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Aroma of Fresh Strawberries Is Enhanced by Ripening over Red versus Black Mulch

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Strawberry (*Fragaria ananassa* Duch) is a high-value food crop, the aroma of which is important in consumer satisfaction. We hypothesized that the concentration of fresh strawberry aroma compounds could be enhanced by growing the berries over a red plastic mulch that was formulated to reflect more far-red (FR) and red light (R) and a higher FR/R photon ratio than is reflected by standard black plastic mulch. Strawberries of the cultivars "Chandler" and "Sweet Charlie" were grown in trickle-irrigated field plots over the two colors of mulch. The berries were harvested when ripe, and aroma compounds were isolated by dynamic headspace sampling. Entrained compounds were quantified by gas chromatography and identified by gas chromatography/mass spectroscopy. Twenty-three compounds were identified, and most of them were aliphatic esters. Strawberries that ripened over the red mulch during periods of sunny weather had significantly higher concentrations of aroma compounds than berries grown over the black plastic mulch. Total levels of aroma compounds from berries grown over red plastic mulch were higher for both Chandler and Sweet Charlie. We postulate that FR and R in light reflected from the red mulch acted through the natural phytochrome system to modify gene expression enough to result in greater concentration of aroma compounds in fresh strawberries.

KEYWORDS: Strawberry; *Fragaria ananassa*; aroma; colored mulch; ester; photomorphogenesis; phytochrome; terpenoid; volatile

INTRODUCTION

Strawberry (*Fragaria ananassa* Duch) is a high-value food crop that is consumed for its pleasant flavor as well as its nutrient content. Although grown by large-scale producers, it is also a favorite crop for many "you-pick" customers and home gardeners who desire early-season fresh fruit. The crop is commonly grown in a raised-bed culture system using trickle irrigation and plastic row covers. These practices keep the berries clean, conserve water, and control weeds with less herbicides. Black is the most commonly used color of plastic mulch.

It is well-known that plants need light for photosynthesis, and that they respond morphologically to various colors such as red (R), far-red (FR), and blue (B) light in controlled environments (*1*−*3*). More recently, it was shown that source-sink allocation and chemical composition of plants can be altered in the field by manipulating the spectral distribution of light reflected from nearby plants, plant residue on the soil surface, color of natural soil, and colored mulches on the soil surface (*4*−*6*).

A recently developed red plastic mulch, for example, combines the benefits attributed to black plastic mulch with the further benefit of growth regulatory effects of reflected FR and R light and a higher FR/R photon ratio (*6, 7*). Yields of tomatoes (*Lycopersicon esculentum* Mill.) and strawberries were increased over this specially formulated red plastic when compared to yields over standard black plastic (*7, 8*). The authors concluded that the R and FR photons reflected from the red mulch entered developing parts of the plants and caused them to allocate more photosynthate to developing fruit due to action of the natural phytochrome system within the growing plants.

After it was shown that strawberry (cv. Chandler) yield could be increased when the berries developed over the red mulch versus the black, and human taste testers indicated a preference for strawberries grown over the red mulch (*8*), many of the berries were frozen and stored at −65 °C for further analyses of flavor and nutrient content. Following several months of storage, berries that ripened over the red plastic were crushed and evaluated for concentration of aroma compounds emitted by the thawed berries. Those that had ripened over red mulch during periods of sunny weather produced significantly higher levels of aroma compounds (*9*).

Although the influence of growing Chandler strawberries over the red versus standard black mulch on concentrations of flavor and aroma compounds in frozen berries was evident (*9*), we needed to evaluate fresh (unfrozen) berries of Chandler and another cultivar to determine whether the response was specific only to frozen Chandler strawberries, or whether the response would also occur in fresh berries and in another cultivar. Therefore, the objectives of the present study were to determine (a) whether color of mulch could influence concentrations of aroma compounds released by fresh strawberries that were prepared for analyses within 15 min after harvest from plants growing over the red versus black mulches, and (b) whether

