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Volatile Precursors and Aroma-Related Enzyme Activities during Fruit Maturation of Muskmelon

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ABSTRACT

Fatty acids and amino acids serve as ester precursors, in addition to lipoxyaenase (LOX; EC 1.13.11.12), alcohol dehydrogenase (ADH; EC 1.1.1.1) and alcohol *o*-acyltransferase (AAT; EC 2.3.1.84) activities, were assessed during maturation of climacteric and non-climacteric muskmelons. The results showed that the levels of the main fatty acids related to aroma formation containing oleic acid, linoleic acid and linolenic acid during fruit development of 'Shannong Golden 1', a climacteric muskmelon were much higher than those in fruits of 'Sweet Delight', a non-climacteric muskmelon. The amino acids contents were very high in 15 days after pollination (DAP) fruit of 'Shannong Golden 1', but dramatically decreased in 30 DAP fruit and reached the lowest levels in mature fruit. But, these amino acids levels almost had no changes during fruit development of 'Sweet Delight'. In contrast to amino acids levels, ADH and LOX activities showed almost the same change trends, however, AAT activities were significantly different during fruit development of these two cultivars. Together, our data suggested that AAT activity, fatty acids and amino acids contents may play important roles and LOX and ADH may be not the limiting factors in ester volatiles production in muskmelon fruit.

Keywords: AAT, ADH, amino acid, *Cucumis melo*, fatty acid, LOX

INTRODUCTION

Sweetness and aroma are the two of the most important factors for fruit quality and consumer preference (Shalit *et al*. 2001), with volatile substances contributing to the flavor of fruit. Melon varieties (*Cucumis melo* L.) differ in physical and chemical attributes. They produce volatile aldehydes, alcohols and especially large quantities of esters. Volatile esters, the most significant contributors to aroma in muskmelon fruit, are generated by esterification of alcohols and acyl-CoA derived from both fatty acid and amino acid metabolism, in a reaction catalysed by the enzyme alcohol *o*-acyltransferase.

In fruit maturation, LOX, ADH and AAT are important enzymes responsible for acetaldehyde and ethanol production. It has been suggested that the selectivity properties of the enzymes controlling the reduction (ADH) and ester formation (AAT) steps in ester biosynthesis do not provide the specificity required to explain the composition of the esters obtained from bananas (Wyllie *et al*. 1996). The specificity must be determined therefore by the properties of the remaining enzymes of the pathway and/or by the availability of the necessary substrates.

In our previous study, we indicated that volatiles differ between climacteric and non-climacteric muskmelons and higher volatile ester concentrations were in climacteric muskmelon, but, the non-climacteric muskmelon with very low ester concentrations was absent in aroma (Tang *et al*. 2008). In this work, we monitored fatty acids and amino acids contents, LOX, ADH and AAT activities during muskmelon fruit maturation of climacteric and non-climacteric muskmelons and elucidated that AAT, fatty acids and amino acids played the key roles, but LOX and ADH may were not the limiting factors in determining ester volatiles formation in muskmelon fruit.

MATERIALS AND METHODS

Plant material and tissue sampling

Muskmelons (cvs. 'Shannong Golden 1' and 'Sweet Delight') were grown in a greenhouse in the experimental farm of Shandong Agricultural University in Tai'an, China from Mar. through June 2009, with spacing of 50 cm between plants, 120 cm between rows. Average day/night temperatures were about 30/20°C. Average daylight was about 12 h. Fertilizer was applied at two stages, a preplant broadcast application of 900 kg·ha^{-1} of 14 N-6.1 P-29.9 K, followed by a sidedress application of 150 kg·ha^{-1} N at flowering stage. Irrigation by furrows was applied as needed. Freshly opened female flowers were tagged on the day of hand-pollination to identify fruit of known age and one fruit per plant was allowed to develop. Different developing stages (15, 30, and 45 DAP) and mature fruit were harvested. Fifty-five DAP was considered commercial maturity for these two cultivars. Five fruit from each stage were used for fatty acids, amino acids and enzymes (LOX, ADH and AAT) activities assay. The experiments were repeated three times.

