Development of Aroma Volatiles and Color during Postharvest Ripening of 'Kent' Strawberries

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Abstract. 'Kent' strawberries were harvested at red, pink, and white stages of development, and stored at 15C in the light. Fruit were sampled over a 10-day period and evaluated for volatile production and surface color. Volatile production by red and pink fruit peaked after 4 days of storage. Maximum volatile production by red fruit was 8- and 25-fold greater than maximum production by pink and white fruit, respectively. Aroma volatiles were not detected in the headspace over white berries until 4 days following harvest after which volatile production increased through the tenth day of storage. Changes in the surface color of white berries during postharvest ripening coincided with the production of volatiles. In another experiment, red, pink, and white 'Kent' strawberries were stored for 3 days at 10 or 20C in the dark or light. Fruit were then evaluated for volatile production, weight loss, anthocyanin content, and surface color changes. White berries produced volatile esters after 3 days of storage at 20C in the light. Both light and temperature influenced the relative production of the volatiles produced by pink fruit. Fresh weight loss, color change, and anthocyanin content were temperature and light dependent.

The strawberry is a nonclimacteric fruit in which ripening is characterized by softening, anthocyanin synthesis, synthesis of flavor, a decrease in acidity, and an increase in sugar content (Abeles and Takeda, 1990; Hulme, 1971; Woodward, 1972). Fruit harvested slightly underripe will ship better and have a longer shelf-life than fully ripe fruit (Mitchell et al., 1964). These less mature berries are firmer, will maintain their shine longer, and decay less (Pritts et al., 1976; Skrede, 1984), but are usually poorer in overall flavor than fully ripe berries (Smith and Heinze, 1958).

The flavor and aroma of strawberries are important quality attributes that influence consumer acceptability. The relative importance of aroma compounds to the flavor of fresh strawberries has been rated by many investigators (Dirinck et al., 1981; Hirvi, 1983; Larsen and Poll, 1992; Pérez et al., 1992; Schreier, 1980). While the volatiles contributing to aroma vary among cultivars, some of the most important contributors to aroma include ethyl butanoate, 2, 5-dimethyl-4-hydroxy-3(2H)-furanone, ethyl hexanoate, methyl butanoate, linalool, and methyl hexanoate.

Investigations of changes in the profile of volatile compounds during strawberry maturation are limited. Yamashita et al. (1977) monitored the conversion of added pentanal to pentanol, pentyl acetate, and pentyl butanoate during maturation of 'Hoko' strawberries. Immature fruit, 5 days after flowering, converted added pentanal to 1-pentanol. But no ester formation was observed. However, the production of 1-pentyl acetate and 1-pentyl nbutanoate increased dramatically between 30 and 40 days after flowering. Ito et al. (1990) analyzed volatile components in headspace vapors of an unpicked strawberry fruit and reported an increase in the total amount of the volatile compounds produced during ripening of the berry. The volatiles produced were mainly esters, including large amounts of methyl acetate and methyl

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butanoate. Pérez et al. (1992) found that both the number and concentration of volatiles produced by 'Chandler' strawberry fruit increased as fruit ripened. Ethyl hexanoate, ethyl butanoate, methyl butanote, and methyl hexanoate were the predominate volatiles present in fully ripe 'Chandler' fruit.

Strawberries harvested underripe can develop full red color in darkness and the process is temperature dependent (Austin et al., 1960; Kalt et al., 1993; Smith and Heinze, 1958). Light slightly increases speed and degree of coloration at 24C (Austin et al., 1960). However, the postharvest development of aroma in underripe fruit has not been described.

The objectives of this study were to 1) describe the postharvest production of aroma volatiles by strawberries harvested at different stages of maturity, 2) correlate changes in volatile production with color development, and 3) determine the influence of light and temperature on aroma volatile production and color development.

