

The Effect of Methyl Jasmonate Vapour on Some Characteristics of Fruit Ripening, Carotenoids and Tomatine Changes in Tomato (*Lycopersicon esculentum* Mill.)

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Abstract

Tomato ripening in normal red-fruited cultivar (Fiorin) was delayed by treatment with methyl jasmonate (JA-Me) vapour. A visual scoring system for describing tomato ripening was used. Surface of fruits exposed to JA-Me vapour, increased in yellow and decreased in red as determined by HunterLab colour meter. JA-Me significantly altered the firmness of fruits after 21 days storage. Vapour of JA-Me enhanced the level of β -carotene in outer part (peel with 3 mm pericarp tissue) of fruit, while it had no effect in peeled fruit pericarp. JA-Me treatment decreased the level of lycopene in outer part and pericarp tissue, however, in outer part lycopene content decreased at a higher rate than in pericarp. Amount of tomatine in fruits treated with JA-Me had enhanced four-fold in outer part and by 62% in peeled fruit pericarp as compared with the control.

Key words: *Lycopersicon esculentum*, methyl jasmonate, tomato fruits, ripening, firmness, carotenoids, tomatine.

INTRODUCTION

Jasmonic acid and methyl jasmonate are endogenous growth substances identified in a wide variety of plant species (Meyers et al., 1984). The biological effects from exogenous applications have been creating substantial interest (Parrish et al., 1990). Some physiological processes during the ripening of tomatoes are affected by methyl jasmonate (JA-Me). JA-Me inhibits lycopene, and stimulates β -carotene accumulation during ripening (Saniewski and Czapski, 1983) as well as chlorophyll degradation (Saniewski et al., 1987 a) and ethylene production (Saniewski and Czapski, 1985). JA-Me enhances ethylene production by its

stimulatory effect on the turnover of 1-aminocyclopropane-1-carboxylic acid (ACC) (Saniewski et al., 1987 b, 1987 c). It was found (Saniewski et al., 1987 a) that the stimulatory effect of JA-Me on chlorophyll degradation and on ethylene production in mature green tomatoes was not associated with the induction of polygalacturonase activity, development of which was inhibited by JA-Me in ripening tomatoes. It was also shown that in tomato fruit, JA-Me stimulates polyphenol oxidase and inhibits peroxidase activity (Czapski and Saniewski, 1988), decreases tocopherol (Czapski et al., 1991) and greatly increases linolenic acid content (Czapski et al., 1992). Czapski and Saniewski (1992) found that JA-Me evidently stimulates ethylene production and ACC oxidase activity, more efficiently in nor than in rin tomato mutant fruits.

The ripening process of tomato can be detected by a change in colour from green to red. In addition to the change in colour there is a change in aromatic organic compounds that gives the flavor to ripened fruits. Also ripened fruits are soft and fleshy as a result of changes in pectic substances. Tomatine content decline during tomato fruit ripening (Eltayeb and Roddick, 1984 a) while lycopene synthesis is activated during this process (Sander, 1956, 1958).

The physiological affects of methyl jasmonate observed during the ripening of tomatoes, has largely been studied by applying JA-Me as a lanolin paste. In the present paper JA-Me in the vapour form has been studied as a number of ripening parameters.

MATERIALS AND METHODS

The normal ripening cultivar of tomato used was the red-fruited Fiorin. Tomato plants were grown in a glasshouse during the summer of 1994. Mature green fruits were harvested, rinsed with water and surface dried with tissue. Intact tomato fruits were placed in 18 l sealed glass desiccator which contained on the bottom 1.8 ml liquid methyl jasmonate in 2 ml of ethyl alcohol for easier vapourization of JA-Me. Control fruits were treated similarly but only with ethyl alcohol. During the experiment fruits were kept at room temperature (about 20°C) in natural light. Each treatment consisted of 10 fruits.

Assessment of ripeness

A visual scoring system of Simons (1969) as modified by Vickery and Bruinsma (1973) for describing tomato ripening was used as detailed in Table 1. Fruits were scored daily for ripeness and treated and control fruits were all removed for analyses after 21 days, when the control fruits were fully ripe.

Surface colour determination

Surface colour was determined using ColorQuest HunterLab colour meter fitted with a 25 mm diameter aperture. For each fruit, four colour readings of Hunter's

values a (redness) and b (yellowness) were taken at different positions on the surface.

Fruit firmness

Fruit firmness was tested (Holt, 1970) using the Warner Bratzler meat shear with an Instron Food Testing Instrument model 1140. Each individual fruit was inserted into a triangular opening in the blade and cut in half by the blade between two rectangular bars. The peak force required to shear the fruit at constant speed was recorded.

