Inhibitory Effect of Methyl Jasmonate on the Volatile Ester-Forming Enzyme System in Golden Delicious Apples

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Golden Delicious apples, after removal from controlled atmosphere, were stored in the dark at 25 °C in an atmosphere containing vapors of methyl jasmonate (8 ppm), and the evolution of aroma components was studied. The sum of ester concentrations of treated apples, in relation to that of controls, decreased by more than 50% after 6 days of treatment. Intact apples held in atmospheres containing acetic acid and hexanol vapors resulted in more than a 2-fold increase in the sum of hexyl ester concentration in the headspace. Hexyl ester formation was inhibited 50–90% by methyl jasmonate.

INTRODUCTION

The typical flavor compounds of fruits are not exhibited during growth, nor are they usually present at the time of harvest. Flavor in fruits, such as bananas, pears, and apples, is produced during a short period related to the climacteric rise in respiration. An important part of apple production, in most countries, is storage in controlled atmosphere (CA) each year. With the improvement of storage technology, apples are maintained with good appearance long after harvest, but the lack of aroma of apples stored under CA conditions is well-known. Guadagni et al. (1971) established that apple fruits from a commercial CA store were defective in ester production after transfer to air. Willaert et al. (1983) objectively proved that there is a significant flavor decrease in apples and a deficiency of ester production after long CA storage.

As flavor quality is a dynamic process and it is accepted that the esters are synthesized enzymatically by combining acid and alcohol moieties, the possibility of enhancing the aroma of stored apples by feeding exogenous precursors has been studied by several groups (Knee and Hatfield, 1981; De Pooter et al., 1983, 1987; Berger and Drawert, 1984; Bartley et al., 1985). Another possibility could be to directly stimulate the ester-forming enzymes.

Jasmonic acid and methyl jasmonate have been suggested as new phytohormones for a number of reasons including their ubiquitous occurrence in the plant kingdom (Meyer et al., 1984), biological activity at low concentrations (Saniewski and Czapski, 1983; Saniewski et al., 1987; Raghavendra and Reddy, 1987), and interaction with other hormones (Ueda and Kato, 1982).

The present investigation was conducted to study the effect of methyl jasmonate atmosphere on the evolution of aroma compounds of Golden Delicious apples ripened in standard conditions after removal from controlled atmosphere.

EXPERIMENTAL PROCEDURES

Materials. Golden Delicious apples were obtained from a local supplier from January to June (1990). The apples were used directly or stored in cold rooms (4 °C) before use.

Whole Fruit Experiments. Carefully color matched fruits, with similar internal ethylene concentration, were randomly divided into batches of four fruits (ca. 650 g), placed in glass jars (ca. 5 L), and held in a dark room at 25 °C. In the experiment with methyl jasmonate, 15 mg was placed in a watch glass at the bottom of the jar and for 4 h the jar was tightly closed. In these conditions, methyl jasmonate concentration in the jar atmosphere, calculated by headspace on column GC–MS–SIR was 8 ppm. After incubation, the jar was opened to allow gaseous exchange with the atmosphere. Control fruits were handled similarly without methyl jasmonate.

Sampling Technique of Headspace Volatiles. Apples were placed in desiccators (±8 L) housed within a thermostated water bath (25 °C). The vessel was continuously flushed with nitrogen (99.9% pure) (333 mL/min). For sampling, a standard charcoal tube (ORBO-32, Supelco) was attached to the outlet of the desiccator (sample time 4 h). Extraction of the trapped headspace volatiles was carried out with carbon disulfide (0.3 mL).

Gas Chromatography. The volatiles were separated in a HP5890 gas chromatograph, equipped with a flame ionization detector and a 30 m × 0.25 mm i.d. glass capillary column Supelcowax 10. Operating conditions were as follows: N_2 carrier gas 1 mL/min; injector and detector 250 °C; injection volume 2 μL splitless; column was held for 15 min at 40 °C and then programmed at 2 °C/min to 160 °C. Identification of compounds was made by means of gas chromatography–mass spectrometry using a MS-30-VG. Structures were assigned by comparison of the spectra with those of authentic standards. Quantitative determinations were obtained using methyl octanoate as external standard. Known quantities of this compound were added to carbon disulfide before extraction of the volatiles. Quantitation was performed by normalization of the values obtained from the integrator against that of methyl octanoate; peak areas were expressed as nanograms of volatile per gram of apple per 80 L of headspace.