Materials and Methods

Experiment 1. In the first experiment, designed to determine postharvest production of aroma volatiles and color changes at different stages of maturity, decay-free and regular-shaped 'Kent' strawberries with the calyx attached were harvested at white, pink, and red stages of maturity and randomly divided into experimental treatments. Each treatment consisted of three replicates of 10 berries for each of the maturity groups. The berries were stored in a growth chamber (model E8VH; Conviron) at 15C and 75% relative humidity. Continuous light (200 μ mol·m²·s⁻¹) was supplied by a combination of fluorescent (75%) and incandescent (25%) sources. Light intensity was monitored using a solar monitor (LI-1776; LI-COR, Lincoln, Neb.). Berries were sampled after 0,2,4,6,8, and 10 days of storage for volatile ester production and surface fruit color (tristimulus calorimeter and visual rating).

Experiment 2. The second experiment was designed to determine the influence of postharvest light and temperature conditions on aroma volatile production and color development. Berries were collected as described above and given two temperature treatments, 10 and 20C, using two growth chambers. Within each growth chamber, half the berries were exposed to light, as described above, and the other half was kept in the dark using sheets

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of foil above the sample trays. Berries were evaluated at harvest and after 3 days of storage for production of volatile compounds, weight loss, surface color (tristimulus calorimeter and visual rating), and anthocyanin content.

Volatile sampling. Ten berries were sealed in a l-liter glass jar with a Teflon lid and held at 20C. Purified air was flushed through the jar at a rate of 100 ml·min⁻¹ for 30 min to allow the volatiles in the jar to equilibrate. Then the outlet air was passed through a 100×6.4 -mm-o.d. glass adsorbent trap containing 120 mg of Tenax-GR 20/35 for 5 min (500 ml). Immediately following the sample collection, the trap was removed from the jar, sealed in a glass culture tube with a Teflon-lined cap, and held at -20C until analyzed.

Volatile analysis. Volatiles absorbed on the traps were analyzed using a gas chromatography-mass spectroscopy system (Magnum; Finnigan-MAT, San Jose, Calif.) equipped with a purge and trap concentrator (LSC 2000; Tekmar Co., Cincinnati). Traps were placed in the concentrator and desorbed for 4 min at 200C into a liquid-CO₂-cooled injector. The injector was held at -65C for 4 min during the sample resorption and then immediately heated to 225C. The sample was analyzed on a DB-5 30-m \times 0.25-mm column with a film thickness of 0.25μ and the column flow rate was 1.1 ml·min⁻¹ of He. The column temperature was held at 35C for 5 tin, increased to 220C at a rate of 4C/min, and held at 220C for 10 min. The transfer line and ion trap temperatures were 240, and 220C, respectively. The ion source temperature was 220C and ionization was at 70eV. Spectra were acquired over the range of 32 to 200 m/z at 1 scan/sec. Identification of methyl butanoate, ethyl butanoate, methyl hexanoate and ethyl hexanoate was carried out by comparing mass spectra and retention time with authentic compounds. Identification of remaining compounds were based on mass spectral library data. Quantitation of volatile peaks was done using external standards.

Color measurement. Color on opposite sides of the 10 berries in each replicate was evaluated using *Commission Internationale de l'Éclairage* L*, a*, b* color space coordinates obtained with a tristimulus calorimeter (Chroma Meter CR-200; Minolta, Ramsey, N.J.) supplied with an S-mm-diameter measuring port, diffuse illumination, and a 0° viewing angle. The calorimeter was calibrated with a white standard calibration plate. Calorimetric values for chroma (intensity) and hue angle (color) were calculated as chroma = $(a^2 + b^2)^{1/2}$ and hue angle= $\cos^{-1}[a/(a^2 + b^2)^{1/2}]$ (Little, 1975).

The amount of red color of each strawberry fruit was scored using a subjective 1–5 scale: 1 = 0% to 20%, 2 = 21% to 40%, 3 = 41% to 60%, 4 = 61% to 80% and 5 = 81% to 100%.

Anthocyanin measurements. Five berries were homogenized and l-g samples of homogenate were used for anthocyanin measurements. Samples were dissolved in a solution of 3 ml 0.1% HCl in methanol and extracted overnight in the dark. One milliliter of the pigment extract was stored at -70C until analyzed. Monomeric anthocyanin content in extracts from berries was determined spectrophotometrically using a microplate reader. Monomeric anthocyanin concentration was calculated based on the extinction coefficient for pelargonidin-3-glucoside (Wrolstad, 1976).