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Table 1. Headspace Concentrations (nanograms per gram of fresh weight) of Aroma Compounds Isolated from Fresh Strawberries that Ripened over Red versus Black Mulch during Periods of Sunny Weather[a]

	culvitar			
	chandler		sweet charlie	
compound	red	black	red	black
A. Aliphatic Esters				
methyl butyrate	175 ± 15.9*	87.7 ± 14.4	166 ± 30.2	111 ± 25.5
ethyl butyrate	512 ± 79.8*	135 ± 15.7	566 ± 94.6*	247 ± 72.5
ethyl 2-methylbutyrate	19.5 ± 2.2	25.3 ± 2.2	20.1 ± 2.5	17.7 ± 3.0
2-methylbutyl acetate	48.0 ± 3.2*	34.3 ± 2.4	24.8 ± 1.5	25.2 ± 1.9
butyl acetate	65.7 ± 16.2*	15.6 ± 2.0	11.0 ± 2.2	8.4 ± 2.0
methyl hexanoate	438 ± 30.8*	274 ± 23.4	674 ± 60.1	502 ± 106
butyl butyrate	34.5 ± 6.9*	16.1 ± 1.2	24.1 ± 4.9	16.8 ± 1.4
ethyl hexanoate	474 ± 53.1*	228 ± 31.6	787 ± 101*	470 ± 99.2
hexyl acetate	154 ± 8.9*	105 ± 5.6	210 ± 23.5	181 ± 20.5
(*Z*)-3-hexenyl acetate	40.3 ± 4.1	36.5 ± 1.8	62.2 ± 7.3	62.3 ± 6.6
(*E*)-2-hexenyl acetate	168 ± 8.8*	124 ± 8.5	191 ± 18.2	178 ± 23.1
methyl octanoate	3.5 ± 0.3	3.0 ± 0.3	3.0 ± 0.6	2.5 ± 0.4
hexyl butyrate	6.6 ± 1.5*	1.6 ± 0.2	10.2 ± 2.1*	3.3 ± 0.9
B. Aliphatic Aldehydes and Alcohols				
hexanal	48.5 ± 2.7	44.5 ± 4.8	55.1 ± 3.9	62.9 ± 3.9
(*E*)-2-hexenal	23.6 ± 1.9*	15.1 ± 2.3	30.2 ± 5.0	27.6 ± 2.7
hexanol	9.8 ± 0.8	9.7 ± 0.5	14.8 ± 2.0	12.6 ± 0.6
(*Z*)-3-hexenol	3.4 ± 0.2	3.8 ± 0.2	4.3 ± 0.6	5.1 ± 0.4
(*E*)-2-hexenol	11.7 ± 0.6	11.3 ± 0.6	13.5 ± 1.1	12.5 ± 0.8
C. Terpenoids				
α-pinene	45.5 ± 3.6	49.7 ± 5.1	29.5 ± 1.2	42.0 ± 7.4*
2-carene	0.9 ± 0.2*	0.4 ± 0.1	2.2 ± 0.7	1.9 ± 1.4
limonene	2.0 ± 0.8	1.3 ± 0.5	0.6 ± 0.2	0.7 ± 0.3
linalool	58.2 ± 6.9*	41.2 ± 2.1	256 ± 20.0	245 ± 72.1
nerolidol	16.7 ± 2.6*	5.6 ± 2.9	335 ± 19.3	270 ± 39.6

[a] Data represent the mean of nine determinations ± standard error of the mean. Within each cultivar, means followed by an asterisk indicate significantly higher levels (analysis of variance, $P = 0.05$).

concentrations of aroma compounds of two popular cultivars (Chandler and Sweet Charlie) would respond similarly to ripening over the red versus standard black mulches.

MATERIALS AND METHODS

Plant Material and Growing Conditions. Strawberry (cv. Chandler and Sweet Charlie) plants were grown in trickle-irrigated plots of Norfolk loamy sand (Typic Kandidults) in outdoor plots at the Coastal Plains Soil, Water and Plant Research Center near Florence, SC. The subplots were covered with standard black plastic mulch or with red plastic mulch [manufactured by Sonoco Products Co., Hartsville, SC, and marketed as Selective Reflective Mulch (SRM-Red) by Ken-Bar, Inc., Reading, MA]. Growth and developmental responses to R and FR over the red plastic could be compared to those that occurred over the standard black plastic because the red and the black surfaces differ in the amounts of R and FR (without influencing the amount of blue light, which can also affect morphogenesis) reflected to the developing parts of sungrown plants. That is, the standard black plastic reflected about five percent of the B, R, and FR that impinged on it, while the red plastic reflected about five percent of the B but higher amounts of R and FR (the amount detected depends on distance from the reflecting surface). Also, the red plastic reflected a FR/R ratio that was higher than the ratio in incoming sunlight at the same time and place.

