Characterization and Occurrence of Lipoxygenase in Bell Peppers at Different Ripening Stages in Relation to the Formation of Volatile Flavor Compounds

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Extracts of green bell peppers (*Capsicum annuum* cv. Mazurka) were analyzed for the presence of lipoxygenase (LOX). A pH optimum of 5.5–6.0 was obtained, and enzyme activity was 100% and 51% inhibited by 10 mM *n*-propyl gallate and 340 μM 5,8,11,14-eicosatetraynoic acid (ETYA), respectively. When bell peppers ripened from green to completely red, LOX activity decreased 70%. Also, the composition of volatile breakdown products from unsaturated fatty acids changed drastically upon maturation. Four typical patterns for these volatile products could be distinguished during ripening; some changed similarly to LOX activity. In both green and red bell pepper homogenates, the addition of linoleic acid increased considerably the levels of hexanal and hexanol, whereas the levels of (*Z*)-3-hexenal and (*E*)-2-hexenal were markedly enhanced by the addition of linolenic acid. When ETYA was added to these systems, the enhancing effects of both substrates were drastically reduced. A biochemical pathway was proposed for the formation of volatile bell pepper compounds upon tissue disruption.

Keywords: *Capsicum annuum; bell pepper; flavor; lipoxygenase; linoleic acid; linolenic acid*

INTRODUCTION

Physical disruption of plant tissue results in the production of volatile compounds from unsaturated fatty acids, which are responsible for characteristic taste and aroma notes of the plant material (Grosch, 1982). The involvement of lipoxygenase (EC 1.13.11.12; LOX) in the formation of these volatile flavor compounds has been reported in many plant food products such as bananas (Tressl and Drawert, 1973), apple (Schreier and Lorenz, 1982), cucumber (Grosch and Schwarz, 1971; Galliard and Phillips, 1976; Galliard et al., 1976), tomato (Schreier and Lorenz, 1982; Galliard and Matthew, 1977; Galliard et al., 1977), raw beans (De Lumen et al., 1978), mushrooms (Tressl et al., 1981; Grosch, 1987), and tea (Gonzalez et al., 1972; Hatanaka and Harada, 1973). The enzymatic formation of volatile compounds from unsaturated fatty acids depends on a number of variables. Shiiba et al. (1991) examined substrate specificity of wheat germ LOX isoenzymes and demonstrated higher activity for linoleic acid compared to linolenic acid. Grosch (1982) and Gardner (1989) suggested that several LOX (iso)enzymes are specific for the production of either C-9 and/or C-13 hydroperoxides. Furthermore, hydroperoxide lyases can specifically cleave C-9 and/or C-13 hydroperoxides to form aldehyde fragments (Galliard and Phillips, 1976; Galliard et al., 1976, 1977; Galliard and Matthew, 1977; Sekiya et al., 1983). Other enzymes such as isomerases (Phillips et al., 1979) and alcohol dehydrogenases (Grosch and Schwencke, 1969; Stone et al., 1975) can modify the primary formed volatile compounds. Consequently, a broad variety of volatile compounds can be created by the primary action of LOX.

LOX activity has been previously observed in both green and red fruits of *Capsicum annuum* varieties (Pinsky et al., 1971; Minguez-Mosquera et al., 1993) and also in seeds of red bell pepper (Daood and Biacs, 1986). Wu and Liou (1986) proposed the involvement of LOX in volatile flavor formation in bell pepper, because addition of stannous chloride considerably reduced the amount of C_6 aldehydes and alcohols. Recently, Luning et al. (1994a,b) suggested that the composition and odor characteristics of C_6 aldehydes and alcohols may be partly responsible for the distinct aroma differences observed between green and red bell peppers. Additionally, Hatanaka et al. (1993) showed that olfactory characteristics of purified saturated and unsaturated C_6 aldehydes and alcohols were distinctively different. Fruity, sweet, and fresh odor notes were dominant for the 2-hexenals and 2-hexenols, while (*Z*)-3-hexenal and (*Z*)-3-hexenol have a strong spicy and grassy green odor.

To investigate the role of LOX in the formation of volatile flavor compounds of bell peppers, the enzyme was extracted from green fruits and partially characterized. Changes in LOX activity and composition of volatile compounds were monitored during maturation of bell peppers. The effects of specific LOX substrates (linoleic and linolenic acid) and a specific LOX inhibitor (5,8,11,14-eicosatetraynoic acid) on the volatile composition of homogenates of green and red bell peppers were analyzed.

MATERIALS AND METHODS

Bell Pepper Samples. *C. annuum* cv. Mazurka was cultivated as previously described (Luning et al., 1994a). Fruits were selected from different plants in early morning at different stages of maturity, depending on the type of experiment. Samples were collected at 6 weeks (mature green)

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after fruit set for partial characterization of bell pepper LOX. The fruits were cut into four pieces, seeded, and then immediately immersed in liquid nitrogen and stored at −80 °C prior to enzyme extraction. Furthermore, to study LOX activity during maturation, fruits were collected at four ripening stages, i.e., 4 (immature green), 6 (mature green), 8 (turning), and 10 (completely red) weeks after fruit set and stored as described above. Analysis of volatile compounds was carried out with samples harvested at seven maturation stages, i.e., 4, 5, 6, 7, 8, 9, and 10 weeks after fruit set, of which stages 6 (mature green) and 10 (completely red) were also used for substrate/inhibitor addition experiments. Collected fruits were stored at 13 °C (maximal 3 days) prior to analysis of volatile compounds by a dynamic headspace technique.

Preparation of Bell Pepper LOX Extracts. Two different samples were prepared, i.e., a crude (CR extract) and an ammonium sulfate precipitation extract (AS extract). Frozen bell peppers were homogenized, and 100 g of subsamples was mixed with 200 mL of 0.05 M phosphate buffer (pH 7) in a Waring blender at 4 °C. The homogenate was stirred at 4 °C for 30 min and then centrifuged at 20000*g* for 20 min at 4 °C. The supernatant was used as CR extract. The AS extract was obtained by precipitation of the CR extract with ammonium sulfate between 25% and 75% saturation. Then, the pellet was sedimented at 20000*g* for 20 min and suspended in 20 mL of 0.05 M potassium phosphate buffer (pH 7). The suspension was dialyzed overnight at 4 °C against 0.05 M potassium phosphate buffer (pH 7) with one change of buffer. Finally, the dialyzed ammonium sulfate fraction was centrifuged at 15000*g* for 5 min to obtain the AS extract.