Preparation of Crude Extract Lipoxygenase. The apple pieces (core removed but peel intact) (50 g) were soaked in 30 mL of 50 mM sodium phosphate buffer (pH 6.8) containing 4 mM dithiothreitol, 0.2% Triton X-100, and 12 g of hydrated polyvinylpolypyrrolidone and then homogenized with an Ultra-Turrax three times for 20 s. The slurry obtained was filtered through two layers of cheesecloth and centrifuged at 25000*g* for 15 min. The pellet was discarded and the supernatant used as crude extract.

Lipoxygenase Assay. Lipoxygenase activity was determined by continuously monitoring the formation of conjugated diene at 234 nm (Axelrod et al., 1981) and expressed as micromoles of hydroperoxide formed per minute.

RESULTS AND DISCUSSION

A typical gas chromatogram of the headspace of Golden Delicious apples used in this work is depicted in Figure 1. The peak numbering is related to identification in Table I. Peaks numbers 17–19 are not included in further discussions but were identified as 4-(methoxyallyl)benzene, a terpene (MW 204), and α-farnesene, respectively.

In relation to eating quality of apples, it is commonly accepted that consumption would occur about 10 days after removal from controlled atmosphere. In this work,

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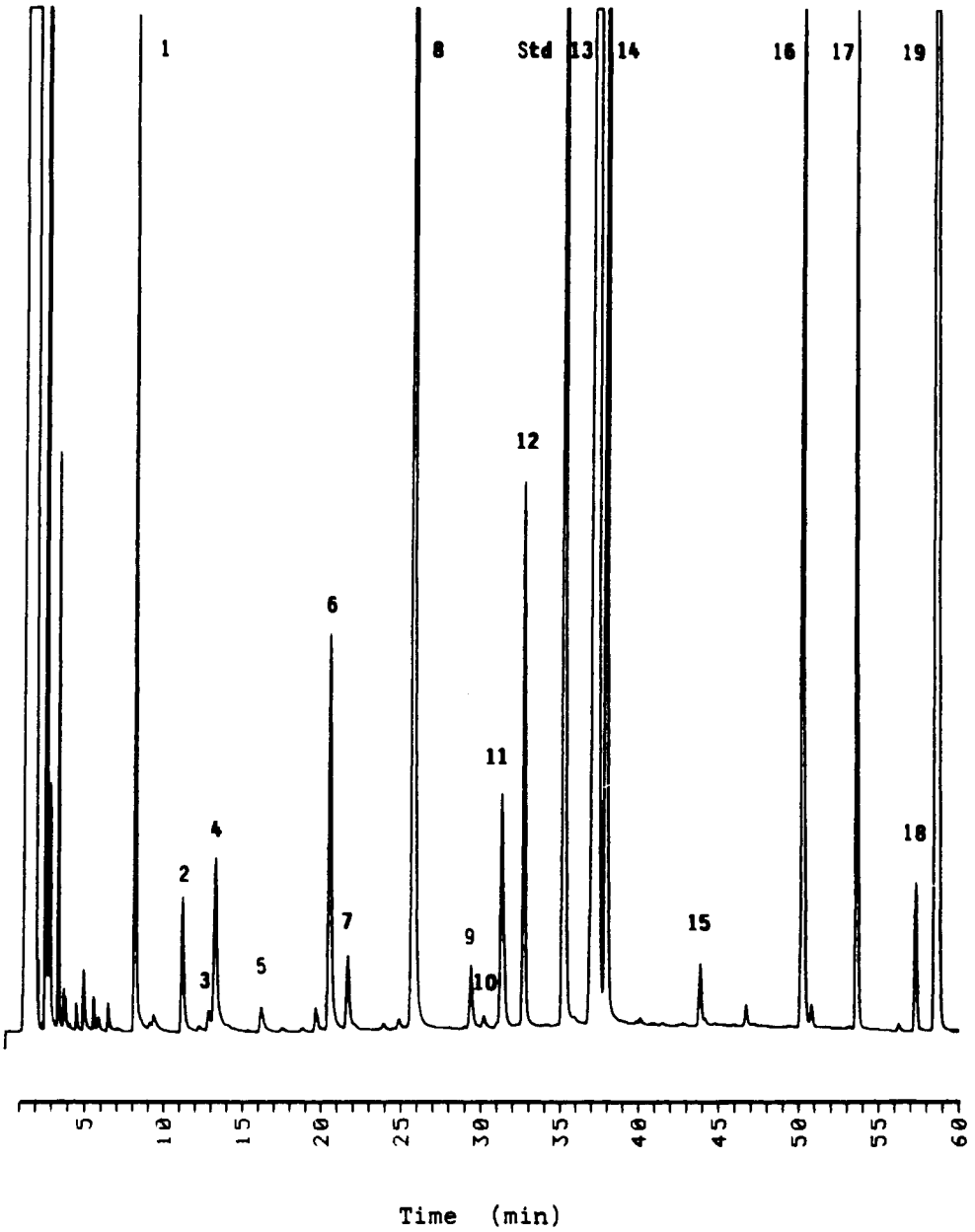