Statistical analysis. Statistical analysis of data was conducted using the analysis of variance procedure in Genstat (Payne et al., 1993). Analysis of volatiles produced by berries at different maturity stages was performed after logarithmic transformation of data. In the second experiment the three factors>maturity, storage temperature, and light—were arranged in a split-plot experimental design with three replications. Temperatures were assigned to the growth cabinets, and maturity and light treatments were randomized within each growth cabinet. Only results that were significant at P < 0.05 are discussed, except where noted.

Results and Discussion

Experiment 1. Volatile production was greatest from fruit that were harvested red ripe (Fig. 1). During the postharvest storage period the release of volatiles from these fruit increased about 7-fold, peaking after 4 days. At harvest, pink fruit released only about 1% of the volatile concentration released by red ripe fruit. Volatile production of pink fruit also peaked after 4 days at 15C, producing volatiles at a concentration similar to that of freshly harvested red fruit, but only 14% of the red fruit production after 4 days. No volatiles were detected from white fruit until 4 days after harvest. Volatile production from white fruit increased slowly and at 10 days after harvest reached about 20% of the volatile concentration produced by freshly harvested red ripe fruit.

In addition to the quantitative differences observed in total volatile production, qualitative differences in esters produced were also observed (Fig. 2). At harvest, eight volatile esters were detected in red ripe fruit, while pink fruit only produced methyl butanoate and a trace of methyl hexanoate, and white fruit produced no detectable volatile esters. During postharvest ripening under light at 15C, concentrations of all 8 esters increased in the red fruit, and six of the eight peaks reached a maximum after 4 days. Both 3-methylbutyl acetate and methyl 3-methylbutanoate reached their highest concentration after 6 days. In addition to these volatiles, small concentrations of hexyl acetate, hexyl butanoate, propyl hexanoate, octyl butanoate, andhexyl hexanoate were detected in the headspace over red fruit after 6 days at 15C. After 4 days of postharvest ripening, headspace concentrations of the eight major volatile esters from pink fruit were similar to those of freshly harvested red fruit, although pink fruit had slightly higher levels of 3-methylbutyl acetate and methyl-3methylbutanoate. Detectable levels of hexyl butanoate, octyl butanoate, and hexyl hexanoate were also present. However,

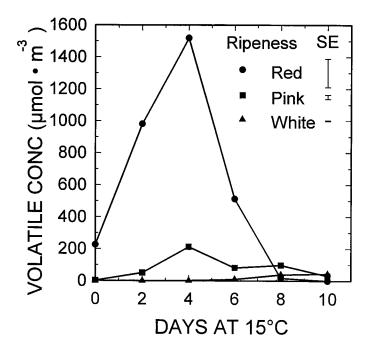


Fig. 1. Total concentration of volatile compounds detected in the headspace above 'Kent' strawberry fruit harvested at three ripenesses and held at 15C in continuous light for 10 days. Each point represents the mean concentration from three 10-fruit samples equilibrated for 30 min in a 1 -liter jar under a flow of 100 ml·min⁴ of purified air. Error bars represent the standard error of the mean (SE) with n = 3 and df = 10

concentrations of the major volatiles did not continue to increase to the high levels seen in red fruit following 2 or 4 days of postharvest ripening. After 6 days of ripening, white berries were able to produce detectable amounts of all eight major esters as well as octyl butanoate and hexyl hexanoate. During 10 days of postharvest ripening the main volatile produced by white berries was ethyl butanoate, whereas the major esters in pink and red berries were methyl and ethyl butanoate.