Analysis of fatty acid

Fatty acid was extracted according to the method described by Hou (2004), with slight modifications. Boring fruit at 105°C before extracted to protect fatty acid from enzymes catalyzing. Methylesterification of fatty acid was carried out by mixing 10 ml benzene and petroleum ether (v/v, 1: 1), adding 10 ml 0.4 N KOH-methanol (v/v, 1: 1) when extract dissolved fully into the mixture, placing static for 15 min after oscillating fully, and then adding 10 ml distilled water to the solution, the upper solution was analyzed by gas chromatography (Finnigan Corp., San Jose, Calif.).

Analysis of amino acid

Determination of amino acids was dried at 60°C, powered after the weight kept stability, added 6 mol/L HCl at 110°C for 22 h. The

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Short Communication

amino acid was analyzed by the Hitachi 835-50 Type High-Speed Amino Acid Analyzer (Japan, Hitachi Ltd.).

Extraction and assay of aroma-related enzyme activities

LOX, ADH and AAT activities on crude enzyme extracts were performed as described by Lara *et al*. (2003). Total protein in the enzyme extract was determined according to the method described by Bradford (1976), with modifications (BioRad Protein Assay kit) according to the manufacturer's instructions, using BSA as a standard.

RESULTS AND DISCUSSION

LOX may play a major role in determining the formation of straight-chain volatile compounds by catalyzing fatty acids in muskmelon fruit

Fatty acids serve as ester precursors, catabolised through two major pathways, β-oxidation and the lipoxygenase system (Sanz *et al*. 1997). The enzyme lipoxygenase catalyses hydroperoxidation of polyunsaturated fatty acids, the preferred substrates in plants being linoleic (18:2) and linolenic (18:3) acids. Lipoxygenase products are further metabolised by the fruit through a number of different pathways (Porta and Rocha-Sosa 2002), one of them mediated by hydroperoxide lyase (HPL) and involving conversion into aldehydes, alcohols and volatile esters. In this study, the levels of the main fatty acids related to aroma formation containing Oleic acid, Linoleic acid and Linolenic acid showed almost the same change trend during fruit development of 'Shannong Golden 1' and 'Sweet Delight'. The levels of these three fatty acids increased during the early fruit development and reached the maximum levels in 45 DAP fruit, but decreased significantly in the mature fruit. However, the levels of these three fatty acids in 'Shannong Golden 1' were much higher than those in fruit of 'Sweet Delight' (**Fig. 1**). Similarly, Lipoxygenase activity showed a significant increase in 45 DAP fruit of 'Shannong Golden 1' and 'Sweet Delight' as well (**Fig. 3A**), indicating LOX may play a major role in determining the formation of straight-chain volatile compounds in muskmelon fruit.