Preparation of Substrate. A modification of the method of Surrey (1964) was used for preparation of substrate. A stock solution of 16 mM of linoleic acid (Sigma, St. Louis, MO) and 1.5% (v/v) Tween 20 (Sigma) in sodium tetraborate buffer (pH 9) was prepared; N_2 was flushed continuously during preparation to maintain oxygen-free conditions. Substrate was stored under nitrogen at 4 °C in the dark for a maximum of 4 days.

Enzyme Assay. LOX activity was measured with a biological oxygen monitor system using the standard oxygen probe and batch assembly (YSI Inc., Yellow Springs, OH). Assays were performed at 30 °C in aerated 0.2 M potassium phosphate buffer (pH 6) giving a final volume of 3 mL. Substrate was incubated at 30 °C while all LOX extracts were stored on ice (<4 °C) prior to assay. Oxygen consumption by the enzyme extract was monitored for 5 min, and the reaction was initiated by the addition of 0.5 mL of substrate, giving a final concentration of 2.67 mM linoleic acid. O_2 consumption was followed for 5−10 min and calculated as percent decrease of oxygen in air saturation per minute. Enzyme activities were converted into rate of O_2 consumption, expressed as nanokatals per milliliter of sample assayed, assuming only LOX activity. (Katal is defined as 1 mol of substrate consumed or product formed by the enzyme per second under defined conditions.)

Calculation of Katals. Net LOX activity = [(O_2 uptake upon addition of substrate) − (O_2 uptake by LOX extract) − (water) controls]. The reaction cell contains 2 mL of aerated buffer, 0.5 mL of LOX extract, and 0.5 mL of substrate. Assuming that substrate is totally O_2 free and that dissolved O_2 concentration relative to air-saturated water at 30 °C = 236 nmol of O_2/mL (Chappell, 1964). Then, 100% saturation = 236 nmol of O_2/mL × 2 mL of buffer (in reaction cell) = 472 nmol of O_2 in reaction cell and the oxygen consumption rate (nkat/mL sample) = {[(236 × volume buffer) × (net % decrease air saturation/min) × 2]/60}; 2 is the factor used to calculate per milliliter of sample and 60 is the factor used to calculate per second.

LOX pH Profile. Oxygen uptake was monitored at pH 5, 5.5, 6, 6.5, and 7 using 0.2 M potassium phosphate buffer and at pH 4, 4.5, 5, and 5.5 using 0.2 M sodium acetate/acetic acid buffer. In each experiment 0.50 mL of water and 2.25 mL of buffer were added to 0.25 mL of AS extract. Controls were run by omitting enzyme and/or substrate. The experiment was repeated twice.

LOX Inhibition Experiments. Stock solutions of 1.7, 3.4, and 6.8 mM 5,8,11,14-eicosatetraynoic acid (ETYA; Sigma) were prepared in 100% ethanol and diluted 10-fold with 0.2 M potassium phosphate buffer (pH 8) to lower the inhibitory effects of ethanol. The AS extracts (1 mL) were preincubated for 5 and 10 min with 1 mL of ETYA solution, giving final concentrations of 85, 170, and 340 μM ETYA. Activity was assayed using 0.5 μL of preincubated AS extracts, and the reaction was initiated by the addition of 0.5 μL of substrate. Controls were run for buffer, ethanol, and inhibitor.

A stock solution of 1.5 M *n*-propyl gallate (Sigma) was prepared in ethanol and diluted with ethanol 10 and 100 times. Each solution was further diluted 1:5 with water to reduce the inhibitory effect of ethanol, giving final solutions of 3, 30, and 300 mM, respectively. Amounts of 0.25 mL of AS extract and 0.1 mL of inhibitor solution were added to 2.15 mL of buffer (pH 6), giving final concentrations of 0.1, 1, and 10 mM *n*-propyl gallate, respectively, in the reaction cell. Controls were run with 0.1 mL of buffer instead of inhibitor. AS extracts were preincubated with the inhibitor for 5 min in the reaction cell. The assay was started upon the addition of 0.5 mL of substrate. Controls were run for buffer, ethanol, and inhibitor. All experiments were repeated twice.

Analysis of LOX Activity during Bell Pepper Maturation. Fruits of four ripening stages were collected at 4, 6, 8, and 10 weeks after fruit set, and CR extracts were prepared as described above. The reaction cell contained 2.0 mL of 0.2 M aerated potassium phosphate buffer (pH 6), 0.5 mL of CR extract, and 0.5 mL of 16 mM linoleic acid (three replicates per sample). Protein was measured according to the method of Bradford (1976), using BSA as standard, to calculate specific enzyme activity. Controls were run with extraction buffer instead of CR extract. The entire experiment was repeated three times.

Isolation of Volatile Bell Pepper Compounds. Five bell peppers were collected at seven ripening stages and cut into small pieces. Portions of 10 g (four replicates) were weighed and homogenized with 50 mL of water for analysis of volatile compounds. Volatiles were trapped on Tenax TA (100 mg, 35/60 mesh) as described previously (Luning et al., 1994a) with a minor modification. Samples were flushed with 30 mL/min purified nitrogen for 1 h at room temperature. The time series was repeated twice.

The effects of substrates and inhibitor on the composition of volatile compounds were analyzed in green (collected 6 weeks after fruit set) and red bell peppers (10 weeks). Control bell pepper samples were prepared by homogenization of, respectively, 20 g of sliced green and 20 g of sliced red fruits with 50 mL of 0.05 M potassium phosphate buffer (pH 6). Linoleic or linolenic acid [0.5 mL of 0.0134 mM; 1% (v/v) Tween 20] with or without ETYA (1 mL of 17 mM) were added to 0.05 M potassium phosphate buffer (total volume 50 mL) prior to homogenization of the sliced bell peppers. After homogenization of the samples, they were immediately transferred to closed flasks (500 mL) and gently stirred at 30 °C for 30 min. Subsequently, the flasks were connected to the dynamic headspace isolation system, and volatiles were trapped on Tenax TA as described previously (Luning et al., 1994a) with a minor modification. Samples were flushed with 30 mL/min purified nitrogen for 30 min at room temperature. Control samples on artifacts were made of only 50 mL of buffer and of buffer with, respectively, 0.5 mL of linoleic acid, 0.5 mL of linolenic acid, and 1 mL of ETYA (total volume 50 mL). The artifact control samples were similarly treated as the bell pepper samples and analyzed on the GC column to control for the presence of volatile compounds. Substrate and inhibitor addition experiments were repeated four times.

