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Methyl Jasmonate Vapor Promotes β-Carotene Synthesis and Chlorophyll Degradation in Golden Delicious Apple Peel

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Abstract. Methyl jasmonate (JAMe) vapors (≤8 ppm) for 4 h at 25°C dramatically increased Golden Delicious apple peel β-carotene synthesis by nearly threefold to 35 ng/mm^2, while control fruits remained nearly constant at 11 ng/mm^2 during the 10 day measurement period. Chlorophyll *a* and to a lesser extent chlorophyll *b* and lutein degradation were accelerated by JAMe treatment, but all showed some recovery after 6 days. Peel chlorophyll *a*:*b* ratio held almost constant at 4.2–4.5 in control fruits during 10 days, while JAMe-treated apple chl *a*:*b* ratio decreased linearly to 2.9 during 10 days.

Jasmonic acid (JA) and its methyl ester (JAMe) are endogenous growth substances (putative hormones) identified in a wide variety of plant species (Meyer et al. 1984) whose physiological functions have not yet been fully elucidated (Staswick 1992, Hamberg and Gardner 1992), but whose biological effects from exogenous applications have been creating substantial interest among plant scientists (Parthier 1990). Among these effects are a marked degradation of chlorophylls (Weidhase et al. 1987), inhibition of lycopene synthesis, and stimulation of β-carotene synthesis in tomatoes (Saniewski and Czapski 1983). These treatments utilized JAMe applied in a lanolin paste. The volatility of JAMe at moderate temperatures (25°C) has led us to attempt vapor treatments of fruits (Olías et al. 1991) and to examine effects on ripening parameters, including peel pigments.

Workman (1963) showed that there were three classes of pigments responsible for the color changes in apples: flavonoids (in the red varieties), chlorophyll, and carotenoids. Further research (Galler and Mackinney 1965, Valadon and Mummery 1967, Knee 1972, Gorski and Creasy 1977) has led to specific identification of carotenoid pigment subclasses, and more recently Gross et al. (1978, 1979) reported mature Golden Delicious apples to have 40–45% violaxanthin, 10–30% neoxanthin, 10% carotene, and 9–10% lutein with only minor amounts of other carotenoids.

During the maturation and subsequent storage of apple fruits, carotenoids generally increase, while the chlorophylls decrease (Gross et al. 1978), such that the fruits acquire the characteristic yellow colors of this variety. Studies with mango (Jacob et al. 1970) indicated a loss of lutein but an increase in carotene during ripening. It has been claimed that banana skins (von Loesecke 1929) and some varieties of apples (Valadon and Mummery 1967) maintain a constant carotene level as chlorophyll is degraded during ripening. The rind or peel of fruits is frequently the region of higher carotenoid concentration. The concentration in apple peel, for example, is five times that of the flesh.

Due to our current interest in the effects of exogenously applied JAMe vapors on ripening parameters, such as aroma development (Olías et al. 1992), ethylene production (Olías et al. 1991), and color changes, the objective of this paper was to study the effects of low concentration vapor applications of JAMe on peel pigments of mature, intact Golden Delicious apple fruits.

Materials and Methods

Golden Delicious apples were obtained from local (Seville, Spain) commercial wholesale markets. These were carefully color-matched and sorted to remove any fruits with defects at the