The distribution of methyl and ethyl esters in fresh strawberries is variable and appears to depend on cultivar, storage time, and storage atmospheric composition. The volatile esters of freshly harvested, ripe 'Kent' strawberries were composed of 93% methyl esters. Methyl esters comprised 60%–80% of the volatiles produced by ripe 'Gorella', 'Jesco', 'Senga Gigana', and 'Sivetta' strawberries (Dirinck et al., 1981), and 98% of those produced by 'Hoko-wase' strawberries (Ueda, 1992). Ethyl esters are predominant in some cultivars. Ripe 'Chandler' strawberries produced 60% of all volatiles as ethyl esters (Pérez et al., 1992), and 'Configra' fruit produced about 60% ethyl ester (Dirinck et al., 1981). During the postharvest ripening period the ratio of ethyl to methyl esters increased in 'Kent' fruit harvested at all three maturities. After 4 days, ethyl esters emitted from red fruit increased from 7% to about 44% of the volatiles. As white fruit began to produce esters, the ethyl esters were dominant. This difference could be due to a low rate of methyl alcohol biosynthesis or a high level of ethanol in the white berries. The postharvest ripening period may also increase ethanol content relative to methanol since the ethyl ester content of both red and pink fruit increased throughout storage. Storage of strawberries under atmospheres of <0.25%O₃ stimulate ethanol synthesis and the production of ethyl esters in 'Chandler' fruit (Ke et al., 1994). The high levels of ethyl esters Pérez et al. (1992) found in 'Chandler' fruit could have been

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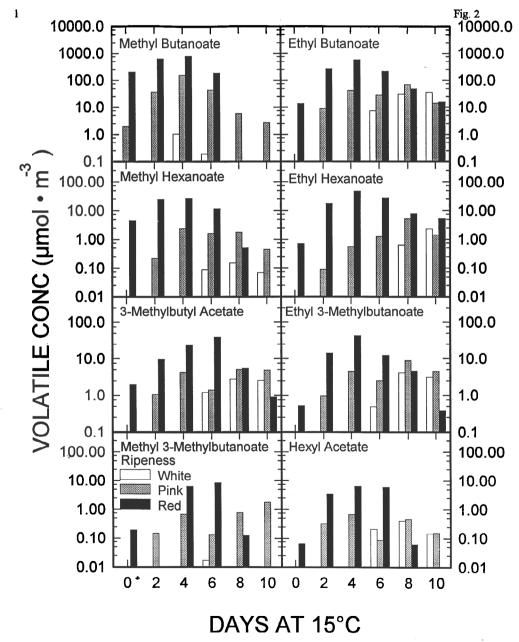


Fig. 2. Concentration of volatiles detected in the headspace above 'Kent' strawberry fruit harvested at three ripenesses and held at 15C in continuous light for 10 days. Each bar represents the mean concentration from three 10-fruit samples equilibrated for 30min in a 1-liter jar under a flow of 100 ml·min⁴ of purified air.

influenced by the 4 h the fruit were held under N_2 while volatiles were being trapped for analysis.

Larsen and Poll (1992) determined the aroma values (aroma value = concentration/threshold) of major volatiles of 'Senga Sengana' strawberries and found ethyl butanoate as the most aromatic compound followed by ethyl hexanoate and methyl butanoate. Taking into consideration these rankings, the red strawberries in this experiment were the most aromatic at harvest and their aroma increased during the first 4 days at 15C. This increased release of volatiles as the red fruit aged could be the result of increased synthesis and accumulation of volatile esters in the fruit tissue and/or a decrease in the resistances to the diffusion of these compounds out of the fruit due to senescence of the tissues. The similarity of aroma compounds emitted from pink fruit 4 days after harvest to those of freshly harvested red fruit would suggest that the flavor of these fruit approach that of ripe fruit during a postharvest ripening period. While white fruit were able to develop the ability to synthesize aroma esters after harvest, the low concentrations of these compounds indicate that their flavor would not be acceptable as a ripe strawberry.

Based on visual ratings, pink and white berries developed full red color 4 and 6 days after harvest, respectively, when held in light at 15C (data not shown). Strawberry brightness (L value, Fig. 3A) began to decrease just after harvest and became constant 4 days after harvest for pink and 6 days after harvest for white berries. Color intensity (chroma, Fig. 3B) values for red and pink berries

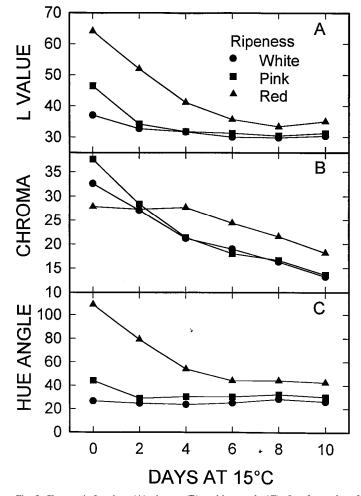


Fig. 3. Changes in L values (A), chroma (B), and hue angle (C) of surface color of white, pink, and red strawberries during 10 day of storage at 15C under continuous light. Standard errors of the mean were 0.69,0.71, and 1.90 for L values, chroma, and hue angle, respectively (n = 3, df = 10).