Test parameters:

Crosshead drive speed: 200 mm min⁻¹

Chart drive speed: 100 mm min⁻¹

Force range : 500 N full scale.

Halves of individual fruit were frozen and kept at -25°C until analysis for carotenoids and tomatine.

Analysis of carotenoids

One half of the frozen fruit was taken for β -carotene and lycopene determination. Carotenoids were analysed separately in peel with 3 mm pericarp tissue (outer part) and in peeled fruit pericarp. The latter was homogenized in a Waring blender. Four grams of homogenate or 4 g of peel with 3 mm pericarp tissue were ground in a mortar with pestle, using an acetone and hexane mixture (2 : 1 v/v). The homogenate was vacuum filtered through Whatman No 1 filter paper and washed repeatedly until colourless. Next steps of the analysis were made according to the procedure described previously (Saniowski and Zapiski, 1983). Carotenoids were determined in a minimum of four replications per treatment.

Analysis of tomatine

Tomatine was determined in the other halves of the frozen tomato fruits, separately in peel with 3 mm pericarp tissue and in peeled fruit pericarp. The latter was homogenized in a Waring blender. Tomatine was extracted from 1 g sample of homogenate and from 1g of peel with 3 mm pericarp by a procedure modified from Eltayeb and Roddick (1984 a, b). The sample was extracted using a mortar with pestle, in 5 ml 96 % (v/v) methanol containing 2 % (v/v) acetic acid. After 16 h, the homogenate was centrifuged at 16000g for 20 min and the residue was re-extracted twice more with 5 ml 64 % (v/v) methanol for 5 and 2 hours. The tomatine contained in combined supernatants was hydrolyzed by refluxing with equal volume of 2 N hydrochloric acid. After cooling, the hydrolysate was adjusted to pH 10 with concentrated ammonia, and tomatidine was extracted twice with 30 ml of chloroform. Chloroform extract, was dried over anhydrous sodium sulfate and concentrated at 45°C on rotary evaporator under vacuum to about 0.5 ml and then it was transferred with chloroform to 2 ml volume in glass stoppered graduate tube. Aliquots of 0.2-0.5 ml of tomatidine extract was evaporated to dryness and then silylated

dissolved in 0.2 ml mixture of chloroform, N-trimethylsilylimidazole (TMSI) and N-trimethylsilyldiethyl-amine (TMSDEA) (2 : 1 : 1). Silylation was performed in tightly capped silylation vials by heating at 70°C for 0.5 h. After the mixture cooled to 20°C, 1 µl of sample was injected into a Hewlett-Packard 5890 series II gas chromatograph equipped with a 5971A mass selective detector, both working in GC/MSD/ChemStation system. Trimethylsilyl ether of tomatidine was analysed on a HP-1 capillary column (12.5 m x 0.2 mm). Identification of tomatidine was achieved by comparison retention time and examining in the SCAN mode mass spectrum of particular peak with trimethylsilyl (TMS) ether of authentic tomatidine standard (purchased from Sigma). The mass spectrum similarity was expressed as match quality, using the probability based matching algorithm. The mass spectral database of TMS-tomatidine standard was stored in a computer in our own created library. Amount of tomatidine was calculated from standard curve of TMS-tomatidine standards measured in the SIM mode using 4 characteristic mass ion (120.10, 121.15, 122.15, 234.20). Recovery of tomatine added to tomato pericarp homogenate samples was evaluated. These samples were extracted and analysed as usual, to determine percent recovery. Halves of tomato fruit were analysed at least in triplicate per treatment.

RESULTS

Control fruits ripened after 17 days. At this time untreated fruits had a score of 13, while in JA-Me treated samples only hints of colour development were apparent (Table 1). Fruits treated with JA-Me vapour had barely changed from their initial (green) appearance after 15 days, at which time the controls were pink all over. Untreated fruits took 21 days to ripen fully (score 15), while at the same time the samples treated with JA-Me were only orange-pink (score 9.2) (Table 1).

Methyl jasmonate vapour had a substantial effect on the surface colour of tomato fruits after 21 days of storage (Table 2). Fruits exposed to JA-Me decreased in red and increased in yellow colour. The difference in Hunter's a and b between control and JA-Me treated fruits was more pronounced for a ratio of the a to b readings (Table 2). This ratio is a convenient and accurate method of expressing the colour of tomato juice within the brightness and chromaticity ranges normally encountered (Anonymous, 1958). The a/b ratio was over 1.7 times higher for the control fruits than for JA-Me treated samples.

