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Differential responses of four biosynthetic pathways of aroma compounds in postharvest strawberry (*Fragaria* × *ananassa* Duch.) under interaction of light and temperature

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ABSTRACT

Light and temperature are two of the most important factors regulating postharvest strawberry aroma. To date the majority of research has been concentrated on the contribution of either light or temperature factors in isolation. In the present study, we investigated integrated effects of light and temperature on the formation of characteristic aromas during postharvest strawberry ripening process. Most volatiles including volatile esters, volatile furanones, and volatile terpenes showed increasing trends, whereas volatile benzenoids showed decreasing trends during postharvest ripening. Biosyntheses of volatile esters and volatile benzenoids were mainly affected by interaction of temperature and dark, whereas formation of volatile furanones and volatile terpenes were mostly influenced by temperature and dark, respectively. This study provided evidence of regulation of strawberry aroma by dual factors for the first time, and characterized a comprehensive profile of formations of strawberry aromas in response to light and temperature during postharvest ripening.

1. Introduction

Strawberry aroma is an important quality attribute that influences acceptability of consumer. Strawberry aroma is also one of the most complex fruit aromas with more than 350 volatile compounds (Bood & Zabetakis, 2002; Van de Poel, Vandendriessche, Hertog, Nicolai, & Geeraerd, 2014), in which only about 20 volatiles actually contribute to aroma and flavor of strawberry (Forney, Kalt, & Jordan, 2000). There are quantitative and qualitative differences of volatile compounds among different cultivars of ripe strawberries. A 35-fold difference in the quantity of volatiles evolved from different cultivars at ripe strawberries (Forney et al., 2000). The aromas of cultivars of 'Configra' and 'Chandler' were dominated by ethyl esters comprising 80% and 60%, respectively. While in fruits of 'Hokowase', 'Kent', 'SengaGigana', and 'Annapolis', methyl esters accounted for more than 70% of the total volatiles (Dirinck,

Abbreviations: AAT, alcohol acyltransferase; CA, *trans*-cinnamic acid; CHP1 (reference gene), conserved hypothetical protein; CNL, cinnamate: CoA ligase; DAHPS, phospho-2-dehydro-3-deoxyheptonate aldolase; DMMF, 4-methoxy-2,5-dimethyl-3(2*H*)-furanone; DMHF, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone; NES, Nerolidol synthase; OMT, O-methyltransferase; PAL, phenylalanine ammonia-lyase; QR, quinoneoxido reductase.

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Depooter, Willaert, & Schamp, 1981; Forney & Jordan, 1995; Miszczak, Forney, & Prange, 1995; Ueda & Bai, 1993). Although the volatiles contributing to strawberry aroma vary with cultivars, esters such as ethyl butanoate, ethyl hexanoate, methyl butanoate, and methyl hexanoate, furanone derivatives such as 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), and 4-methoxy-2,5-dimethyl-3(2*H*)-furanone (DMMF), and terpenes such as linalool and nerolidol, are relatively constant factors that contribute to strawberry aroma (Miszczak et al., 1995). Recently, some attempts have been made to identify and characterize genes involved in the volatiles contributing to strawberry aroma. The last step in the biosynthesis of volatile butanoates and hexanoates is catalyzed by alcohol acyltransferase (AAT). The *strawberry alcohol acyltransferase* (*SAAT*) and *alcohol acyltransferase* (*FaAAT2*) genes have been identified and functionally characterized to be involved in the formation of volatile esters in strawberry (Aharoni et al., 2000; Cumplido-Laso et al., 2012). DMHF and DMMF are deriving from sugar metabolism (Roscher, Schreier, & Schwab, 1997). *Fragaria* × *ananassa quinoneoxido reductase* (*FaQR*) is involved in the last biosynthetic pathway leading to DMHF (Raab et al., 2006), and *Fragaria* × *ananassa O-methyltransferase* (*FaOMT*) encodes an O-methyltransferase that is responsible for DMMF biosynthesis (Lavid et al., 2002; Wein et al., 2002), and the variation in mesifurane content (Zorrilla-Fontanesi et al., 2012). The monoterpene linalool and the sesquiterpene nerolidol are the main volatile terpenes in cultivated strawberry species fruits. A *nerolidol synthase* (*FaNES1*) gene is characterized to be involved in strawberry terpene formation (Aharoni et al., 2004).