Figure 1. Typical gas chromatogram of Golden Delicious apple volatiles collected by dynamic headspace.

fruits were transferred from CA to air for ripening during 2 weeks at 25 °C in the dark, with or without methyl jasmonate (blank). As flavor quality is a dynamic process, the evolution of volatiles as a function of ripening time was followed. The concentrations of the carboxylic esters and hexanol in the headspace, expressed as nanograms per gram of fruit per 80 L of gas flushed, are shown in Table I.

From the point of view of objective flavor quality measurement, the sum of released volatiles in the headspace can be used as an aroma quality parameter. The total volatile esters for the different ripening periods is shown in Figure 2. Examination of this figure obviously indicates that after a short time (3 days) in dark at 25 °C a slight increase in aroma production is observed in both samples [a similar pattern has been reported by Willaert et al. (1983)], but after 6 days an evident distinction can be made between samples: the total volatile esters in methyl jasmonate treated apples is clearly less than in the blank, and the loss of esters was more than 50%. These data suggest an inhibition of the volatile ester-forming enzyme system by methyl jasmonate. Low ester contents could be caused by a lack of precursors, low esterifying activity, or high esterase activity. A full discussion of these changes is beyond the scope of this publication; only some aspects of the individual aroma compounds will be discussed.

From Table I is clear that carboxylic esters in which the alcohol moiety is hexanol are quantitatively important compounds in apple aroma, accounting for about 70% of the total volatile compounds. The evolution of hexanol and of the sum of hexyl esters in the headspace of Golden Delicious apples after treatment with methyl jasmonate is illustrated in Figure 3. The lower levels of esters in methyl jasmonate treated fruits seem to be a consequence of low rates of alcohol synthesis. Apples stored in low-oxygen atmospheres (2% O_2) were shown to have similar behavior (Knee and Hatfield, 1981). Alcohols such as hexanol are produced (Paillard, 1979) from fatty acids supplied to excised apple tissue, presumably by β-oxidation followed by reduction in two stages from acyl-coenzyme A to the corresponding aldehyde and the aldehyde to alcohol. Hexanol could also be derived by reduction of hexanal, which is a fragment resulting from the oxidative cleavage of linoleic acid (Tressl and Drawert, 1973). In this work we

Table I. Volatiles in the Headspace of Golden Delicious Apples Treated with Methyl Jasmonate