began to drop just after harvest. Chroma for white berries was constant for the first 4 days after harvest and then tended to decrease. Hue angles (color) for red fruit were constant during the whole period of storage, while the hue angle of pink berries did not change after 2 days of storage (Fig. 3C). Hue angle of white berries decreased in the first 6 days after harvest, and then remained unchanged.

Pink fruit developed full red color at the same time they reached their maximum volatile production. Changes in color of white berries also coincided with the production of volatiles. Volatile production by white berries began to increase 6 days after harvest when they were fully colored, according to the visual rating and changes in L and hue angle (Fig. 3 A–C). At this time, white berries produced the maximum number of volatiles.

Experiment 2. Both temperature and light affected volatile production by pink fruit; however, there was no consistent influence on all 'compounds (Table 1). Production of methyl-3methylbutanoate and 3-methylbutyl acetate was higher at 20 than 10C, whereas the opposite occurred for ethyl butanoate. Light was more consistent in stimulating volatile production as seen with ethyl hexanoate and 3-methylbutyl derivative esters (Table 1). Temperature and light may affect specific biosynthesis pathways in strawberry. The precursors of alcohol for ester synthesis are thought to come from the oxidation of long-chain fatty acids through the B-oxidation pathway (Bartley, 1985; Paillard, 1979). Methyl-branched carboxylic acids and alcohols are presumably formed from the methyl branched amino acids leucine, isoleucine, and valine (Tressl et al., 1970). The finding that light and temperature increased 3-methylbutyl derivative esters may result from an effect of these factors on any biosynthetic step (synthesis, transamination, or decarboxylation) of ester formation from these amino acids. In white fruit, light and temperature stimulated methyl and ethyl butanoate production, resulting in concentrations of 0.668 and 0.374 μ mol·m⁻³, respectively after 3 days of storage at 20C in light. No other volatiles were detected in the headspace over these fruit. White berries held at 20C in the dark or at 10C in the light or dark did not produce any detectable aroma volatiles. The effect of temperature and light on volatile production by red fruit was inconclusive in this experiment.

Color development of 'Kent' strawberries depended on temperature and light conditions (Table 2). This effect was especially pronounced for white berries, where color development was greatest in the light, and at 20C compared with 10C. The effects of temperature and light were apparent using either subjective visual determinations or objective measurements of L (brightness), chroma (intensity), and hue angle (color) values (Table 2). Visually, there was no difference in receptacle surface color between white and red berries after 3 days of storage at 20C in light. The presence of achenes lighter in color on white than on red fruit was probably responsible for differences in L and hue angle readings for these berries. Our study indicates that both temperature and light can influence anthocyanin synthesis in 'Kent' strawberries (Table 2). Pronounced stimulation of anthocyanin synthesis in light was observed for white and pink berries stored at 20C and for red berries stored at 10C. Considering that anthocyanins are the main contributor of color in strawberries, these data further support the stimulating effect of light on color development in berries noted above.

Several authors have reported a stimulating effect of temperature and a negligible effect of light on postharvest color development of nonfully colored berries (Austin et al., 1960; Kalt et al., 1993; Smith and Heinze, 1958). Kalt et al. (1993) observed that light increases red color development in white 'Blomidon' berries

Table 1. Influence of storage temperature and light on the concentration (μ mol·m⁻³) of volatile esters emitted by pink strawberries after 3 days of storage. Values represent the mean concentration in three 1-liter jars each containing 10-fruit samples equilibrated for 30 min under a flow of 100 ml·min⁻⁴ of purified air.

