

Title Biosynthesis of branched-chain esters in ripening apple fruit: implications of ¹³C-labeled acetate incorporation in vivo

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Citation Abstracts Book, 6th International Postharvest symposium, 8-12 April 2009, Antalya, Turkey. 256 pages.

Keyword Apple; spectrometry; ester

Abstract

We studied the incorporation of 20 mM ¹³C- labeled acetate (1-¹³C, 2-¹³C, ¹³C₂) into esters and ester precursors synthesized by peel discs of 'Jonagold' and 'Red Delicious' apple [*Malus sylvestris* (L.) Mill. var. domestica (Borkh.) Mansf.] fruit. The incorporation of ¹³C into headspace volatiles was analyzed by gas chromatography coupled with mass spectrometry (GC/MS). Following analysis of volatiles, apple discs were freeze dried and derivatized acids were also quantified using GC/MS. Labeled acetate was incorporated into isoleucine and esters containing 2-methylbutanoate, a degradation product of isoleucine. In addition, significant label was detected in propanoate esters, with a minor degree of incorporation of isotope in butanoate and pentanoate esters. Importantly, the acids citramalic acid and citraconic acid were also extensively labeled. The latter data suggests that the biosynthesis of isoleucine in ripening apple may, in part, be via a previously uncharacterized pathway that uses starting products pyruvate and acetyl-CoA. The hypothesized alternative pyruvate pathway will be discussed as it relates to isoleucine biosynthesis and propanoate and 2-methylbutanoate ester production.