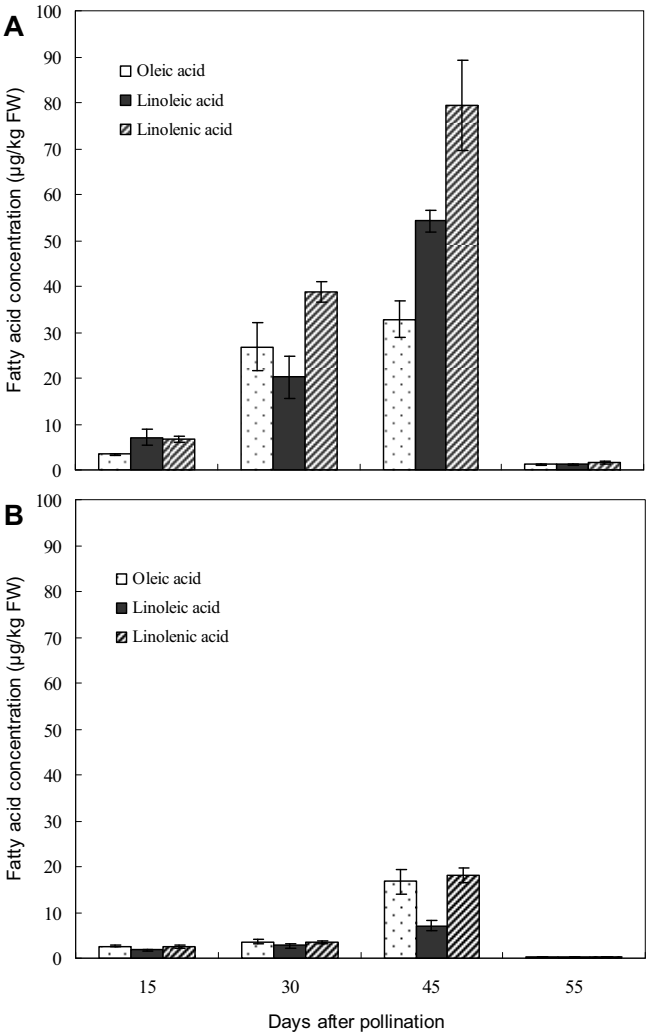

Fig. 1 Fatty acid concentration during fruit development of cvs. 'Shannong Golden 1' (**A**) and 'Sweet Delight' (**B**). Fifteen, 30, 45 and 55 d after pollination (DAP) fruit were harvested, respectively. Fifty-five DAP is considered commercial maturity. The experiment was repeated three times and five fruit were used for each replicate. Bars indicate SE.

AAT is a limiting factor in determining the ester volatiles formation in muskmelon fruit

Branched-chain volatile compounds, important in the aroma of many fruit, are derived from branched-chain amino acids leucine, isoleucine and valine (Tressl and Drawet 1973; Wyllie and Fellman 2000). The conversion into branched-chain alcohols proceeds through the production of an α-keto acid by an aminotransferase, and may involve as well an α-keto acid decarboxylase and alcohol dehydrogenase. AAT can act subsequently upon the alcohol pool contributed by this and other pathways to produce esters. The actual composition of the resulting esters could be controlled by both the selectivity and activity of the enzymes involved or by the substrate availability in this pool. ADH is an important enzyme responsible for ethanol production. It has been suggested that the selectivity properties of the enzymes controlling the reduction (ADH) and ester formation (AAT) steps in ester biosynthesis do not provide the specificity required to explain the composition of the esters obtained from bananas (Wyllie *et al*. 1996).

In our previous study, we indicated that volatiles differ between climacteric and non-climacteric muskmelons and higher volatile ester concentrations were in 'Shannong Golden 1' fruit, a climacteric muskmelon, but, 'Sweet Delight', a non-climacteric muskmelon with very low ester concentrations was absent in aroma (Tang *et al*. 2008). In this study, we measured the levels of Cystine, Valine, Isoleucine, Leucine, Tyrosine and Phenylalanine participating in volatile formation during fruit maturation of these two different climacteric-type varieties. The result showed that these six amino acids contents were very high in 15 DAP fruit of 'Shannong Golden 1', but dramatically decreased in 30 DAP fruit and reached the lowest levels in mature fruit. These amino acids levels almost had no changes during fruit development of 'Sweet Delight'. Interestingly, the levels of these amino acids in fruit of 'Sweet Delight' were definitely lower that those in fruit of 'Shannong Golden 1' before fruit mature, but very similar and no big difference in mature fruit (**Fig. 2**). In contrast to amino acids levels, ADH activity showed almost the same change trend during fruit development of these two cultivars (**Fig. 3B**), suggesting ADH may be not a limiting factor for the accumulation of volatile compound in muskmelon fruit. In contrast to ADH, AAT activities were significantly different during fruit development of these two cultivars. AAT activities increased dramatically at the later stage of 'Shannong Golden1' fruit development, but almost no changes during fruit development of 'Sweet Delight' (**Fig. 3C**), suggesting AAT may play important roles in ester volatiles production in muskmelon fruit.

In conclusion, the results presented here demonstrate that AAT activity, fatty acids and amino acids contents may play important roles and LOX and ADH may be not the limiting factors in ester volatiles production in muskmelon fruit.