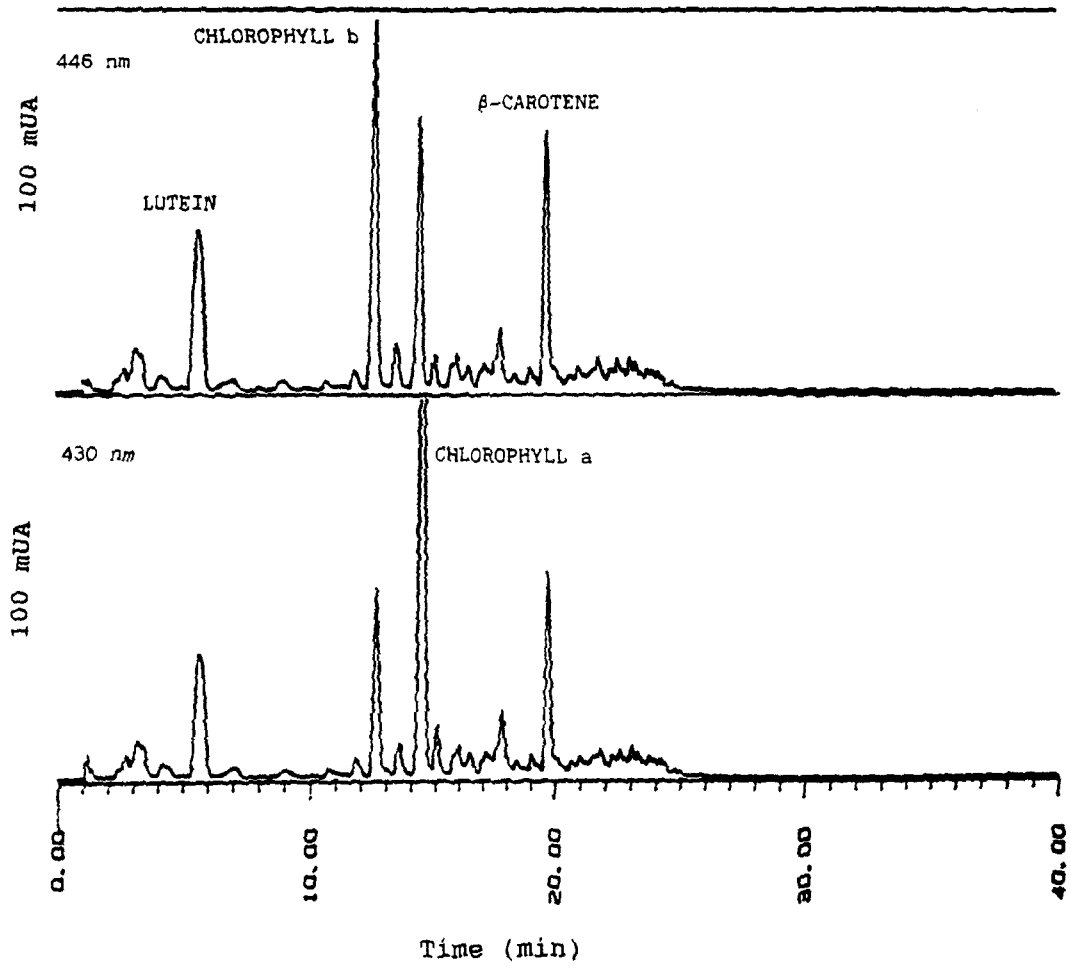

Fig. 1. *HPLC chromatogram of Golden Delicious apple peel pigments extracted with dimethylformamide.*

beginning of each experiment to assure uniformity. Jasmonic acid methyl ester was Lot 30, ref. no. 13124.87.06 purchased from Firmenich (Basel, Switzerland).

All experiments were carried out in triplicate in the dark at 25°C. Five intact apples (~1 kg) were placed in 4 L glass jars with a small watch glass on the bottom which contained 15 mg of liquid JAMe. The jars were sealed, and placed in the dark for 4 h to allow JAMe to volatilize into the atmosphere of the jar. Control fruits were treated similarly, but without JAMe. After 4 h of initial JAMe vapor treatment, the jars were opened, a lid allowing gas exchange was attached, JAMe was left in the jars, and they were returned to darkness for the duration of the experiment. Sufficient jars were prepared to allow fruits to be sampled every 2 days for up to 2 weeks. Jasmonic methyl ester concentrations in the jars were measured by GC-MS-specific ion monitoring at m/e 224. After 4 h at 25°C, the volatile JAMe concentration reached 8 ppm in the sealed jars.