Postharvest strawberry aroma is mainly affected by storage conditions. Light and temperature are two of the most important factors regulating the quality components in postharvest strawberry. Forney et al. (2000) found that ethyl butanoate and ethyl hexanoate content increased at 1 °C of storage temperature, while methyl butanoate and methyl hexanoate contents increased at 15 °C. Volatile production from strawberry fruit is also affected by light condition. Miszczak et al. (1995) showed that storage in light (200 μ mol m^{-2} s^{-1}) for 3 days at 10 or 20 °C increased the production of ethyl hexanoate, 3-methylbutyl acetate, ethyl 3-methylbutanoate, and methyl 3-methylbutanoate in pink 'Kent' strawberries. To date the majority of research has been concentrated on the contribution of either light or temperature factors in isolation. Very few studies on integrated effects of light and temperature on volatiles in strawberry fruits is available (Miszczak et al., 1995). Furthermore, no information on regulation mechanism of biosynthetic pathways of aroma compounds in postharvest strawberry by dual factors of light and temperature is available. Several key questions remain to be answered. (1) Do different biosynthetic pathways of aromas in postharvest strawberry have same or different responses to dual factors of light and temperature? (2)What is the regulation mechanism of aromas by the dual factors? To answer these questions, we used strawberry cultivar 'Sweet Charlie', which contains the characteristic aromas in strawberry fruits. We investigated the integrated effects of light and temperature on the aromas, and key genes involved in the formations of aromas. Finally we proposed a schematic model of regulation of formation of aromas by the factors.

2. Materials and methods

2.1. Treatments on strawberry fruits

'Sweet Charlie' strawberry fruit cultivated in Guangzhou (Guangdong province, China) was harvested at the following development stages: white fruits, white fruits with red achenes (1/2 red), turning stage fruits (3/4 red), and full-ripe red fruits. To study the regulating mechanisms of aromas by light and temperature, white fruits were harvested and exposed to light and dark conditions under 15 °C and 25 °C, respectively, for 7 days. The strawberries were stored in two growth chambers under 80% relative humidity, and at 15 °C and 25 °C, respectively. Within each growth chamber, half of the strawberries were exposed to light, which supplied by a light emitting diode (LED) light source (white light, 12,000 K, 3500 lx, Shenzhen FHT Electronics Technology Co., Ltd.). The other half of strawberries were kept in the dark box. Strawberries were sampled after 0, 2, 5, and 7 days, respectively.

2.2. Collection and analyses of emitted aromas from strawberry fruits

Aromas from whole and intact strawberry fruits were collected using solid phase micro extraction (SPME). Three strawberries were sealed in a 500 mL beaker per sample, and collected by SPME (2 cm 50/30 mm DVB/Carboxen™/PDMS Stable Flex™) for 15 min. The aromas absorbed on the SPME were analyzed using a gas chromatograph-mass spectrometer (GC–MS) QP2010 SE (Shimadzu Corporation, Japan) equipped with a SUPELCOWAX™10 column (Supelco Inc., 30 m × 0.25 mm × 0.25 μm). The injector temperature was 230 °C, splitless mode was used with a splitless time of 1 min, and helium was carrier gas with a velocity 1.0 mL/min. The GC oven was maintained at 40 °C for 3 min. The temperature of the oven was programmed at 4 °C/min to 240 °C, and kept at this temperature for 15 min. The mass spectrometry was operated with full scan mode (mass range *m*/*z* 40–200).

2.3. Extraction and analyses of internal aromas of strawberry fruits

To analyze internal aromas of strawberry, 3 g crushed frozen tissue of strawberry fruits were extracted with 10 mL of dichloromethane containing ethyl *n*-decanoate as an internal standard overnight under dark condition, centrifuged (2000*g*, 4 °C, 5 min), and then dried over anhydrous sodium sulfate. The resulting extract was concentrated to 1 mL by nitrogen, and then 1 μL of the concentrated extract was subjected to GC–MS, which method was described as above.

2.4. Extraction and analyses of glycosidically conjugated volatiles of strawberry fruits

Enzymatic hydrolysis combined with GC–MS analysis were employed to determine glycosidically conjugated volatiles in strawberry fruits. The method was similar with the reference (Gui et al., 2015). Five hundred mg (fresh weight) of strawberry fruit tissues (finely powdered) were extracted with 2 mL pre-cold methanol by vortexing for 2 min and ultrasonic extraction for 10 min. For phase separation, the extracts were added with 2 mL pre-cold chloroform and 0.8 mL pre-cold water. The resulting upper layer was dried, and dissolved in 1 mL water. The resulting solution was added with 30 mg PVPP, stood for 90 min, centrifuged (16,400*g*, 4 °C, 10 min), and repeated with the supernatant. The final supernatant was loaded to an Amberlite XAD-2 column (1 mL) and eluted with 5 mL water, 5 mL pentane: dichloromethane (2:1), and 5 mL methanol. The methanol eluent was dried and redissolved in 500 μL of 50 mM citric acid buffer (pH 5.6) containing β-primeverosidase and β-glucosidase, and reacted at 37 °C for 14 h. Afterwards, 144 mg of sodium chloride was added to the reaction solution, and stood for 15 min followed by adding 5 nmol of ethyl decanoate as an internal standard. The solution was extracted by 500 μL dichloromethane, centrifuged, and the dichloromethane fraction was dried over anhydrous sodium sulfate. The resultant solution was subjected to GC–MS analysis, which condition was same as described above.