Gas Chromatography Analysis. Volatile compounds were analyzed as described previously (Luning et al., 1994a). The gas chromatograph (Carlo Erba GC Vega 6000, Interscience, Breda, The Netherlands) was equipped with a flame ionization detector (FID). The oven temperature was programmed from 40 to 150 °C at 2 °C/min and subsequently heated to 250 °C at 10 °C/min. Combined gas chromatography and mass spectrometry of volatile compounds was carried out as described previously (Luning et al., 1994a).

Statistical Analysis. Data of volatile compounds were subjected to analysis of variance (ANOVA, Genstat 5) to determine least significant differences (lsd) among ripening

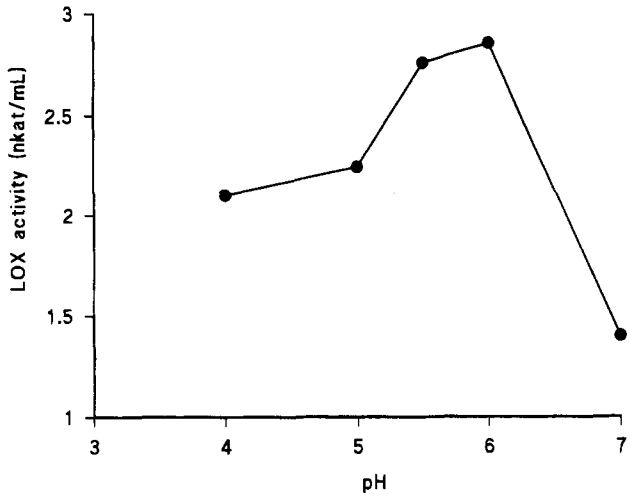

Figure 1. pH profile of bell pepper lipoxygenase (LOX) activity (cv. Mazurka) in ammonium sulfate precipitation extracts (AS extract) with linoleic acid as substrate.

stages and to determine lsd among the volatile composition of bell peppers with and without addition of substrate and/or inhibitor.

RESULTS AND DISCUSSION

In this study the occurrence of LOX activity in fresh bell peppers (*C. annuum* cv. Mazurka) and its role in the formation of volatile flavor compounds were investigated.

Lipoxygenase Activity in Bell Peppers. AS extracts of green bell pepper fruits were used to study the pH-profile and the effect of inhibitors. The AS extract showed activity with linoleic acid over a broad pH range, pH 4.0–7.0, and a maximum oxygen consumption at pH 5.5–6.0 (Figure 1). The observed activity optimum at pH 6.0 is typical for type-2 LOX and is comparable to those of cucumber, pH 5.5 (Wardale and Lambert, 1980), pea, pH 6.0 (Yoon and Klein, 1979), and broad bean, pH 6.0 (Al-Obaidy and Siddiq, 1981). Minguez-Mosquera et al. (1993) reported a maximum activity at pH 6.5 for LOX isolated from bell pepper cv. Bola and cv. Agridulce, using the spectrophotometric assay. Furthermore, the activity of the AS extract was quite high at the lower pH end of the curve, approximately 75% of the maximal activity at pH 6.0, but it declined sharply at the higher pH end (Figure 1). Similar skewed pH profiles have been noticed for several other lipoxygenases (Al-Obaidy and Siddiqi, 1981; Ganthavorn and Powers, 1989; Boyes et al., 1992; Leoni et al., 1985).

Two known LOX inhibitors, i.e., *n*-propyl gallate and ETYA, were used to investigate their inhibitory effect on the AS extract of green bell peppers. The antioxidant *n*-propyl gallate is a universal inhibitor of lipoxygenase activity (Schewe et al., 1986). Table 1 indicats that the percentage inhibition of enzyme activity was 54%, 82%, and 100% at concentrations of 100, 1000, and 10 000 μM *n*-propyl gallate, respectively. Al-Obaidy and Siddiqi (1981) reported 43% and 100% inhibition of broad bean LOX at 10 and 100 μM *n*-propyl gallate, respectively. Kristie and Thomson (1989) suggested that the effectiveness of *n*-propyl gallate as an inhibitor of tomato LOX in a crude system depended on the assay used. At a concentration of 1000 μM *n*-propyl gallate, the percentage inhibition was 100% with the spectrophotometric method but only 60% with the polarographic assay. The acetylenic fatty acid ETYA is known to be a

Table 1. Inhibitory Effects[a] of *n*-Propyl Gallate and 5,8,11,14-Eicosatetraynoic Acid (ETYA) on Lipoxygenase Activity in AS Extract of Green Bell Pepper

inhibitor	inhibitor concn (μM)	preincubation time (min)	% inhibition[b]
n-propyl gallate	100	5	54
	1000	5	82
	10000	5	100
ETYA	85	5	0
		30	0
	170	5	17
		30	30
	340	5	35
		30	51

[a] Average of two experiments; mean coefficient of variation of *n*-propyl gallate assay is 3.6% and of ETYA assay is 5.1%. [b] Maximal concentration of ethanol is 5% in the ETYA solutions.