Applied JA-Me vapour significantly delayed softening of tomato fruits after 21 days of storage (Table 2). The force required to shear JA-Me treated fruits was 1.8 times higher than that for control.

Results of β -carotene and lycopene contents in fruit tissues are presented in Table 3. JA-Me treatment enhanced 2.7 times β -carotene content in peel with 3 mm pericarp tissue, but had no affect in peeled fruit pericarp. JA-Me vapour reduced lycopene content as compared to control more in the outer part of fruits than in

peeled fruit pericarp. Parallel determination of carotenoids from peel with 3 mm pericarp tissue and fruit pericarp revealed much higher levels of β -carotene and lycopene in outer part of fruit than in pericarp, both in control and JA-Me treated fruits.

Spiking experiments of tomato homogenate with authentic tomatine gave a mean recovery of 92 % of added alkaloid at the level of 12 μg per 1 g fresh weight and the limit of detection was 0.2 μg per 1 g fresh tissue.

Comparison of the level of tomatine in the control and JA-Me treated fruits revealed remarkable differences (Table 4). After 21 days, amount of tomatine in fruits treated with vapour of JA-Me had reduced tomatine loss four-fold in peel with 3 mm pericarp tissue and by 62 % in peeled fruit pericarp as compared with control. Distribution of tomatine in fruit for both control and JA-Me treated samples revealed substantial differences; much higher alkaloid level in outer part than in peeled fruit pericarp (Table 4).

Table 1

The effect of methyl jasmonate vapour on the ripening of harvested mature green tomato fruits (expressed in mean ripeness scores \pm SD)

Storage time (days)	Control	JA-Me
0	1.0 \pm 0.0	1.0 \pm 0.0
10	5.4 \pm 1.4	1.7 \pm 0.6
15	10.2 \pm 2.1	3.0 \pm 0.7
17	13.0 \pm 0.9	5.0 \pm 1.4
18	13.8 \pm 1.0	6.0 \pm 1.1
19	14.2 \pm 0.5	8.2 \pm 0.9
21	15.0 \pm 0.5	9.2 \pm 1.1 (orange-pink)

Score:

- 1 – Entirely green
- 2 – Green with translucent streaks in sutures at blossom end
- 3 – Breaker; faint yellow-pink in sutures at blossom end
- 6 – Pink in sutures extending approximately half way to stem end
- 8 – Colour in sutures almost to stem end
- 10 – Pink all over, no green
- 13 – Table ripe, entirely red, feels firm
- 15 – Fully ripe, intense red, feels soft, peels easily

Table 2

The effect of methyl jasmonate vapour on surface colour (expressed as Hunter's colour values) and firmness (expressed as a value of force required to shear the fruit) after 21 days of storage of tomatoes

Treatment	Hunter's colour values			Firmness (N)
	a (redness)	b (yellowness)	a/b	
Control	28.0 a	16.2 b	1.74 a	74.7 b
JA-Me	20.1 b	20.5 a	0.99 b	132.7 a

Means within columns followed by the different letters are significantly different by Newman – Keuls test at $P = 0.05$

Table 3

The effect of methyl jasmonate (JA-Me) vapour on β -carotene and lycopene contents ($\mu\text{g}\cdot\text{g}^{-1}$) in tomato fruits after 21 days of storage

Fruit tissue	β -Carotene		Lycopene	
	Control	JA-Me	Control	JA-Me
Peel with 3 mm pericarp tissue	2.90 c (100)*	7.78 b (268)	39.80 a (100)	7.36 b (18)
Peeled fruit pericarp	0.64 e (100)	0.69 e (108)	2.56 c (100)	1.18 d (46)

* in parentheses % from the control (100 %)

Means followed by the same letter are not significantly different by Newman – Keuls test at $P = 0.05$

Table 4

The effect of methyl jasmonate (JA-Me) vapour on tomatine content in tomato fruit after 21 days of storage

Fruit tissue	Tomatine content ($\mu\text{g}\cdot\text{g}^{-1}$)		
	Initial (green)	Control	JA-Me
Peel with 3 mm pericarp tissue	58.3	2.19 b (100)*	9.05 a (413)
Peeled fruit pericarp	45.8	0.40 d (100)	0.65 c (162)

* in parentheses % from the control (100 %)

Means followed by the different letters are significantly different by Newman – Keuls test at $P = 0.05$