Peel disks (triplicate samples) were cut with an 8 mm diameter cork borer, 15 disks were placed in a test tube, covered with 3 ml dimethylformamide (DMF), air bubbles were removed, the tubes were sealed, covered with foil, and held at 4°C for 24 h in the dark. The DMF was collected, replaced by a second 3 ml of DMF, and the disks extracted an additional 24 h at 4°C. The combined DMF extracts were filtered through a 0.45 μM microfilter, and triplicate samples were ready for HPLC analysis of pigments. HPLC analysis was carried out on a Hewlett-Packard Model 1090 HPLC, with Model 85B controller, diode-array UV-Vis detector, and model 9121 data processor. The column was a 2 mm × 250 mm Beckman Ultrasphere ODS (C18) 5 μM, operated at 30°C, fitted with a 100 μl injection loop in a Rheodyne valve. Two-step gradient elution utilized solvent A:acetonitrile/H_2O (90:10) and B:ethyl acetate, programmed at 0.5 ml/min, starting with 10% B at time zero increasing to 20% B in 5 min, then to 100% B at 30 min and thereafter. Diode array detection was set from 320–600 nm, with specific monitoring at 430 and 446 nm. Post-run data processing allowed specific spectra of individual peaks to assist in identification. Pigment concentrations were calculated based upon calibration curves with commercial standards and expressed as ng pigment/mm² of peel. Calculated molar extinction coefficients were found to be in good agreement with those published in the literature (Davies 1976, Khachik et al. 1986, Mínguez-Mosquera and Garrido-Fernández 1986).

Results

Figure 1 shows the HPLC separation of Golden Delicious apple peel pigments extracted by the mild DMF procedure used in this study. Every pigment

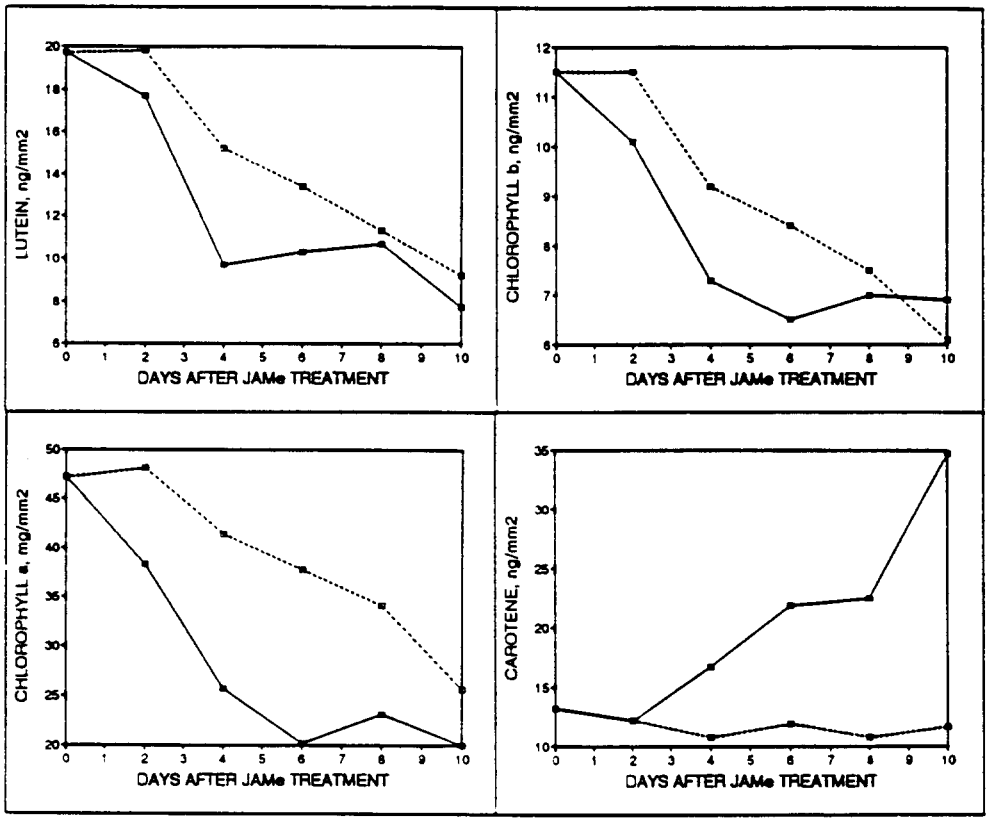

Fig. 2. Concentration in the four major pigments in Golden Delicious apple peel following JAMe treatment: 8 ppm JAMe in the atmosphere(—); untreated control(· · ·).

was identified by comparison to its characteristic spectra and by similarity in the retention time after spiking the pigment standards to the extracts. Surprisingly, no violaxanthin was detected in the apple peels by the methods used in this study. This contrasts sharply with other reports (Gross 1979, Gross and Eckhardt 1978, Gross and Lenz 1979) which indicated violaxanthin up to 45% in Golden Delicious apple peel. This is not due to differences in extraction by DMF compared to acetone which was used in previous studies. However, since our samples were not subjected to saponification or preparative TLC on silica, this could account for the differences. The recent paper by Kimura et al. (1990) points out that saponification can lead to significant carotenoid and xanthophyll changes, qualitatively as well as quantitatively.