peak	volatile,[a] ng (g of fruit)$^{-1}$ (80 L of headspace)$^{-1}$	blank (days after treatment)					treated (days after treatment)			
		0	3	6	10	13	3	6	10	13
1	butyl acetate	230.0	52.5	55.0	92.7	83.8	27.1	3.6	11.6	4.7
2	2-methylbutyl acetate	50.8	45.8	53.3	71.3	100.1	20.6	2.3	9.7	7.9
3	propyl butanoate	3.9	3.1	1.7	1.9	4.1	1.7	0.8	1.5	2.0
4	butyl propanoate	7.8	9.1	4.6	3.3	12.8	1.9	1.1	2.6	4.4
5	pentyl acetate	8.8	8.8	7.0	8.4	7.9	5.7	0.9	1.3	1.4
6	butyl butanoate	30.0	24.0	14.7	15.7	21.8	23.3	4.1	9.4	7.2
7	butyl 2-methylbutanoate	16.7	19.3	14.7	11.9	19.5	13.3	1.2	5.2	4.3
8	hexyl acetate	235.6	169.7	120.6	129.0	129.8	106.9	12.6	12.5	10.0
9	pentyl butanoate	1.9	6.2	2.8	2.9	4.1	6.6	0.8	2.0	3.0
10	pentyl 2-methylbutanoate	1.2	3.4	2.5	2.3	3.8	3.5	0.6	1.5	2.1
11	hexyl propanoate	16.5	52.9	19.1	15.6	22.9	31.7	2.3	2.8	3.8
12	hexanol	4.5	6.7	3.9	8.8	13.1	7.0	1.2	3.7	4.7
13	hexyl butanoate and butyl hexanoate	112.9	261.2	113.8	132.1	147.7	287.8	62.5	116.7	116.0
14	hexyl 2-methylbutanoate	55.7	202.2	131.2	167.5	237.5	182.7	42.1	101.6	107.3
15	hexyl pentanoate	2.5	8.4	4.8	3.8	4.7	5.3	2.3	3.3	4.4
16	hexyl hexanoate	26.1	107.1	65.4	47.3	54.3	110.4	32.4	38.7	33.6

[a] Amounts are expressed in nanograms of volatile per gram of apples per 80 L of dynamic headspace. Methyl octanoate was used as external standard for GC analysis.

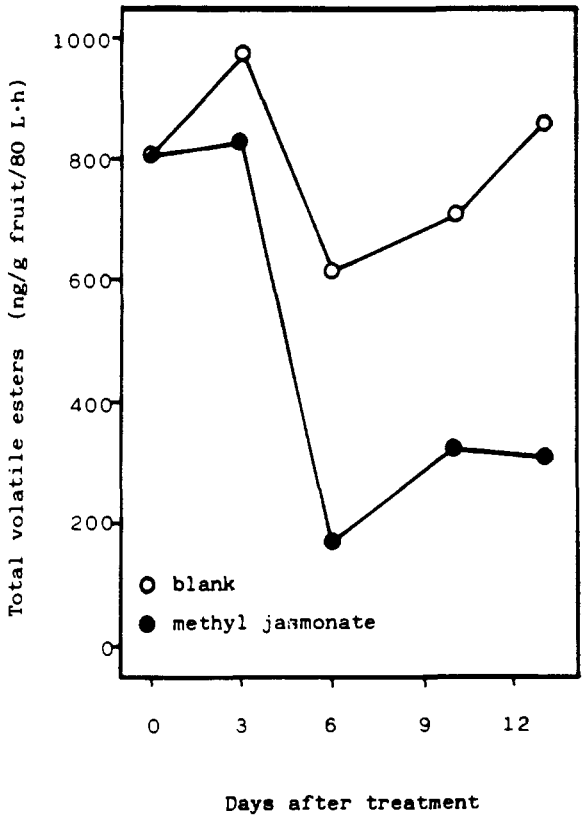

Figure 2. Ester formation by Golden Delicious apples during treatment with methyl jasmonate and evolution of total apple volatiles.

