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Ethylene treatment induces changes in folate profiles in climacteric fruit during postharvest ripening

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ABSTRACT

Avocados, bananas, papayas, and tomatoes are typical climacteric fruits that are usually harvested at the mature green stage. Their ripening is later triggered by exogenous ethylene as a common postharvest practice; however, the effect of ethylene on the folate content of fruit has not been studied. Folates are part of the vitamin B complex and their consumption is essential for proper human development. In this work, changes in folate profiles and contents during postharvest ripening, both with and without ethylene treatment, were evaluated in four climacteric fruits: papaya, tomato, banana, and avocado. Non-induced postharvest ripening increased folates in ripe papaya fruit, while it did not affect total folate contents in avocados. Significant fluctuations in total folate in tomatoes and bananas were observed throughout ripening; however, total folates in ripe fruit returned to their initial mature green values. Ethylene treatment also affected the folate pools in the fruit differently, causing a 24% and 51% increase in ripe tomatoes and bananas, respectively, a 26% decrease in papayas, and no change in avocados compared to non-treated ripe controls. Ethylene treatment affected the accumulation of 5-CH_3-THF in all fruits; this folate derivative is involved in ethylene biosynthesis. This work shows that ethylene treatment and postharvest ripening affect fruit folate levels and derivatives in a species-specific manner. This knowledge shows that postharvest treatments can help improve the folate accumulation in plant foods for fresh consumption.

1. Introduction

The nutritional quality of fruit has been shown to be dependent on genotype, preharvest environmental conditions, maturity at harvest, and postharvest handling (Kader, 2008). Ethylene is the gaseous hormone that coordinates climacteric fruit (e.g., banana, papaya, tomato, mango, avocado) ripening when produced endogenously or when applied exogenously (Klee and Giovannoni, 2011). Application of ethylene is often used in the commercial postharvest handling of climacteric fruit to trigger the ripening of mature green (MG) fruit during storage (Kader, 2002). The effects of postharvest ethylene treatment on the sensory, quality, and nutrient attributes of several fruits have been studied, including vitamins A and C (Watada, 1986). However, to date, there are no studies that include vitamin B9, tetrahydrofolate (THF) and its derivatives, known as folates. Moreover, there is little information on folate dynamics in fruit during development and ripening. Changes in the folate levels of tomatoes when ripening on the vine are the best documented, but the available data has not been conclusive (Basset et al., 2002; Iniesta et al., 2009; Periago et al., 2009; Tyagi et al., 2015). Information regarding other fleshy fruits is even more limited; we recently reported that total folate levels in papayas increased during postharvest ripening (Ramos-Parra et al., 2013a). However, none of the aforementioned studies have taken into consideration the common practice of using exogenous ethylene to ensure the rapid and homogeneous ripening of climacteric fruit.

In addition to the well-recognized association between folate malnutrition with neural tube defects (NTD) and megaloblastic anemia, inadequate folate intake has been associated with higher risks of cardiovascular diseases, Alzheimer's dementia, and certain types of cancer (Bailey et al., 2015). The recent growing interest in the importance of an adequate intake of this vitamin highlights the need for a better understanding of how external factors affect our

Abbreviations: ACC, aminocyclopropane-1-carboxylic acid; ACO, ACC oxidase; ACS, ACC synthase; Br, breaker; DAT, days after treatment; FW, fresh weight; LR, light red; MG, mature green; ns, not significant; NTD, neural tube defects; 1C, one carbon; RR, red ripe; RH, relative humidity; R, ripe; RS, ripening stage; SAM, S-adenosylmethionine; T, treatment; Tr, turning; OR, overripe; THF, tetrahydrofolate; 5-CH_3-THF, 5-methyl-THF; 5,10-CH=THF, 5,10-methenyl-THF; 5-CHO-THF, 5-formyl-THF.

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main dietary sources—green leafy vegetables, legumes, and fruit (Scott et al., 2000). Because fruit is often consumed fresh, common folate losses due to oxidation and leaching associated with processing are not of concern (Delchier et al., 2013). However, the contribution of fruit to dietary folate might be affected by the same factors known to influence the levels of other nutrients found in fruit.

Folates participate as cofactors in 1-carbon (1C) transfer reactions in organisms; these reactions are involved in several metabolic functions, such as DNA methylation and the biosynthesis of amino acids (methionine, glycine, and serine), nucleic acids, and S-adenosylmethionine (SAM) (Fig. 1; Hanson and Gregory III, 2011). In plants, folates are needed during photorespiration and chlorophyll synthesis; SAM, a 1C product, is also required for the synthesis of plant-derived metabolites such as ethylene, nicotinamide, and polyamines (Sauter et al., 2013; Ravanel et al., 2011). The biosynthesis of ethylene and 1C metabolism share a common metabolic step through the action of the enzyme methionine synthase (Fig. 1A). Therefore, we hypothesized that ethylene treatment could impact folate levels in climacteric fruit. Methionine synthase utilizes 5-methyl-THF (5-CH_3-THF) as a methyl donor to synthesize methionine and to regenerate the methyl group of SAM after methylation reactions (Ravanel et al., 2004). Aminocyclopropane-1-carboxylic acid (ACC) is produced by ACC synthase (ACS) using SAM as a substrate, after which it is oxidized further by ACC oxidase (ACO) to synthesize ethylene (Sauter et al., 2013).