Figure 2 shows the effects of JAMe on peel lutein, chlorophyll *a*, chlorophyll *b*, and β-carotene. Peel lutein decreased from 20 ng/mm^2 to less than 10 ng/mm^2 during 10 days at 25°C. The rate of loss was accelerated by the JAMe treatment. Beta-carotene dramatically increased from 11 ng/mm^2 to 35 ng/mm^2 in JAMe-treated apples, whereas control apple peel β-carotene remained essentially unchanged at 11 ng/mm^2 over 10 days. Chlorophyll *a* and *b* in the controls were degraded at almost the same rate (4.5% and 4.3% per day, respectively) but JAMe treatment accelerated chlorophyll *a* loss by about 35% and chlorophyll *b* loss by about 20% compared to control fruits.

Discussion

Carotenoids are normal constituents of the photosynthetic apparatus of chloroplasts and are, therefore, present in green fruit tissues prior to maturation. Maturation does not always involve an accumulation of carotenoids—many varieties of apples, pears, grapes, and olives belong to this group (Goldschmidt 1983). Thus, the color change of ripe fruit from green to yellow may be partly explained by the unmasking of existing carotenoids as chlorophylls are degraded.

Golden Delicious apple control fruits held for 10 days in the dark at 25°C had decreased lutein, and chlorophyll *a* and *b*, about 43% less than at the start of the experiment. When JAMe vapors were enclosed in the atmosphere for 4 h on the first day, this very low (8 ppm) gaseous concentration caused some dramatic changes in apple peel pigments,

stimulating a substantially greater (66%) loss of the above compounds. Surprisingly, there appeared to be recovery of chlorophyll synthesis in the dark 6 days after the JAMe treatment began; thus, the effect of the initial JAMe vapor treatment on chlorophyll was only transitory.

Most notable was the increase in β-carotene synthesis. Initially, there was a 2 day lag in JAMe stimulated β-carotene synthesis; but once started, β-carotene increased linearly by almost 3 ng/mm^2 peel/day over the following 8 days, when the experiment was terminated. The same stimulatory effect on β-carotene synthesis by JAMe was found in treated tomato fruit (Saniewski and Czapski 1983). Control apple peel β-carotene concentrations remained essentially constant, which agrees with the earlier reports by Valadon and Mummery (1967).

The complement of carotenoids that an organism can synthesize is under genetic control; however, the amount, and to some extent, the type of carotenoid actually synthesized, can be influenced by a number of factors. These include nutritional factors, light, temperature, and the presence of certain chemicals. The genetic control of carotene biosynthesis has been extensively studied in some higher plants. In tomato fruits, for instance, at least four major genes have been identified (Darby 1978). Understanding how these genes are switched on and off, however, remains for future research.

Growth regulators have been used as powerful tools to improve fruit coloration; in this respect, it is well known that ethylene accelerates chlorophyll degradation (Purvis and Barmore 1981) as well as the synthesis of carotenoids (Steward and Wheaton 1972). Applications of JAMe vapors to Golden Delicious apples have been shown to significantly stimulate ethylene formation (2.5- and 4.6-fold in the cortical and peel tissues, respectively) (Olías et al. 1991). This concomitant effect on ethylene and carotenoids was found in both studies and raises the question as to whether JAMe directly stimulates carotenoid biosynthesis or indirectly via ethylene stimulation which then affects carotenoids. On the other hand, there is evidence suggesting that carotenoids are biosynthetic precursors of abscisic acid (ABA) through lipoxygenase action (Parry and Horgan 1991a,b). In a previous work we found an increase in lipoxygenase activity in JAMe-treated apples (Olías et al. 1992) that could also support a possible interaction between JAMe and ABA.

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