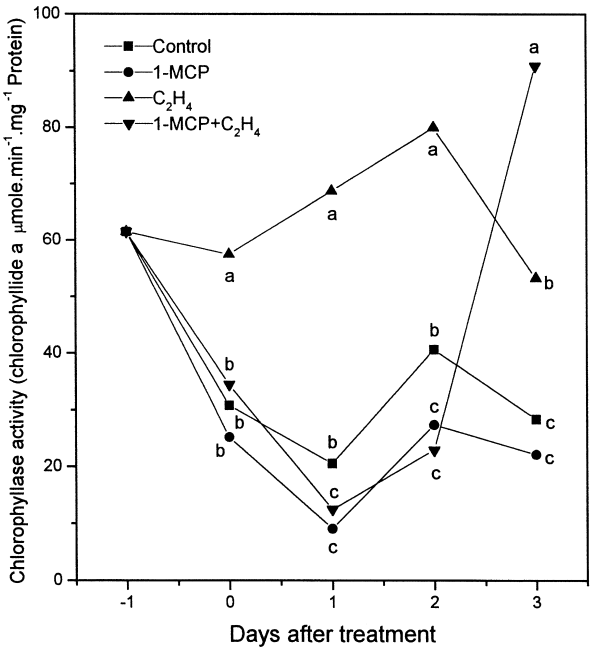

Figure 4. Chlorophyllase activity (chlorophyllide a μmole·min^{-1}·mg^{-1} Protein) in broccoli branchlets. Branchlets were treated with air (control); 1 μL·L^{-1} 1-MCP for 14 hr at 20 °C (1-MCP): 1000 μL·L^{-1} ethylene for 5 hr at 20 °C (C_2H_4); 1-MCP then C_2H_4 (1-MCP + C_2H_4). Branchlets were held at 20 °C in the dark for up to three days after treatment. Means (n = 3) followed by different letters are significantly different ($p < 0.05$) within the same analysis date.

Discussion

Broccoli color changes assessed colorimetrically and presented as h° were similar to those reported previously (Fan and Mattheis 2000; Tain et al. 1994). Treatment of broccoli florets with 1-MCP inhibited Chl degradation and the accompanying reduction in h° values. The data indicate a strong relationship (r = 0.9375) exists between total Chl content and h° in broccoli florets. The relationship between Chl a content and h° (r = 0.9550) is higher than that between Chl b and h° (r = 0.8638). 1-MCP prevents ethylene action by competing for the ethylene receptor (Sisler et al. 1990). Previous studies have reported that 1-MCP treatment of broccoli inhibits the degradation of Chl and delays the onset of degreening (Fan and Mattheis 2000; Ku and Wills 1999). However, Chl catabolism in plant tissue can proceed via several different degradative pathways, and the mechanism by which 1-MCP delays Chl degradation in broccoli is not clear.

LOX catalyzes peroxidation of polyunsaturated fatty acids to various primary and secondary oxidation products (Hildebrand 1989); and Chl degradation has been related to the level of lipid peroxidation products in plant tissue (Yamauchi and Watada 1991). Other reports indicate degreening and Chl loss occurring during storage in controlled (Gong 1993) or modified atmospheres (Zhuang et al. 1995) is accompanied by decreased LOX activity. Similar results in our study also do not support a relationship between LOX activity and degreening of broccoli.

Chlase activity can be induced by exogenous ethylene to accelerate the degradation of Chl in citrus fruit, parsley leaves (Amir-Shapira et al. 1987) and spinach leaves (Yamauchi and Watada 1991). Similarly, Chlase activity is higher following ethylene treatment of broccoli; and the increase in Chlase activity is inhibited by 1-MCP treatment. However, the activity of Chlase in control florets decreased coincident with degreening during holding at 20 °C indicating Chlase activity alone may not be required for Chl degradation in broccoli florets.

Ethylene can induce POD activity (Gahagan et al. 1968; Ku et al. 1970) and increased POD activity may also contribute to enhanced ethylene synthesis (Boyer and de Jaegher 1986). Chl in plant tissue can be degraded by the peroxidase–hydrogen peroxide system (Yamauchi and Minamide 1985). Abeles et al. (1988)

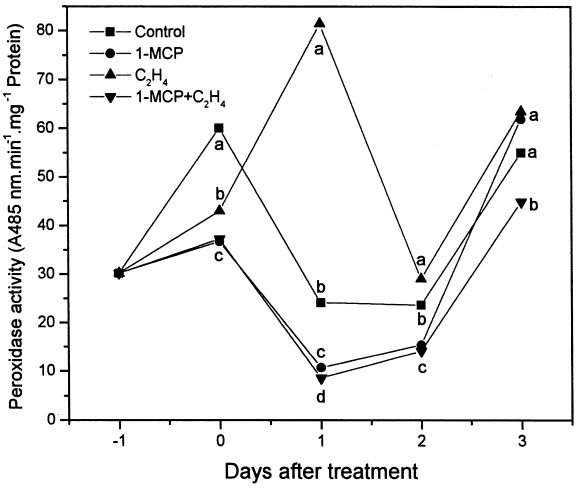

Figure 5. Peroxidase activity (A485 $nm \cdot min^{-1} \cdot mg^{-1}$ Protein) in broccoli branchlets. Branchlets were treated with air (control); 1 $\mu L \cdot L^{-1}$ 1-MCP for 14 hr at 20 °C (1-MCP): 1000 $\mu L \cdot L^{-1}$ ethylene for 5 hr at 20 °C (C_2H_4); 1-MCP then C_2H_4 (1-MCP + C_2H_4). Branchlets were held at 20 °C in the dark for up to three days after treatment. Means (n = 3) followed by different letters are significantly different ($p < 0.05$) within the same analysis date.

found two PODs induced by ethylene in cucumber cotyledons and both forms of POD degraded Chl in vitro. In broccoli, stimulation of POD activity by ethylene is inhibited by 1-MCP, and POD activity is also inhibited by 1-MCP alone. POD activity and Chl degradation are highly correlated in broccoli stored in air or high CO_2 (Gong 1993). Additionally, ethanol (0.2 $\mu L \cdot L^{-1}$) or acetaldehyde (54 $\mu L \cdot L^{-1}$) in air reduces ethylene production and inhibits POD activity and yellowing of broccoli (Abe et al. 1995; Gong 1993). POD activity in broccoli florets decreases when endogenous ethylene action and presumably synthesis is inhibited. As Chlase activity decreases during broccoli degreening, Chl degradation in broccoli may be degraded via the POD-hydrogen peroxide pathway and this system appears to be closely regulated by ethylene. In conclusion, a clear association between LOX activity and Chl degradation is not evident in broccoli. Degreening and increased Chlase and POD activity are delayed by 1-MCP indicating Chl metabolism via these systems is regulated by ethylene action.

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