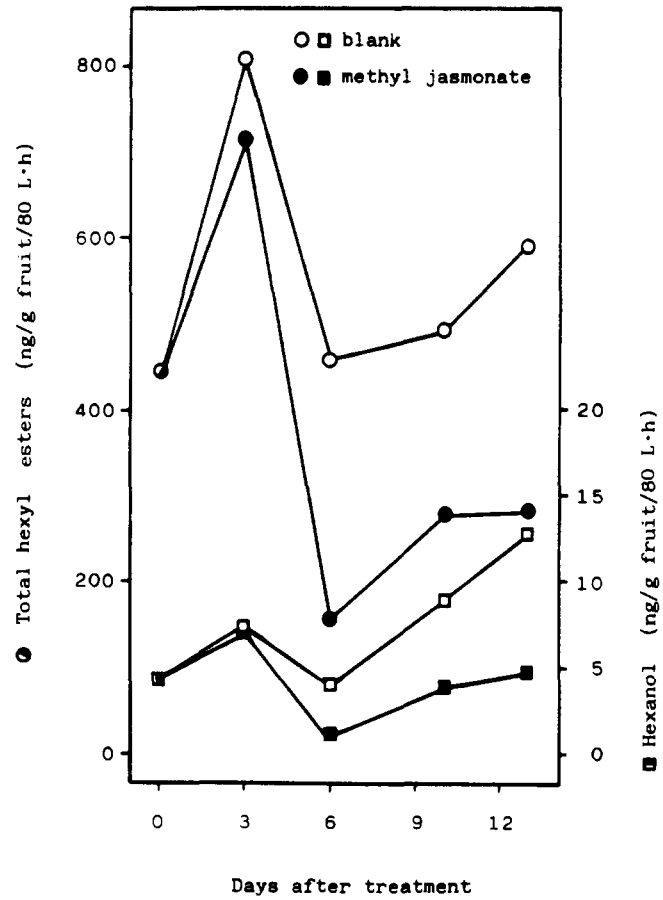

Figure 3. Evolution of total hexyl esters and content of hexanol in headspace of Golden Delicious apples treated with methyl jasmonate.

only tested this oxidative pathway. The biosynthesis of hexanol, from the oxidative cleavage of linoleic acid, implicates the action of several enzymes (Tressl and Drawert, 1973; Galliard et al., 1977): lipoxygenase, hydroperoxide lyase, and alcohol dehydrogenase. A preceding hydrolysis action to release the acids from phospholipids has been suggested, but it is questionable whether this is necessary in vivo (Galliard, 1975). Kim and Grosch (1979) found that lipoxygenase from Golden Delicious apples converted linoleic acid into the 13-hydroperoxyoctadeca-9,11-dienoic acid, the hexanol precursor. In crude extracts from apples treated with methyl jasmonate we found an almost similar level of lipoxygenase activity as in the blank; even a slight increase in lipoxygenase activity can be noticed in treated apples,

Table II. Lipoxygenase Activity in Apples Treated with Methyl Jasmonate

	days after treatment				
	0	3	6	10	13
blank	5.62[a]	5.75	5.18	4.80	6.47
methyl jasmonate	5.62	6.65	7.62	5.90	7.27

[a] Total activity unit. One unit of activity is defined as the amount of enzyme catalyzing the formation of 1 μmol of hydroperoxide/min.

as Table II shows. Consequently, the inhibition of hexanol biosynthesis appears more likely to be due to hydroperoxide lyase or alcohol dehydrogenase or the β-

Table III. Effect of Methyl Jasmonate on Formation of Hexyl Esters by Golden Delicious Apples Incubated with Acetic Acid (95 ng/g of Fruit) and Hexanol (75 ng/g of Fruit)

	(A) long CA storage apples			(B) short CA storage apples		
volatile,[a] ng (g of fruit)$^{-1}$ (80 L of headspace)$^{-1}$	blank	acetic acid + hexanol	methyl jasmonate + acetic acid + hexanol	blank	acetic acid + hexanol	methyl jasmonate + acetic acid + hexanol
hexyl acetate	30.7	67.5	4.9	113.4	445.0	152.2
hexyl propionate	7.4	15.9	1.2	22.7	77.8	21.9
hexyl butanoate and butyl hexanoate	63.1	157.6	68.3	498.6	918.7	518.6
hexyl 2-methylbutanoate	35.4	79.5	20.1	123.9	417.2	126.5
hexyl pentanoate	3.8	5.3	1.8	6.5	9.5	4.8
hexyl hexanoate	39.9	75.8	28.5	75.7	140.8	86.4
hexanol	2.9	9.6	4.3	20.1	60.3	40.5

[a] Amounts are expressed in nanograms of volatile per gram of apples per 80 L of dynamic headspace. Methyl octanoate was used as external standard for GC analysis.

oxidation system. At present we are moving on to study both of these possibilities.