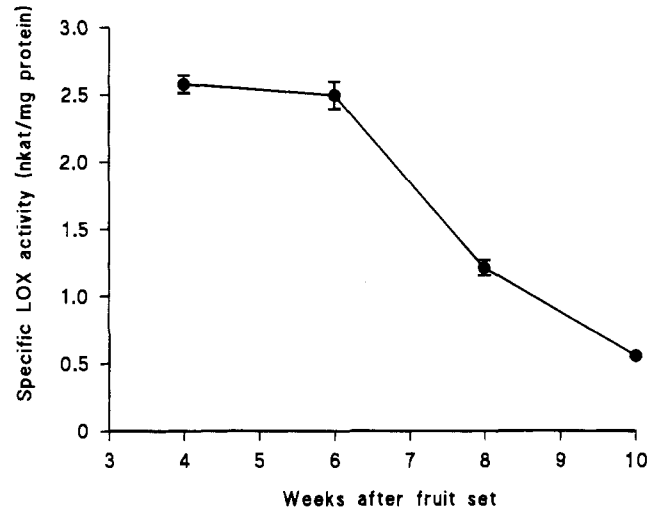

Figure 2. Lipoxygenase (LOX) activity in crude extracts (CR extract) at different stages of bell pepper maturation (cv. Mazurka). Bars represent standard deviation (n = 3).

powerful inhibitor of lipoxygenases and is recommended as a selective criterium for true lipoxygenase activity (Schewe et al., 1986, 1987). Preliminary experiments with soybean LOX type-2 (Sigma) showed that 100 μM ETYA inhibited enzyme activity 85% after 5 min of incubation. However, ETYA was less effective in inhibiting LOX activity in the AS extract of green bell pepper (Table 1). There is no influence of ETYA on enzyme activity at a concentration of 85 μM; however, activity decreased 30% and 51% after preincubation for 30 min with 170 and 340 μM ETYA, respectively (Table 1). No total inhibition was achieved, which may be due to residual bell pepper LOX. It was not possible to carry out inhibition assays at ETYA concentrations above 340 μM, because of insolubility. In addition, other enzymatic oxidation reactions may be responsible for the residual oxygen uptake, since the AS extract is a crude protein preparation.

CR extracts were prepared to monitor enzyme activity of cv. Mazurka at four different ripening stages. Figure 2 shows that LOX activity remained high at the immature (4 weeks after fruit set) and mature green (6 weeks) stages of development, and then it decreased significantly at the turning (8 weeks) and red (10 weeks) stages of maturation. Activities of the latter were approximately 45% and 30% of that of green bell peppers (4–6 weeks), respectively. Pinsky et al. (1971) observed that LOX activity in red bell pepper was approximately 50% of that of the green stage, and Minguez-Mosquera et al. (1993) noticed a decrease of

more than 50% upon ripening from green to turning stage. Recently, Lomnitski et al. (1993) reported that soybean LOX type-2 activity toward linoleic acid was decreased 70% by 1 μM β-carotene at pH 6.5. Luning et al. (1994a) showed that the content of carotenoids (expressed as β-carotene) in cv. Mazurka increased from 0.6 to 1.5 g/kg of dry matter when bell pepper color turned from green to red. Probably, the increase in carotenoids influences bell pepper LOX activity as reviewed by Siedow (1991).

Development of Volatile Compounds during Bell Pepper Ripening. The enzymatic formation of volatile flavor compounds from unsaturated fatty acids upon tissue disruption has been demonstrated for numerous vegetables (Tressl et al., 1981; Whitfield and Last, 1991). In this study, a selected number of volatile compounds, which may be derived from the breakdown of unsaturated fatty acids, were analyzed in homogenates of bell peppers collected at seven maturation stages. Nine aldehydes, five alcohols, one ketone, and one furan were identified and quantified, of which hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenol, (*Z*)-2-hexenol, and hexanol were present in considerable amounts depending on the stage of maturation (Table 2). The composition of volatile compounds changed drastically during ripening and some particular patterns could be distinguished, as illustrated in Figure 3. Pattern A shows a significant ($P < 0.05$) decrease between 4 and 6 weeks (from immature to mature green); between 6 and 8 weeks no marked changes occur, but after 8 weeks the level dropped significantly ($P < 0.05$). This pattern is typical for the volatile compounds hexanal and hexanol (Table 2). Pattern B shows high levels in the green stages (4–6 weeks), which decreased significantly ($P < 0.05$) between 7 and 8 weeks, when bell pepper color turned from green to red. This pattern represents the development of (*Z*)-3-hexenal, (*Z*)-3-hexenol, (*Z*)-2-pentenal, (*Z*)-2-pentenol, (*E*)-2-pentenal, (*E*)-2-pentenol, and 1-penten-3-one during bell pepper maturation (Table 2). In contrast, the third pattern (C) exhibits a significant ($P < 0.05$) increase between 7 and 8 weeks, followed by a significant ($P < 0.05$) decrease after 9 weeks, representing the changes of (*E*)-2-hexenal, (*E*)-2-hexenol, and (*Z*)-2-hexenal during ripening (Table 2). The remaining compounds, 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal, showed a maximum level at 8 weeks (pattern D), which declined sharply thereafter. Comparing changes in LOX activity (Figure 2) with changes in volatile composition (Figure 3) during bell pepper maturation indicates that the volatile compounds belonging to pattern B showed a similar rapid decrease between 7 and 8 weeks as LOX activity, whereas volatile compounds belonging to pattern C showed a rapid increase.

Influence of Exogenous Linoleic and Linolenic Acids on the Composition of Volatile Compounds. LOX substrates were added to bell pepper homogenates for determining the influence of LOX on the formation of volatile compounds. The effects of the addition of linoleic or linolenic acid on the composition of volatile compounds of mature green (collected 6 weeks after fruit set) and completely red (10 weeks) bell pepper homogenates are shown in Tables 3 and 4, respectively.

In green bell pepper homogenates, linoleic acid significantly ($P < 0.05$) increased the levels of hexanal, hexanol, 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal, and (*Z*)-3-hexenal (Table 3). The addition of linolenic acid to the same homogenates resulted in significantly ($P < 0.05$) higher levels of (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-2-hexenal, (*Z*)-2-pentenal, (*E*)-2-pentenal, (*Z*)-2-pentenol, (*E*)-pentenol, and 1-penten-3-one. However, the alcohols (*Z*)-3-hexenol and (*E*)-2-hexenol did not change significantly (Table 3). Although the inherent volatile composition of green and red peppers is markedly different (Table 2), the effects of linoleic and linolenic acid on red pepper volatiles (Table 4) were quite similar to the ones reported for green fruits (Table 3). In red bell pepper homogenates, linoleic acid caused significant ($P < 0.05$) increases in hexanal and hexanol but was less effective in raising the levels of 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal, as compared to in the green fruits (Tables 3 and 4). The addition of linolenic acid resulted in a larger increase of (*E*)-2-hexenal, (*Z*)-2-hexenal, and (*E*)-2-hexenol than recorded for green bell pepper. Headspace analysis of artifact control samples for buffer, ETYA, and linoleic and linolenic acids did not show detectable amounts of the volatile compounds, which increased/decreased upon addition of the inhibitor and substrates to green or red bell pepper homogenates (Tables 3 and 4).