Black polyethylene mulch (1.5 m wide), trickle-irrigation tubing, and bromomethane fumigation were all applied to the field plots in a single operation in mid-August. This operation also included raising the beds and covering the edges of the plastic with soil to keep it in place. The mulch-covered beds were 90 cm wide and 15 cm high. They remained undisturbed until early October when the red plastic was put in place, holes were punched, and strawberries were transplanted. Plants of both cultivars were purchased at the same time from Aarons Creek Farm (Buffalo Junction, VA). There were six replicated plots of black versus red plastic mulch. The red-mulched subplots were obtained by taping a layer of red plastic over the existing layer of black plastic just before the holes were punched. The within-row plant spacing was 30 cm, and the rows were 1.8 m apart. This spacing gave a high probability that strawberry fruit developing and ripening over the red mulch would receive R and FR reflected from the mulch surface. Soil temperatures 10 cm below the two colors of mulch were about 0.5 °C warmer under the black plastic than under the red (as used in this study) at noon on a sunny day.

Sample Preparation and Collection. Collections were performed on a "push−pull" apparatus described by Loughrin and Kasperbauer (*10*). It had six chambers for samples, each consisting of a 500-mL sidearm Erlenmeyer flask. Bracts were removed from the strawberries within 15 min after harvest, and the fruits were quartered lengthwise. About 35 g of fruit was placed into each sampling chamber along with 10 μg each of cumene (*iso*-propylbenzene) and hasmigone (2-(hex-2-enyl)-cyclopentanone) dissolved in 50 μg of $CHCl_3$ as internal standards. Collection traps consisting of 0.635 cm o.d. glass tubing containing 50 mg of Super Q absorbent (Supelco Inc., Bellefonte PA) enclosed within glass wool plugs were rinsed with 2 mL of high purity hexane prior to attachment.

Entrained compounds were eluted from the traps with 320 μL of an 80:20 mixture of high purity hexane and CH_2Cl_2. Aliquots of 1 μL were injected onto a gas chromatograph (GC; Varian model 3800, Varian Assoc., Walnut Creek, CA) operated in splitless mode for one min and equipped with a 60 m × 0.32 mm Supelcowax-10 column with a 0.5-μm film thickness. Gas chromatographic operating conditions were as follows: injector, 220 °C; oven temperature, 50 °C for 1 min then programmed at 3 °C per min to 180 °C; flame ionization detector, 240 °C; and helium carrier linear flow velocity, 21 cm per sec. Compounds were measured against the cumene and hasmigone standards within relative retention time windows, and results were expressed as ng per g fresh weight of fruit contained in the sampling vessels.

For each cultivar, aroma compounds were collected from samples of fresh strawberries that ripened over red versus black plastic mulch on three sunny days. On each of the harvest dates, three samples of ripe berries were collected from each color of mulch for a total of nine samples for each cultivar and each color of mulch. Because there were fewer overcast days during the harvest period, fruits were collected on 2 days of overcast weather, taking three samples each of fruits grown over each color of mulch for a total of six samples for each cultivar and each color of plastic mulch.

Compound Identification. Gas chromatography/mass spectroscopy was performed on a GC equipped with a 30 m × 0.25 mm HP-5 column (Hewlett-Packard, Palo Alto, CA) interfaced to a Hewlett-Packard GCD model 1800B mass selective detector. Injections were made onto the GC in splitless mode for 1 min ,and the mass ion detector used a scanning range of 40−450 amu. Operating conditions for the GC were as follows: injector, 220 °C; and column oven, 40 °C for 1 min then programmed at 3 °C per min to 180 °C. Compound identifications were performed by computer database searches and coelution of authentic samples of compounds on the Supelcowax-10 column. The authentic samples of compounds were obtained from commercial sources. Data were compared by analysis of variance using the SAS system for windows version 6.12 (*11*).

RESULTS AND DISCUSSION

Twenty-three aroma compounds were identified from fresh strawberry fruit. The greatest concentration of aroma compounds consisted of aliphatic esters, with the methyl and ethyl esters of butanoic and hexanoic acids predominating. A number of six-carbon aldehydes and alcohols were also identified, as well as terpene hydrocarbons and alcohols. In general, the yields of aroma compounds were quite similar between the two cultivars. Sweet Charlie, however, produced much higher amounts of the terpene alcohols, linalool, and nerolidol.