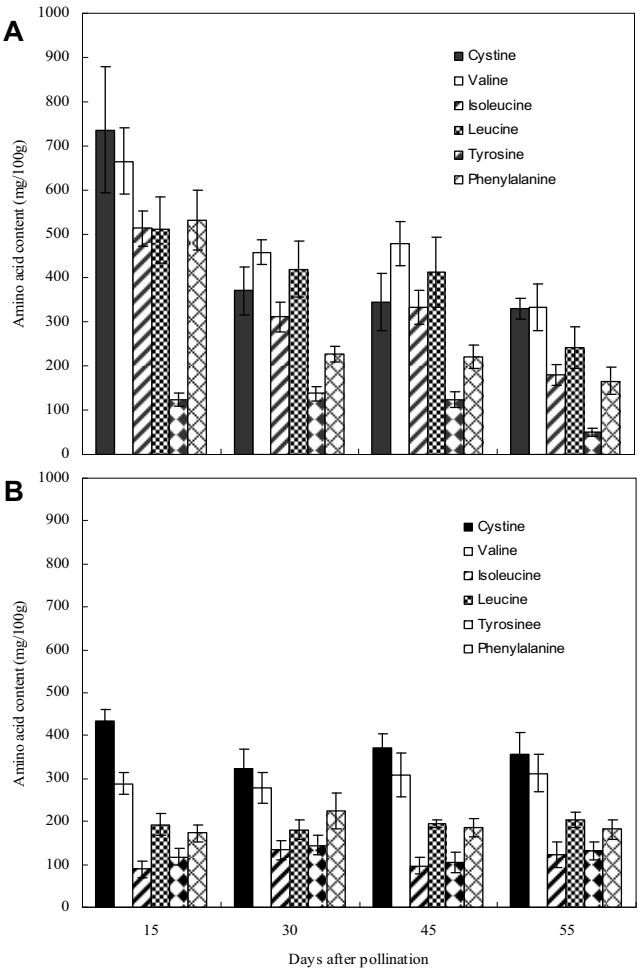

Fig. 2 Amino acid content during fruit development.of cvs. 'Shannong Golden 1' (**A**) and 'Sweet Delight' (**B**). Fifteen, 30, 45 and 55 d after pollination (DAP) fruit were harvested, respectively. Fifty-five DAP is considered commercial maturity. The experiment was repeated three times and five fruit were used for each replicate. Bars indicate SE.

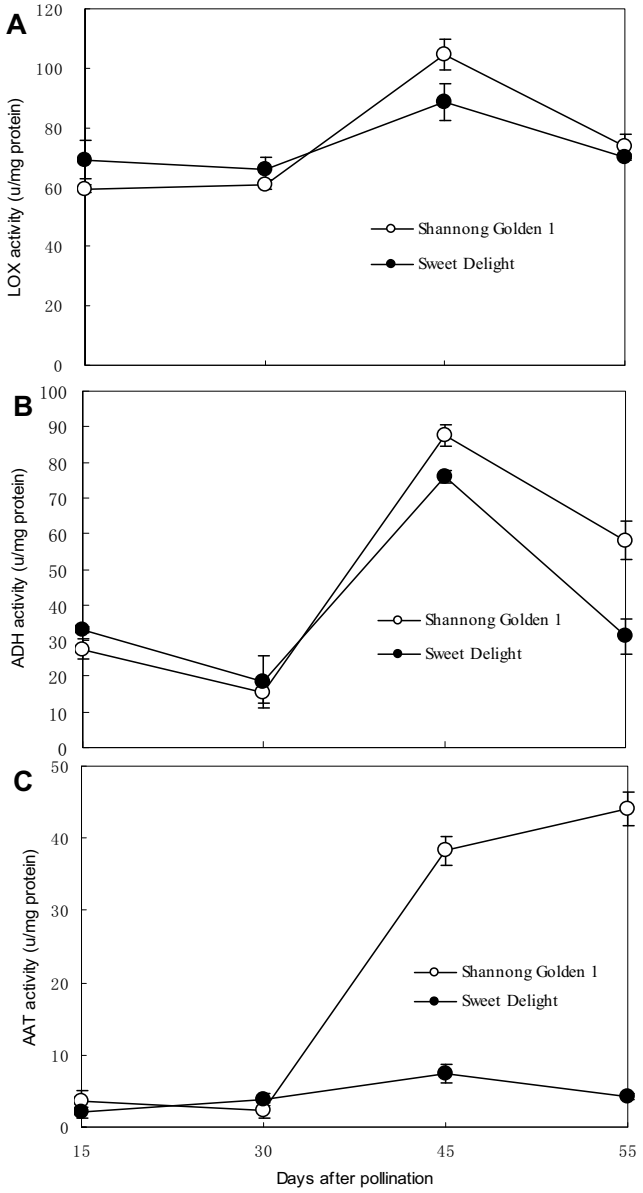

Fig. 3 LOX (**A**), ADH (**B**) and AAT (**C**) activities during fruit development of cvs. 'Shannong Golden 1' and 'Sweet Delight' muskmelons. Fifteen, 30, 45 and 55 d after pollination (DAP) fruit were harvested, respectively. Fifty-five DAP is considered commercial maturity. The experiment was repeated three times and five fruit were used for each replicate. Bars indicate SE.

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