The increase of hexanal and hexanol upon addition of linoleic acid and the increase of (*Z*)-3-hexenal and (*E*)-

Table 2. Average GC Peak Areas[a] of Volatile Compounds Obtained from Dynamic Headspace Gas Chromatography of Homogenates of Bell Peppers Collected at Different Maturation Stages

compound	KI[c]	harvest time[b] 4	5	6	7	8	9	10	pattern[d]
hexanal	1074	194.2	123.9	81.8	72.9	62.6	35.4	12.9	A
1-hexanol	1330	10.8	8.4	4.2	4.6	5.7	4.2	2.1	A
(*Z*)-3-hexenal	1143	107.7	136.9	130.1	128.4	3.9	0.9	0.1	B
(*Z*)-3-hexenol	1361	17.0	24.9	19.1	17.4	2.2	0.5	0.3	B
(*Z*)-2-pentenal	1092	4.2	4.2	3.4	2.7	0.2	0.2	<0.1[e]	B
(*Z*)-2-pentenol	1287	0.3	0.2	0.2	0.2	<0.1	<0.1	<0.1	B
(*E*)-2-pentenal	1116	0.1	0.1	0.1	0.1	<0.1	<0.1	<0.1	B
(*E*)-2-pentenol	1295	1.0	1.0	1.0	0.9	0.6	0.4	<0.1	B
1-penten-3-one	1008	5.9	7.4	7.0	6.8	2.3	1.8	1.0	B
(*E*)-2-hexenal	1210	53.6	44.0	34.8	105.9	301.2	279.9	170.9	C
(*Z*)-2-hexenal	1192	2.0	3.0	2.9	4.3	8.4	7.2	4.8	C
(*E*)-2-hexenol	1380	1.0	1.1	1.1	3.8	19.9	23.8	10.8	C
2-pentylfuran	1230	1.2	1.3	1.0	1.3	2.0	1.3	0.6	D
(*E*)-2-heptenal	1312	0.6	0.7	0.6	0.7	1.1	0.5	0.2	D
(*E*)-2-octenal	1416	0.5	0.5	0.4	0.5	0.6	0.3	0.2	D
(*E*)-2-nonenal	1525	0.4	0.4	0.3	0.2	0.5	0.2	0.1	D

[a] Average peak areas of two time series (Vs); mean coefficient of variation is 13%. [b] Weeks after fruit set. [c] Calculated Kovats indices on CP-Wax CB52 column. [d] Codes correspond with patterns presented in Figure 3. [e] Mean peak area is below 0.1 (Vs), or compound is below detection limit.

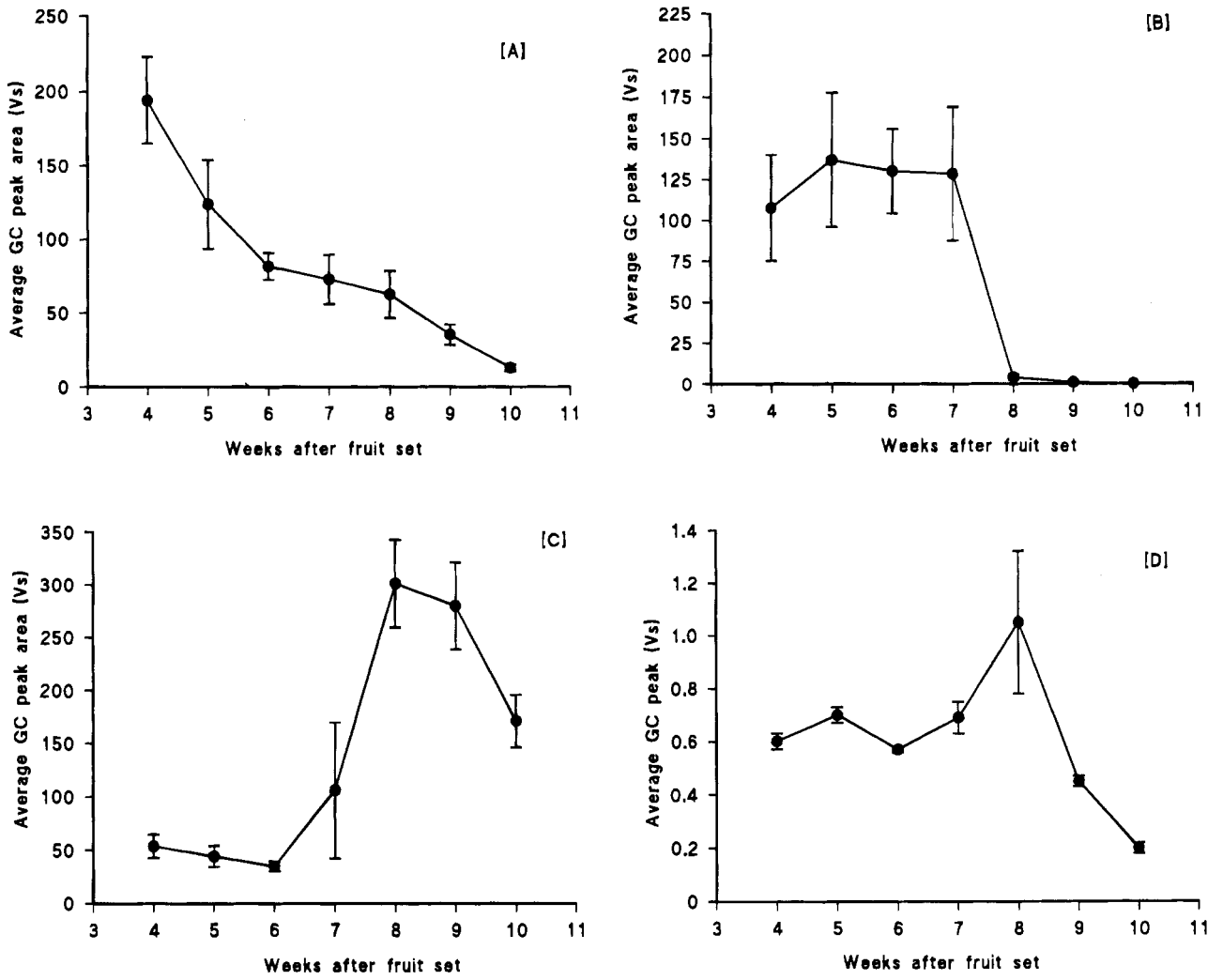

Figure 3. Four distinct ripening-related changes in average gas chromatography peak areas, illustrated by the volatile compounds hexanal [A], (*Z*)-3-hexanal [B], (*E*)-2-hexenal [C], and (*E*)-2-heptenal [D]. Bars represent standard deviation ($n = 4$); other compounds are classified in Table 2.