	10C		20C		SE ^z	
Volatile	Dark	Light	Dark	Light	(significance)	
Methyl butanoate	80	93	60	80	29.9 ^{NS}	
Ethyl butanoate	9.2	20.3	3.5	2.5	$2.22^{T, L \times T}$	
Methyl hexanoate	0.52	1.02	0.75	1.33	0.32 ^{NS}	
Ethyl hexanoate	0.02	0.119	0.031	0.086	0.028 ^L	
3-methylbutyl acetate	0.73	2.02	1.74	4.9	0.674 ^{t, l}	
Ethyl 3-methylbutanoate	0.13	1.25	0.00	1.32	0.367 ^L	
Methyl-3-methylbutanoate	0.12	0.57	0.40	2.30	0.344 ^{t, l}	

²Standard error of the mean with n = 3 and df = 8.

^{IST,L}Nonsignificant at P < 0.05 or significant for T = temperature, L = light.

Table 2. Weight loss, visual color evaluation, L, chroma, hue angle, and anthocyanin content of white, pink, and red 'Kent' strawberries after 3 days of postharvest ripening at 10 or 20C under continuous light or dark.

	Storage		Wt	Visual		Chroma		
	temp		loss	color	L	value	Hue	Anthocyanin
Maturity	(°C)	Light	(%)	(1–5)	value	$(mg \cdot g^{-1} DW)$	value	content
White 10 20	10	No	13.4	1.3	53.0	26.5	98.7	0.08
		Yes	24.0	1.6	51.5	28.1	91.4	0.10
	20	No	20.3	2.2	49.4	25.5	82.2	0.23
		Yes	33.2	4.2	38.2	27.2	46.7	1.17
Pink 10 20	10	No	10.6	4.5	35.9	31.8	33.3	1.47
		Yes	12.4	4.6	34.9	30.0	31.6	1.72
	20	No	11.9	4.9	34.6	29.0	30.7	1.76
		Yes	23.7	5.0	31.9	22.6	25.3	3.56
Red 10 20	10	No	7.6	5.0	30.8	26.7	24.4	2.47
		Yes	13.4	5.0	30.1	24.1	23.9	3.10
	20	No	14.5	5.0	30.6	21.4	23.7	3.44
		Yes	23.9	5.0	30.1	21.5	23.6	3.57
se $(n = 3, df = 35)$			1.49	0.06	0.39	0.87	0.94	0.15
Significance	$e^{i} (P < 0.05)$	L, T, M _q		$T \times L \times M_q$				

^zL= light, T = temperature, M = maturity at harvest; q = linear and quadratic effects.</sup>

more at 10C than at 20C, but the difference was not statistically significant. In our study, light significantly influenced color development of pink and white 'Kent' strawberries, particularly at 20C.

Temperature increased weight loss of berries, probably due to fruit transpiration (Table 2). Light influenced weight loss for white, pink, and red berries, which was especially high at 20C (Table 2). This finding is consistent with data obtained by Kalt et al. (1993). Temperature at the tray level in light was about 1 degree higher than in the dark, and the effect may be related to vapor pressure deficit created by light-induced temperature differences between the light and dark treatments. It is also possible that light could stimulate the opening of stomata on the calvx and possibly on the fruit. The above effects could contribute to the difference in color development and volatile production between berries kept under light and dark conditions. In apples, water loss enhances the production of acetate esters, whereas the rates of alcohol production decreases with increasing rates of water loss (Wills, 1968). In our experiments, an increase in ester production during postharvest ripening of berries may also be partially influenced by their water loss during storage. Weight loss response under different temperature and light conditions may have also modified volatile production.

In conclusion, the ability of 'Kent' strawberries to produce volatiles increased with maturity. After harvest, white berries were able to become fully red and to produce aroma-important volatiles similar to those produced by pink and red berries but at lower concentrations. We observed that both temperature and light affected volatile production, color development, and anthocyanin production during postharvest ripening of white, pink, and red berries. While pink fruit were able to develop an acceptable level of volatile production and color to approach the organoleptic quality of a ripe fruit, white fruit were not. While white fruit appeared ripe based on color, they remained hard, high in acid, low in sugar, and lacked ripe strawberry aroma. Although aroma volatile synthesis began as color developed in white berries, the volatile concentration and composition did not approach that of ripe fruit. A further understanding of the ripening process in the strawberry, and mechanisms that enhance the development of volatile production and other quality parameters, could lead to methods to ripen immature fruit.

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