Papaya, tomato, banana, and avocado fruit were selected in this study to explore folate during ripening. The aim of this work was to determine the effects of postharvest ripening and, for the first time, ethylene treatment on total folate levels and folate species distribution in climacteric fruit.

2. Materials and methods

2.1. Plant material

Papaya (*Carica papaya* cv. Maradol) and banana (*Musa acuminata* AAA cv. Cavendish) fruit at the MG stage were obtained from a local supplier distribution center immediately after they were unloaded from refrigerated trucks (12 °C for papayas and 14 °C for bananas). The sampling was conducted on fruit harvested at just one orchard. Times from harvest to unloading were three days for papayas and two days for bananas. The fruits were harvested when their peels were all green, and they were packed and transported in rigid cardboard boxes to prevent damage. The MG avocado fruits (*Persea americana* cv. Hass) were obtained from a commercial plantation located in Tamazula, Jalisco and transported in boxes at 18 °C; the samples were taken two days after harvest. Tomato seeds (*Solanum lycopersicum* cv. Ailsa Craig) were acquired from the C. M. Rick Tomato Genetics Resource Center (http://tgrc.ucdavis.edu/) and cultivated in the greenhouse facility of ITESM Monterrey, N.L., Mexico. The tomato plants were subjected to standard fertilization and pest control measurements. The fruits were tagged at anthesis and harvested at the MG stage, which in our conditions it occurred after 32 days.

All of the MG fruit were randomly separated into two groups. Fruits from the control groups were allowed to ripen in incubators at 20 °C and 90% relative humidity (RH), and fruits from the experimental groups were treated with ethylene as explained in the next section. All treatments and storage were conducted independently for each type of fruit.

2.2. Ethylene treatments

The MG papaya and banana fruit were treated with 100 $\mu L\,L^{-1}$ and 150 $\mu L\,L^{-1}$ of ethylene, respectively, in a ripening chamber at 20 °C for 24 h. The avocado and tomato fruit were placed in a 70 L container and exposed to 100 $\mu L\,L^{-1}$ of ethylene in balance synthetic air (Praxair, Monterrey, Mexico) for 24 h at 20 °C under a continuous flow (300 $cm^3\,min^{-1}$); the humidity in the chamber was always >90%. After treatment, all of the fruit were allowed to ripen in chambers under the same temperature and humidity conditions as the control groups.

2.3. Ripening stages and fruit sample preparation

The fruit ripening stages were identified according to published parameters and descriptors (Santamaría Basulto et al., 2009; United States Department of Agriculture [USDA], 1975; USDA, 2001; Cox et al., 2004). Sampling was conducted based on both ripening stages and days after treatment (DAT). The first sample was taken immediately after treatment (+24 h). Three whole fruits were taken at each postharvest ripening stage and corresponding DAT, the seeds and peels were removed, and the pulp was cut into 0.5–0.7-cm thick longitudinal wedges, flash-frozen in liquid nitrogen, and kept at −80 °C for folate analysis.

2.4. Folate analysis

Folates were extracted and purified as described previously (Ramos-Parra et al., 2013b), with minor modifications. Briefly, 1 g of fruit tissue (0.2 g for avocado) was ground in liquid nitrogen and extracted in 10 mL of folate-extraction buffer (50 mM HEPES, 50 mM CHES, 10 mM 2-mercaptoethanol, 2% ascorbic acid, pH 7.8) with $CaCl_2$ (4 mM) for pectin precipitation. The samples were

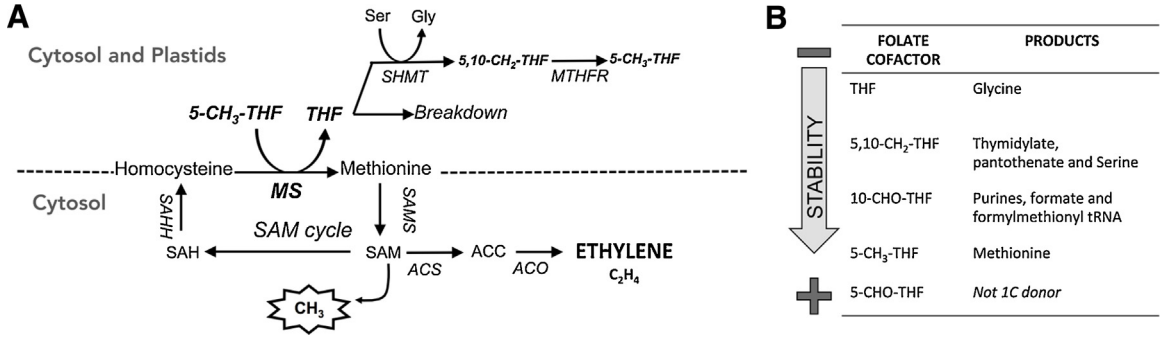

Fig. 1. Schematic representation of folate 1-carbon (1C) metabolism and ethylene crossroads (A). Stability and functions of folate species (B). ACC: aminocyclopropane-1-carboxylic acid: ACO: ACC oxidase; ACS: ACC synthase; Gly: glycine; MS: methionine synthase; Ser: serine; SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine; SAMS: SAM synthase; SHMT: serine hydroxymethyl transferase; THF: tetrahydrofolate; 5,10-CH_2-THF: 5,10-methylene-THF; 10-CHO-THF: 10-formyl-THF. Based on data from Ravanel et al. (2004),Sauter et al. (2013), Hanson and Gregory III (2011).