Table 3. Influence of Linoleic and Linolenic Acids, with and without ETYA, on the Average GC Peak Areas[a] of Volatile Compounds Obtained from Dynamic Headspace Gas Chromatography of Homogenates of Green Bell Peppers

compound	control[b]	linoleic acid added	linolenic acid added	linoleic acid + ETYA added	linolenic acid + ETYA added
hexanal	82.3 ± 14.4	270.1 ± 19.2	99.9 ± 12.0	158.0 ± 24.2	84.9 ± 12.0
hexanol	3.9 ± 0.2	8.3 ± 1.2	2.5 ± 0.4	3.8 ± 1.0	1.7 ± 0.1
2-pentylfuran	0.3 ± 0.1	2.5 ± 0.3	0.2 ± 0.0	3.2 ± 0.3	0.3 ± 0.1
(*E*)-2-heptenal	0.5 ± 0.2	1.3 ± 0.4	0.8 ± 0.1	2.2 ± 0.3	1.7 ± 0.2
(*E*)-2-octenal	0.3 ± 0.1	2.3 ± 0.5	0.5 ± 0.1	4.7 ± 0.2	2.8 ± 0.3
(*E*)-2-nonenal	0.2 ± 0.1	4.0 ± 0.8	0.8 ± 0.1	3.5 ± 0.9	0.6 ± 0.2
(*Z*)-3-hexenal	78.7 ± 13.6	122.4 ± 20.7	179.8 ± 26.6	69.7 ± 6.9	68.2 ± 25.6
(*E*)-2-hexenal	67.2 ± 12.5	75.7 ± 11.6	122.3 ± 2.8	49.7 ± 13.0	73.5 ± 11.1
(*Z*)-2-hexenal	2.1 ± 0.6	2.3 ± 0.2	3.2 ± 0.1	1.8 ± 0.3	1.8 ± 0.4
(*Z*)-2-pentenal	1.1 ± 0.3	1.8 ± 0.5	2.9 ± 0.5	1.3 ± 0.4	1.2 ± 0.0
(*E*)-2-pentenal	0.9 ± 0.2	1.3 ± 0.3	1.6 ± 0.3	0.6 ± 0.1	0.8 ± 0.3
(*Z*)-2-pentenol	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
(*E*)-2-pentenol	0.6 ± 0.2	0.7 ± 0.2	1.8 ± 0.5	0.1 ± 0.0	0.4 ± 0.1
1-penten-3-one	2.8 ± 0.8	4.0 ± 0.7	7.2 ± 1.9	4.0 ± 0.4	6.9 ± 0.2
(*Z*)-3-hexenol	12.2 ± 1.0	10.3 ± 1.5	13.6 ± 2.7	9.2 ± 1.5	10.1 ± 1.4
(*E*)-2-hexenol	0.8 ± 0.3	0.6 ± 0.2	0.8 ± 0.1	0.5 ± 0.1	0.8 ± 0.1

[a] Average peak areas (Vs) of four replicates ± standard deviation. [b] Control of 20 g of sliced green fruits homogenized with 50 mL of 0.05 M potassium phosphate buffer, (pH 6).

2-hexenal upon addition of linolenic acid have been reported for LOX in other vegetables such as tomato (Stone et al., 1975; Galliard and Matthew, 1977) and cucumber (Grosch and Schwarz, 1971; Galliard et al., 1976, 1977; Tressl et al., 1981). The increase of (*Z*)-3-hexenal and (*E*)-2-hexenal upon addition of linoleic acid has been demonstrated also for fresh green beans by Tressl et al. (1981). However, the significant increase ($P < 0.05$) of (*Z*)-3-hexenal in green bell pepper (Table 3) and of (*E*)-2-hexenal in red (Table 4) bell pepper homogenates upon addition of linoleic acid does not confirm the breakdown pathways of this unsaturated acid as proposed by Galliard et al. (1976, 1977), Galliard and Matthew (1977), and Stone et al. (1975). Possibly, some linoleic acid is enzymatically converted to linolenic acid during headspace isolation.

Table 4. Influence of Linoleic and Linolenic Acids, with and without ETYA, on the Average GC Peak Areas[a] of Volatile Compounds Obtained from Dynamic Headspace Gas Chromatography of Homogenates of Red Bell Peppers

compound	control[b]	linoleic acid added	linolenic acid added	linoleic acid + ETYA added	linolenic acid + ETYA added
hexanal	3.9 ± 1.2	21.6 ± 4.0	6.2 ± 1.2	3.8 ± 0.7	3.2 ± 0.5
hexanol	0.9 ± 0.4	3.0 ± 0.5	1.1 ± 0.3	1.9 ± 0.1	0.7 ± 0.1
2-pentylfuran	0.1 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	1.0 ± 0.1	0.1 ± 0.1
(*E*)-2-heptenal	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
(*E*)-2-octenal	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0
(*E*)-2-nonenal	<0.1[c]	0.3 ± 0.1	<0.1	0.3 ± 0.1	<0.1
(*Z*)-3-hexenal	0.8 ± 0.2	2.0 ± 1.2	2.9 ± 0.9	0.2 ± 0.1	1.6 ± 0.2
(*E*)-2-hexenal	22.8 ± 5.9	58.5 ± 15.6	157.5 ± 35.8	17.4 ± 6.7	69.5 ± 17.0
(*Z*)-2-hexenal	0.8 ± 0.2	1.0 ± 0.2	2.8 ± 1.2	0.6 ± 0.2	1.0 ± 0.0
(*Z*)-2-pentenal	<0.1	<0.1	<0.1	<0.1	<0.1
(*E*)-2-pentenal	<0.1	<0.1	<0.1	<0.1	<0.1
(*Z*)-2-pentenol	<0.1	<0.1	<0.1	<0.1	<0.1
(*E*)-2-pentenol	<0.1	<0.1	<0.1	<0.1	<0.1
1-penten-3-one	0.5 ± 0.2	0.6 ± 0.2	1.3 ± 0.3	0.5 ± 0.1	1.4 ± 0.2
(*Z*)-3-hexenol	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
(*E*)-2-hexenol	2.4 ± 0.6	2.5 ± 0.5	7.6 ± 3.1	2.3 ± 0.4	4.2 ± 0.5