DISCUSSION

Some variations in ripening rate occurred for individual fruits. This required to record the score of individual fruit to calculate mean score of all fruits. Despite the variability in ripening rate, subjective scoring system adequately reflected differences in ripening of JA-Me treated and control fruits. The acceptability of this scoring system was also confirmed by Eltayeb and Roddick (1984 b). By slightly modifying some of the scoring characteristics, they could also utilize the system for assessing ripeness in orange- and yellow-fruited cultivars. Delay in fruit ripening caused by JA-Me vapour greatly affected their firmness. It is well known that firmness declined during ripening, corresponding to develop polygalacturonase activity. Polygalacturonase is almost absent in mature green tomato fruits and develops rapidly during ripening (Hobson, 1964; Tucker et al., 1980). Tigchelaar

(1978) and P o o v a i a c h and N u k a y a (1979) suggest that polygalacturonase activity is an essential catalyst of ripening and has a primary role in the initiation of ripening in tomatoes. We found earlier, that JA-Me inhibited the development of polygalacturonase activity in ripening tomatoes (S a n i e w s k i et al., 1987 a). Our previous report indicated, that vapour from 10 μ l and 50 μ l JA-Me markedly stimulated β -carotene accumulation in pale red and red ripe fruits (C z a p s k i and S a n i e w s k i, 1985). The calculated concentration of JA-Me vapour from 50 μ l was 25 μ l per liter. This finding was confirmed in experiments reported here. We calculated the JA-Me vapour concentration to be 100 μ l per liter. This greatly increased β -carotene in the outer part of fruit, while it had no effect in peeled fruit pericarp; development of lycopene was more suppressed in the outer part than in the pericarp. The changes in β -carotene recorded here are similar to those reported by P e r e z et al. (1993) in Golden Delicious apple peel. The effect of JA-Me vapour on carotenoids in tomato fruit raises the question whether JA-Me directly influences carotenoids level by e.g. enzymatic transformation of colourless carotenoids, or indirectly via interaction between JA-Me and other growth regulators (K h u d a i r i and A r b o l e d a, 1971).

In all tomato fruits the tomatine concentration continuously declined from a high level in an early stage of development to a low or negligible level as maturation proceeds (F r i e d m a n et al., 1994). Fruit in which ripening was retarded by incubating under reduced pressure, had a higher alkaloid level than control (E l t a y e b and R o d d i c k, 1984 b). Our results also support this inverse dependence between fruit ripening (pigmentation) and tomatine accumulation. It seems less likely, that JA-Me is involved *per se* in tomatine accumulation and/or degradation processes.

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REFERENCES

- A n o n y m o u s, 1958. Judging quality of tomatoes for processing by objective color evaluation with subjective estimation of defects. Marketing Res. Rep., USDA, No. 235, 1-47.
- C z a p s k i J., S a n i e w s k i M., 1985. Effect of methyl jasmonate on carotenoids in tomato fruits. Gartenbauwiss. 50: 35-37.
- C z a p s k i J., S a n i e w s k i M., 1988. The effect of methyl jasmonate on polyphenol oxidase and peroxidase activities in tomato fruit. Bull. Pol. Ac.: Biol. 36: 127-132.
- C z a p s k i J., S a n i e w s k i M., 1992. Stimulation of ethylene production and ethylene forming enzyme activity in fruits of the non-ripening nor and rin tomato mutants by methyl jasmonate. J. Plant Physiol. 139: 265-268.
- C z a p s k i J., H o r b o w i c z M., S a n i e w s k i M., 1991. The effect of methyl jasmonate on tocopherol content in ripening tomato fruit. Bull. Pol. Ac.: Biol. 39: 41-45.
- C z a p s k i J., H o r b o w i c z M., S a n i e w s k i M., 1992. The effect of methyl jasmonate on free fatty acids content in ripening tomato fruits. Biol. Plant. 34: 71-76.