During periods of sunny weather, fruit collected from plants grown over the red plastic mulch gave a greater concentration of aroma compounds than did fruit collected from plants grown over black plastic (Table 1). This difference was most evident in the case of Chandler and, for both cultivars, in the case of the aliphatic esters. These compounds typically predominate in most fruit essences, and are important in describing overall fruit quality. The concentration of esters produced by fruits grown over the red plastic mulch was more than 90% higher for Chandler and almost 50% higher for Sweet Charlie than for fruits grown over black plastic mulch.

Although there was a tendency for strawberries grown over red plastic to produce higher amounts of unesterified aliphatic compounds and terpenoids, this difference was not as pronounced as those we obtained from frozen (and thawed) fruit of Chandler in a previous study (*9*). In that study, we found that strawberries grown over red plastic mulch produced significantly higher concentrations of unesterified aliphatic compounds, while those grown over black plastic produced significantly higher levels of terpenoids. In neither case, however, were the differences in concentrations as pronounced as in the case of the esters. We also found that ester production by frozen (and thawed) Chandler fruits grown over red plastic mulch was more than 60% higher than that of fruits grown over black plastic mulch. In the present study, amounts of aroma compounds emitted by fresh fruit of the two cultivars grown over red versus black plastic and grouped by biosynthetic class are summarized in Figure 1. Statistical interpretation is presented in Table 2.

Although concentrations of aroma compounds released by ripe berries were higher for Sweet Charlie, concentrations emitted from berries that ripened during overcast weather did not differ significantly over red versus black mulches (Table 3). That is, within each cultivar, the levels of individual compounds were very similar regardless of the color of mulch over which the fruit had ripened, when ripened during overcast conditions. This finding with fresh fruit is consistent with

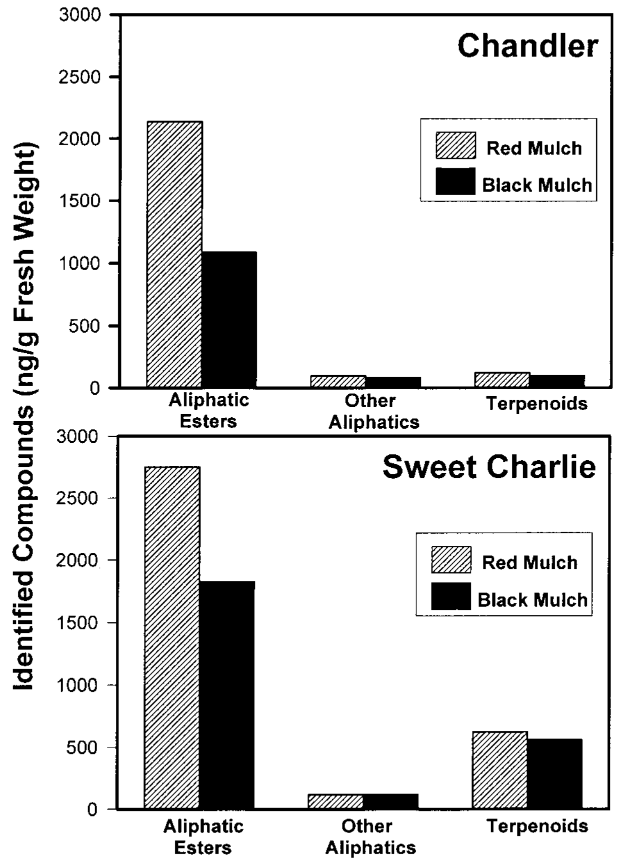

Figure 1. Aroma compounds isolated from fresh strawberries classified according to biosynthetic classes. Berries ripened and were collected during periods of sunny weather. Statistical interpretations of differences are summarized in Table 2.

Table 2. Summary of Statistical Analyses for Color of Mulch, Cultivar, and Color of Mulch by Cultivar Interaction for Aliphatic Esters, Other Aliphatics, and Terpenoids Shown in Figure 1

comparison	aliphatic esters	other aliphatics	terpenoids
mulch color (M)	*[a]	NS	*
cultivar (C)	*	NS	*
M × C	NS	NS	NS

[a] An asterisk (*) indicates that the values differed significantly at $P = 0.05$, and NS indicates the values were not significantly different at $P = 0.05$.

previous observations obtained with Chandler strawberries that were frozen, and thawed, before analyses (*8, 9*). In those studies, it was noted that differences in the flavor of strawberries grown over red versus black plastic mulch were most evident when the berries ripened during periods of sunny weather.