[a] Average peak areas (Vs) of four replicates ± standard deviation. [b] Control of 20 g of sliced red fruits homogenized with 50 mL of 0.05 M potassium phosphate buffer, (pH 6). [c] Mean peak area is below 0.1 (Vs), or compound is not detected.

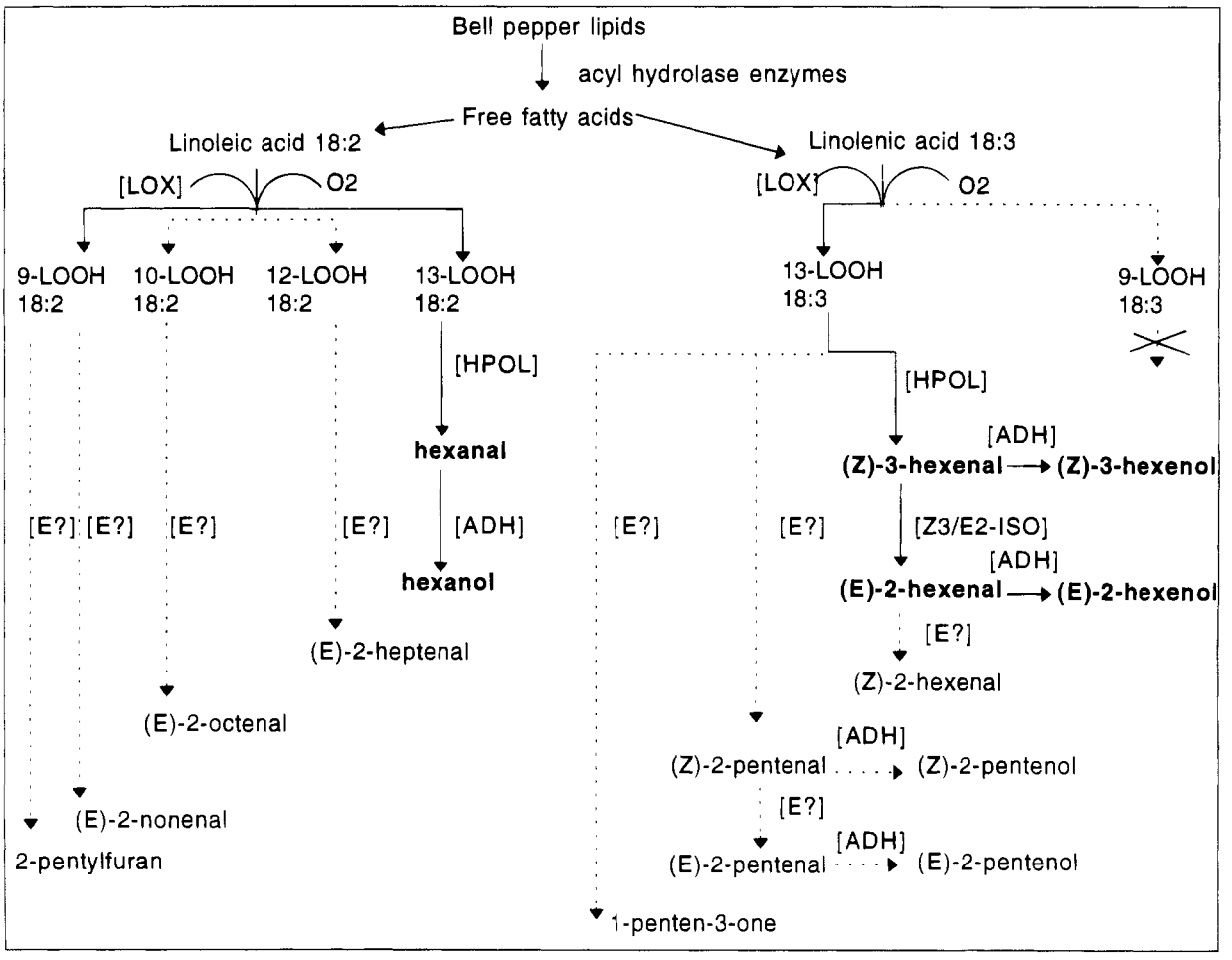

Figure 4. Biochemical pathways proposed for the formation of volatile compounds from linoleic and linolenic acids in fresh bell peppers. Abbreviations: LOOH, hydroperoxide; [LOX], bell pepper lipoxygenase; [HPOL], hydroperoxide lyase; [ADH], alcohol dehydrogenase; [Z3/E2-ISO]; *cis*-3/*trans*-2 isomerase; [E?], possible enzymatic transformation.

The increase of 2-pentylfuran and (*E*)-2-nonenal upon addition of linoleic acid has been reported previously for cucumber homogenates by Tressl et al. (1981), who suggested that labile (*Z*)-3-nonenal was enzymatically converted into 2-pentylfuran and (*E*)-2-nonenal. Gardner et al. (1991) showed that in soybeans seeds (*E*)-2-nonenal derived from the 9-hydroperoxide of linoleic acid, and they proposed that it was converted from (*Z*)-3-nonenal. In our experiments, no (*Z*)-3-nonenal was detected in any of the bell pepper homogenates and possibly this compound was converted into (*E*)-2-nonenal and 2-pentylfuran. Additionally, pea and soybean lipoxygenases generated, among others, 2-pentylfuran on pure linoleic acid (Sessa, 1979).