- Eltayeb E. A., Roddick J. G., 1984 a. Changes in the alkaloid content of developing fruits of tomato *Lycopersicon esculentum* Mill. I. Analyses of cultivars and mutants with different ripening characteristics. J. Exp. Bot. 35: 252-260.
- Eltayeb E. A., Roddick J. G., 1984 b. Changes in the alkaloid content of developing fruits of tomato *Lycopersicon esculentum* Mill. II. Effects of artificial acceleration and retardation of ripening. J. Exp. Bot. 35: 261-267.
- Friedman M., Levin C. E., McDonald G. M., 1994. α -Tomatine determination in tomatoes by HPLC using pulsed amperometric detection. J. Agric. Food Chem. 42: 1959-1964.
- Hobson G. E., 1964. Polygalacturonase in normal and abnormal tomato fruit. Biochem. J. 92: 324-332.
- Holt C. B., 1970. Measurement of tomato firmness with a universal testing machine. J. Texture Stud. 1: 491-501.
- Khudairi A. K., Arboleda O. P., 1971. Phytochrome-mediated carotenoid biosynthesis and its influence by plant hormones. Physiol. Plant. 24: 18-22.
- Meyer A., Miersh O., Buttner C., Dath W., Sembdner G., 1984. Occurrence of the plant growth regulator jasmonic acid in plants. J. Plant Growth Regul. 3: 1-8.
- Parthier B., 1990. Jasmonates: Hormonal regulators or stress factors in leaf senescence? J. Plant Growth Regul. 9: 57-63.
- Perez A. G., Sanz C., Richardson D. G., Olias J. M., 1993. Methyl jasmonate vapor promotes β -carotene synthesis and chlorophyll degradation in Golden Delicious apple peel. J. Plant Growth Regul. 12: 163-167.
- Poovaiah B. W., Nukaya A., 1979. Polygalacturonase and cellulase enzymes in the normal Rutgers and mutant rin tomato fruits and their relationship to the respiratory climacteric. Plant Physiol. 64: 534-537.
- Sander H., 1956. Studien über Bildung und Abbau von Tomatin in der Tomatenpflanze. Planta, 47: 374-400.
- Sander H., 1958. Tomatin ein möglicher Ausgangsstoffe für die Lycopinbildung. Naturwiss. 45: 59.
- Saniewski M., Czapski J., 1983. The effect of methyl jasmonate on lycopene and β -carotene accumulation in ripening red tomatoes. Experientia 39: 1373-1374.
- Saniewski M., Czapski J., 1985. Stimulatory effect of methyl jasmonate on ethylene production in tomato fruits. Experientia 41: 256-257.
- Saniewski M., Urbaneck H., Czapski J., 1987 a. Effects of methyl jasmonate on ethylene production, chlorophyll degradation and polygalacturonase activity in tomatoes. J. Plant Physiol. 127: 177-181.
- Saniewski M., Czapski J., Nowacki J., 1987 b. Relationship between stimulatory effect of methyl jasmonate on ethylene production and 1-aminocyclopropane-1-carboxylic acid content in tomatoes. Biol. Plant., 29: 17-21.
- Saniewski M., Nowacki J., Czapski J., 1987 c. The effect of methyl jasmonate on ethylene production and ethylene-forming enzyme activity in tomatoes. J. Plant Physiol., 129: 175-180.
- Simons D. H., 1969. Possible relationships of ethylene to tomato fruit development and ripening. Ph. D. Thesis, University of California, Davies.
- Tigchelaar E. C., McGlasson W. B., Buescher R. W., 1978. Genetic regulation of tomato fruit ripening. Hort Science 13: 508-513.
- Tucker G. A., Robertson N. G., Grierson D., 1980. Changes in polygalacturonase isoenzymes during the ripening of normal and mutant tomato fruit. Eur. J. Biochem. 112: 119-124.
- Vickery R. S., Bruinsma J., 1973. Compartments and permeability for potassium in developing fruits of tomato *Lycopersicon esculentum*. Mill. J. Exp. Bot. 35: 252-260.

Wpływ par estru metylowego kwasu jasmonowego na niektóre cechy dojrzewania pomidorów oraz zmiany zawartości karotenoidów i tomatyny

Streszczenie

Dojrzewanie zielonych pomidorów odmiany Fiorin było opóźnione przez pary estru metylowego kwasu jasmonowego (JA-Me). Zastosowano wizualną skalę określającą stopień dojrzałości owoców. Pomiar

zabarwienia powierzchni owoców przy użyciu spektrokolorymetru HunterLab wykazały, że pomidory traktowane parami JA-Me były mniej czerwone i bardziej żółte od owoców kontrolnych. Traktowanie parami JA-Me podwyższało zawartość β -karotenu i obniżało poziom likopenu w skórce i przylegającej do niej tkance perykarpu grubości 3 mm. JA-Me nie powodował zmian zawartości β -karotenu ale obniżał poziom likopenu (aczkolwiek w mniejszym stopniu niż w skórce) w perykarpie głębszych warstw owoców. Zawartość tomatyny w pomidorach traktowanych parami JA-Me była 4-krotnie wyższa w skórce i przylegającej do niej 3 mm grubości tkance perikarpu i o 62 % wyższa w głębszych warstwach perikarpu w porównaniu z pomidorami kontrolnymi.