In the present study with fresh strawberries of two cultivars, the total concentration of aroma compounds was significantly higher from fruit that ripened over red plastic mulch during periods of sunny weather (Figure 2). On the other hand, the total concentration of aroma compounds from fruit that ripened during overcast weather over the red plastic mulch was quite similar to that of fruit grown over black plastic mulch. Photocontrol of the pronounced differences in quantity of aroma compounds released by strawberries that ripened over the red (versus standard black) mulch during sunny versus overcast weather is beyond the scope of our experiment. However, it may involve what was referred to about 40 years ago as the high irradiance response of photomorphogenesis. That is, a number of controlled environment studies indicated that presence

Table 3. Headspace Concentrations (nanograms per gram of fresh weight) of Aroma Compounds Isolated from Fresh Strawberries that Ripened over Red versus Black Mulch during Periods of Overcast Weather[a]

	cultivar			
	chandler		sweet charlie	
compound	red mulch	black mulch	red mulch	black mulch
	A. Aliphatic Esters			
methyl butyrate	61.2 ± 14.8	60.5 ± 11.9	82.9 ± 29.0	51.6 ± 23.6
ethyl butyrate	119 ± 10.4	131 ± 11.8	238 ± 81.9	216 ± 95.5
ethyl 2-methylbutyrate	28.8 ± 3.3	26.5 ± 3.1	31.4 ± 3.5	25.9 ±4.2
2-methylbutyl acetate	57.0 ± 4.4	68.0 ± 20.1	26.0 ± 4.0	20.5 ± 1.2
butyl acetate	10.2 ± 0.7	9.1 ± 1.2	8.3 ± 0.8	8.1 ± 0.7
methyl hexanoate	347 ± 9.7*	289 ± 19.6	449 ± 181	319 ± 157
butyl butyrate	17.4 ± 1.4	15.9 ± 1.1	20.6 ± 1.5	17.3 ± 1.4
ethyl hexanoate	160 ± 13.8	213 ± 11.3	372 ± 138	358 ± 185
hexyl acetate	148 ± 34.3	112 ± 6.1	199 ± 22.2	209 ± 25.0
(*Z*)-3-hexenyl acetate	36.6 ± 2.1	26.1 ± 5.2	67.1 ± 4.1	84.5 ± 4.0*
(*E*)-2-hexenyl acetate	137 ± 16.3	146 ± 13.2	223 ± 32.6	214 ± 38.1
methyl octanoate	3.2 ± 0.2	3.1 ± 0.4	3.6 ± 0.5	3.4 ± 0.3
hexyl butyrate	2.0 ± 0.3	1.7 ± 0.4	5.1 ± 2.2	5.0 ± 2.9
	B. Aliphatic Aldehydes and Alcohols			
hexanal	57.7 ± 5.0	54.6 ± 4.2	68.5 ± 5.9	54.6 ± 5.8
(*E*)-2-hexenal	14.0 ± 2.5	18.7 ± 1.3	26.8 ± 8.9	17.3 ± 4.8
hexanol	10.9 ± 0.8	10.9 ± 0.8	15.6 ± 1.5	17.5 ± 2.9
(*Z*)-3-hexenol	3.4 ± 0.5	4.1 ± 0.5	5.6 ± 0.5	7.1 ± 0.7
(*E*)-2-hexenol	13.8 ± 0.7	11.6 ± 1.0	16.2 ± 1.7	16.8 ± 2.2
	C. Terpenoids			
α-pinene	50.4 ± 2.7	45.6 ± 2.8	30.7 ± 3.3	34.5 ± 5.1
2-carene	tr[b]	tr	1.1 ± 0.5	1.1 ± 0.6
limonene	0.9 ± 0.3	0.7 ± 0.2	tr	tr
linalool	59.6 ± 6.7	48.7 ± 5.0	253 ± 23.2	243 ± 15.4
nerolidol	7.5 ± 0.7	8.2 ± 2.1	263 ± 56.9	184 ± 31.5

[a] Data represent the mean of six determinations ± standard error of the mean. Within each cultivar, means followed by an asterisk indicate significantly higher levels (analysis of variance, $P = 0.05$). [b] Indicates that compound occurred at less than 0.5 ng per gram fresh weight.