As stated earlier, low ester contents could also be caused by high esterase activity. Knee and Hatfield (1981) detected a carboxylic ester hydrolase in apple peel tissue, present at picking and during ripening of the fruit in air at 12 °C. From a comparison of hexanol and hexyl esters amounts, shown in Figure 3, it is unlikely that the esterase was activated by methyl jasmonate. On the contrary, the pattern of esterification evolution apparently suggests that the esterifying capacity of treated fruits was inhibited.

Willaert et al. (1983) reported that a clear distinction can be made between aroma evolution during ripening after short storage (2–3 months) and that after long CA storage (6–8 months). They objectively showed that although long-stored apples had excellent appearance and were free of disorders, their flavor quality had decreased significantly. The results of the works developed by Knee and Hatfield (1981), De Pooter et al. (1981, 1987), Willaert et al. (1983), Berger and Drawert (1984), and Bartley et al. (1985) have demonstrated the feasibility of studying the biosynthesis of carboxylic esters by treating fruits with precursors in the vapor phase, since the esterifying site appears to be readily accessible to exogenous alcohols. In our case, to study the esterifying capacity of fruits treated with methyl jasmonate, acetic acid and hexanol were chosen as the precursors. In preliminary experiments with untreated apples, precursor amounts and incubation times were studied. Levels of 95 and 74 ng/g of fruit of acid and alcohol, respectively, and 7 days of incubation at 25 °C in the dark were good conditions to show a marked increase in the concentration of volatile esters. In these circumstances, treated apples enclosed in a glass jar (ca. 5 L), as stated under Experimental Procedures, were supplied with precursors by placing the pure compounds on a watch glass at the bottom of the vessel, and the concentration of volatiles in the headspace was established at the seventh day. De Pooter et al. (1981) proved, in apples treated with propanoic acid and butanoic acid vapors, that the composition of the headspace seems to be dependent not only on the availability of individual substrate but also on the nature of the substrate and on the relative amounts in which they are present. In our experiment, the treatment with acetic acid and hexanol produced alterations in levels of all hexyl esters in the headspace; therefore, in Table IIIA all of them have been included. From these results one may conclude that methyl jasmonate exerts an inhibitory effect on the esterifying system. The hexanol amounts estimated, included in this table, reinforce these conclusions since it was clearly shown that low amounts of hexyl esters were not caused by low levels of alcohols. When short CA storage (2–3 months) apples were treated with methyl jasmonate and precursors, similar results were obtained (Table IIIB) but having a higher level of volatile esters. The headspace hexanol data in Table III suggest some interesting effects of methyl jasmonate. The lower hexanol values for methyl jasmonate treated apples might be explained in two ways. Methyl jasmonate may be acting to modify the cuticular wax in some manner that alters hexanol penetration. Alternatively, penetration might not be affected, but pathways of hexanol utilization, other than esterification into volatile fraction, may be activated by methyl jasmonate.

Results obtained in this work show an inhibitory effect of methyl jasmonate on the esterification system of apples during stages of both high and low esterification capacity. If flavor production is related to senescence, we can conclude from these results that methyl jasmonate is an inhibitor of this process. These observations contrast with the senescent effect observed in unripened apples treated with methyl jasmonate, which produced an earlier climacteric peak, found in our laboratory (unpublished results).

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Registry No. Methyl jasmonate, 1211-29-6; acetic acid, 64-19-7; hexanol, 111-27-3; butyl acetate, 123-86-4; 2-methylbutyl acetate, 624-41-9; propyl butanate, 105-66-8; butyl propanoate, 590-01-2; pentyl acetate, 628-63-7; butyl butanoate, 109-21-7; butyl 2-methylbutanoate, 15706-73-7; hexyl acetate, 142-92-7; pentyl butanoate, 540-18-1; pentyl 2-methylbutanoate, 68039-26-9; hexyl propanoate, 2445-76-3; hexyl butanoate, 2639-63-6; hexyl 2-methylbutanoate, 10032-15-2; hexyl pentanoate, 1117-59-5; hexyl hexanoate, 6378-65-0; butyl hexanoate, 626-82-4; lipoxygenase, 9029-60-1.