The effect of linolenic acid on the level of (*E*)-2-pentenal has been demonstrated by Grosch and Laskawy (1975), who suggested that (*E*)-2-pentenal was generated from 13-hydroperoxides of linolenic acid by soybean LOX. Mattick and Hand (1969) implied that 1-penten-

3-one derived from 13-hydroperoxides of linolenic acid in soybeans.

Influence of ETYA Combined with Linoleic or Linolenic Acid on the Composition of Volatile Compounds. The levels of hexanal, hexanol, and (*Z*)-3-hexanal decreased when ETYA was added to bell pepper homogenates containing linoleic acid, whereas levels of (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-2-hexenal, (*E*)-2-hexenol, (*Z*)-2-pentenal, (*E*)-2-pentenal, (*Z*)-2-pentenol, and (*E*)-2-pentenol decreased when ETYA was added to green and/or red bell pepper homogenates containing linolenic acid (Tables 3 and 4). However, some volatile, such as 1-penten-3-one, 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal, were unaffected or even significantly ($P < 0.05$) increased by the addition of ETYA (Tables 3 and 4). The effect of LOX inhibitors on the formation of volatile compounds has been reported by Wu and Liou (1986). They suggested that stannous chloride reduced the enzymatically formed C_6 aldehydes and alcohols in disrupted bell peppers. In fresh green beans, De Lumen et al. (1978) showed that 20 mM cyanide decreased (*E*)-2-hexenal and hexanal to one-sixth of the control, while practically no volatiles were formed at 100 mM.

Formation of Volatile Bell Pepper Compounds from Unsaturated Fatty Acids. A biochemical pathway has been proposed for the formation of volatile bell pepper compounds from unsaturated fatty acids, based on our results and available literature (Figure 4). Grosch (1982) showed that bell pepper LOX specifically produced 9-hydroperoxides with linoleic acid as substrate. However, the majority of volatile compounds detected in bell peppers were probably formed from 13-hydroperoxides of linoleic and linolenic acid (Tables 3 and 4; Figure 4). Galliard et al. (1977) and Galliard and Matthew (1977) demonstrated that tomato LOX produces mainly 9-hydroperoxides, whereas C_6 aldehydes were only formed from the 13-hydroperoxides. They suggested that a hydroperoxide lyase (HPOL) in tomato specifically cleaved the 13-hydroperoxides. Likewise, bell peppers seem to contain an HPOL cleaving mainly the 13-hydroperoxides to volatile compounds (Figure 4).

Figure 4 shows that only the minor compounds 2-pentylfuran and (*E*)-2-nonenal can be derived from 9-hydroperoxides of linoleic acid as reported earlier (Grosch and Laskawy, 1975; Tressl et al., 1981; Gardner et al., 1991). The major compound, hexanal, is formed from the 13-hydroperoxides of linoleic acid and is converted by an alcohol dehydrogenase to hexanol as demonstrated for tomatoes (Galliard and Matthew, 1977; Galliard et al., 1977). The minor compounds (*E*)-2-octenal and (*E*)-2-heptenal appeared to be formed from, respectively, the 10- and 12-hydroperoxides of linoleic acid (Tressl et al., 1981). Chan et al. (1979) proposed a mechanism for the rearrangement of linoleic acid hydroperoxides leading to interisomerization, which affect the distribution of hydroperoxide isomers obtained and therefore the type of volatile compounds formed.

Figure 4 shows that the volatile compounds which increased upon the addition of linolenic acid are all derived from the 13-hydroperoxides. The formation of (*Z*)-3-hexenal from 13-hydroperoxides of linolenic acid and the conversion to (*E*)-2-hexenal by a *cis*-3/*trans*-2 isomerase have been demonstrated for cucumber fruits (Galliard et al., 1976; Phillips and Galliard, 1978; Phillips et al., 1979); (*Z*)-2-hexenal is possibly formed from its *trans*-form chemically or by another isomerase enzyme. The conversion of 1-penten-3-one from 13-hydroperoxides of linolenic acid has been proposed by Mattick and Hand (1969). Additionally, (*Z*)-2-pentenal could be formed according to the same pathway prior to 1-penten-3-one formation. Other enzymes such as a *cis/trans* isomerase probably convert (*Z*)-2-pentenal to (*E*)-2-pentenal. All aldehydes seemed to be converted to their corresponding alcohols by an alcohol dehydrogenase, as reported for several aldehydes in tomatoes (Stone et al., 1975). Obviously, the aldehydes derived from the 9-, 10-, and 12-hydroperoxides of linoleic acid such as (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal were not converted to their corresponding alcohols.

In conclusion, changes in the composition of volatile compounds during the ripening of bell peppers and different quantitative effects of LOX substrates on the composition of green and red bell peppers indicated that several changes in the pathways of breakdown of fatty acids might occur. Our results demonstrated that LOX activity decreased rapidly when color turned from green to red. Similarly, major compounds (*Z*)-3-hexenal and (*Z*)-3-hexenol decreased, whereas (*E*)-2-hexenal and (*E*)-2-hexenol rapidly increased up to 8 weeks after fruit set. Moreover, the addition of linolenic acid increased (*Z*)-3-hexenal most in the green fruits, whereas in the red fruits the increase of (*E*)-2-hexenal was highest, suggesting an increased *cis*-3/*trans*-2 isomerase activity during ripening. In the completely red fruits (10 weeks), levels of all volatile compounds decreased or disappeared, which may be due to the decreased level of hydroperoxides formed by the lower bell pepper LOX activity.

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Registry No. Supplied by the Author: 1-Penten-3-one, 1629-58-9; 1-hexanal, 66-25-1; (*Z*)-2-pentenal, 1576-86-9; (*E*)-2-pentenal, 1576-87-0; (*Z*)-3-hexenal, 6789-80-6; (*Z*)-2-hexenal, 16635-54-4; (*E*)-2-hexenal, 6728-26-3; 2-pentylfuran, 3777-69-3; (*Z*)-2-pentenol, 1576-95-0; (*E*)-2-pentenol, 1576-96-1; (*E*)-2-heptenal, 18829-55-5; 1-hexanol, 111-27-3; (*Z*)-3-hexenol, 928-96-1; (*E*)-2-hexenol, 928-95-0; (*E*)-2-octenal, 2548-87-0; (*E*)-2-nonenal, 18829-56-6.

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