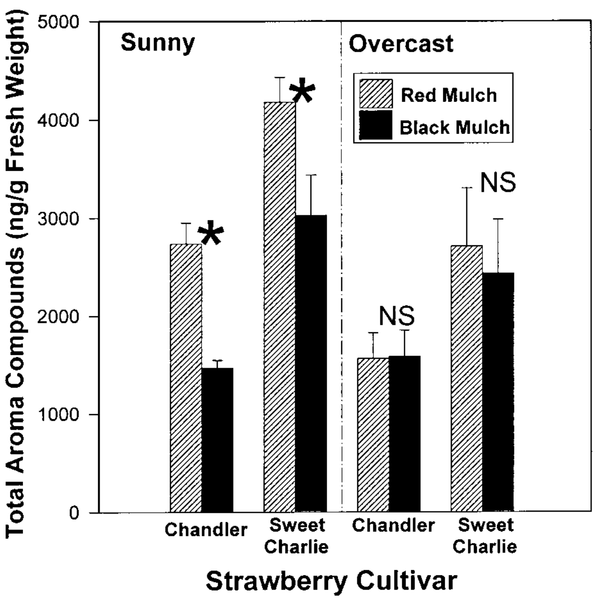

Figure 2. Total yields of identified compounds from two cultivars of fresh strawberries that ripened during periods of sunny versus overcast weather. Data for sunny weather represent the means of nine determinations ± standard error of the mean. Data for overcast weather represent the means of six determinations ± standard error of the mean. Total aroma compounds from strawberries grown over red versus black within each cultivar and marked with an * are significantly different at $P = 0.05$ by analysis of variance.

(or absence) of a period of high-intensity light influenced effectiveness of R and FR (*2*).

The proximal mechanism whereby reflection from red mulch to ripening strawberries during sunny weather serves to enhance aroma production is also beyond the scope of our study. However, it could involve phytochrome activation of the enzymes responsible for production of aroma compounds or could perhaps occur more indirectly through plant growth regulatory effects. As production of aliphatic esters was enhanced by growing the strawberries over red plastic, it might be that esterase activity was directly increased by this treatment. Alternatively, an altered FR/R photon ratio can affect plant responses to and/or production of ethylene, an important growth regulator affecting fruit ripening in both climateric and nonclimateric fruits (*12*, *13*).

Aliphatic esters in fruit are synthesized from short-chain acids and amino acid precursors (*14*, *15*). It is possible that higher levels of esters from strawberries that ripened over red plastic might be due to increased levels of these precursors rather than enhanced esterase activity. Perhaps future research will elucidate the relative importance of the size of precursor pools or level of enzyme activity to the higher concentrations of esters obtained from strawberries grown over red surfaces.

When we examined the aroma compounds produced by frozen Chandler fruit grown over red or black plastic mulch in the previous study (*9*), we found that concentrations of aliphatic esters, such as ethyl butyrate and ethyl hexanoate, released by frozen (and thawed) Chandler fruit were quite similar to the concentrations obtained from fresh fruit in the present work. Frozen fruit, however, produced much greater amounts of the aliphatic aldehydes hexanal and (*E*)-2-hexenal. These compounds, which arise by the action of lipoxygenase on fatty acids and subsequent cleavage of a hydroperoxide intermediate by hydroperoxide lyase (*16*, *17*), arise from a chemical pathway different from those of the other aliphatic compounds (*14*, *15*). These compounds are often indicative of damage in higher plants. Interestingly, the amounts produced of the reduced and esterified forms of these compounds, (*Z*)-3-hexenyl acetate and (*E*)-2-hexenyl acetate, were quite similar in fresh and frozen fruit. Perhaps cellular damage caused by the thawing of the fruit (*9*) increased the levels of the aldehydes relative to that of fresh fruit while damaging the enzymes necessary to reduce and/or esterify them. Fresh strawberry fruits normally have a considerable capacity for the reduction and esterification of aliphatic aldehydes when applied exogenously (*18*). Our findings were opposed to that of Douillard and Guichard (*19*) who found that these aldehydes were more characteristic of fresh than of frozen fruit. Possibly freezing and storing the fruit at −65 °C rather than at a temperature more typical of a commercial freezer served to preserve enzymatic activity in our samples.

From a practical standpoint, variability in the levels of aroma compounds produced by strawberries greatly affects fruit quality and consumer acceptance (*19*−*23*). Because most days were sunny during our study, aroma production in both cultivars was greatly enhanced when the berries were grown over red compared to those grown over black plastic. It is likely, therefore, that strawberries grown over red plastic would usually have a correspondingly greater acceptance by consumers. This was evident in a taste preference survey of more than 50 individuals over a two-year period (*8*, *9*). Perhaps future research will show that the quality of other small fruits can be similarly enhanced.

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