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Sugar and acid interconversion in tomato fruits based on biopsy sampling of locule gel and pericarp tissue

Rob E. Schouten[a,*], Ernst J. Woltering[b], L.M.M. Tijskens[a]

[a] Horticulture and Product Physiology, Wageningen University, Droevendaalsesteeg 1, 6708 PD Wageningen, The Netherlands
[b] Food & Biobased Research, Wageningen University and Research Centre, P.O. Box 17, 6700 AA Wageningen, The Netherlands

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ABSTRACT

This study deals with quantifying sugar and acids levels important for the perceived taste of tomatoes (*Solanum lycopersicum*). Sugar and acids levels were measured repeatedly on the same tomato using tissue samples obtained with a biopsy needle in combination with HPLC protocols. Biopsies of pericarp and locular gel tissue from tomatoes differing in position in the truss, from mature green to ripe red, were taken from a beef- ('Licorossa'), a cocktail- ('Lucino') and a cherry type ('Petit Sweet') cultivar. Tomatoes were stored up to three weeks at three temperatures (12, 19 and 24.5 °C) and biopsy samples were taken every few days. A model regarding the most important processes that interconvert sugars and acids (glycolysis, TCA cycle and gluconeogenesis (GNG)) is proposed. Results of the model calibration showed more breakdown of hexoses in red tomatoes and more conversion of malate into hexoses in green tomatoes. More hexose turnover was found in locular gel than in pericarp tissue. GNG was more important in the cherry type cultivar due to faster hexose and malate breakdown. In the round type cultivar malate levels were higher due to faster citrate breakdown and slower malate breakdown. Starch and sucrose levels did not significantly affect postharvest sugar and acid development. Molecular markers that quantify the kinetic parameters of the model might be important to develop genotypes with better taste performance.

1. Introduction

Next to external quality attributes like colour and firmness (Tijskens and Evelo, 1994) the main motivator for repeated purchase (Schepers and Van Kooten, 2006) of tomatoes (*Solanum lycopersicum*) is the flavour (Kader et al., 1978). Flavour depends on taste and aroma. Aroma compounds are considered important (Kader, 2008), but in the case of tomatoes it is still hard to relate consumer flavour perception with volatile content (Klee and Tieman, 2013). In general, high sugar content and relatively high acid content are required for a favourable taste. High levels of acids and low levels of sugars will produce a tart tomato, while high levels of sugars and low acids will result in a bland taste (Davis and Hobson, 1981). Probably the most important factors affecting the development of tomato flavour are growth conditions (Dorais et al., 2001). The assortment of different tomato types available in supermarkets nowadays; with generally smaller tomato types (e.g. cherry tomatoes) having better flavour than bigger tomatoes (e.g. beef tomatoes) indicates that cultivar has also a significant effect. Tomatoes picked at earlier stages of ripeness, such as immature green and breaker tomatoes were evaluated by panellists as less sweet, more sour and less 'tomato like' compared to table ripe tomatoes (Kader et al., 1977). Storage temperature also affects flavour. Storage of mature green and light pink tomatoes at 5 and 12.5 °C before ripening at 20 °C showed trends that indicate smaller values for sugar/acid ratio, lower sweetness and higher sourness sensory evaluation scores compared to direct ripening at 20 °C (Kader et al., 1978). Maul et al. (2000) showed that varying storage time and storage temperature affects the sweetness and sourness (by sensory evaluation), titratable acidity, glucose and fructose content of table ripe tomatoes. Other postharvest treatments, such as ethylene treatments to accelerate ripening of green tomatoes and controlled atmosphere storage to slow down ripening showed little effects on flavour (Kader et al., 1978).

Surprisingly, the regulation of pathways that govern the conversion of sugar and acids in tomato has not been an area of considerable interest (Carrari and Fernie, 2006). Sucrose is the major photo-assimilate transported from photosynthetic leaves to developing fruit where it is converted into hexoses. The rate of sucrose import might be regulated by the sucrose gradient

* Corresponding author at : Horticulture and Product Physiology, Droevendaalsesteeg 1, 6708 PD Wageningen, The Netherlands. Fax: +31 317 484709.
E-mail address: Rob.Schouten@wur.nl (R.E. Schouten).

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between leaves and fruit (Walker et al., 1978) by sucrose and hexose sensing mechanisms (Koch, 2004). Sucrose enters the tomato fruit mainly during apoplastic unloading early in the development. Apoplastic invertase and sucrose synthase convert sucrose into hexoses (Carrari and Fernie, 2006). Invertase converts sucrose into fructose and glucose; sucrose synthase converts sucrose into fructose and uridine di-phosphate glucose (Koch, 2004). Modern day tomato cultivars are characterised by high hexose accumulation which indicates that sucrose is efficiently hydrolysed into hexoses. Breakdown products of sucrose are mainly used either for glycolysis or for starch synthesis during fruit development. During the preharvest period, starch synthesis and starch degradation are simultaneously taking place with net synthesis until about 24 days post anthesis and net breakdown thereafter (Luengwilai and Beckles, 2009). Peak levels of starch early in development are related to final levels of soluble sugars after postharvest storage. Starch biosynthesis is likely an additional sink for carbon during early fruit development when carbon import is largest (Robinson et al., 1988).

Glycolysis and TCA cycle represent the dominant carbon fluxes in tomato fruit, although the fatty acid-, flavonoid-, pigment-, alkaloid- and isoprenoid pathways are also fuelled by the break down products of sucrose (Carrari and Fernie, 2006). Relatively little is known about the regulation of glycolysis and the conversion of sugars into organic acids in tomatoes (Carrari and Fernie, 2006). Glycolysis is a sequence of ten reactions that starts with glucose. The final product of glycolysis is pyruvate. The respiratory process continues with the mitochondrial reactions of the TCA cycle (Fernie et al., 2004). The TCA cycle is fuelled by pyruvate which, together with oxaloacetate, forms citrate. During the cycle citrate is transformed into malate and, again, oxaloacetate. ^{13}C glucose enrichment experiments at different stages of the growth cycle of tomato cell suspensions showed that 70% of the glucose was used for respiration. Five days after the start of the enrichment experiment ^{13}C citrate and malate accumulated (Rontein et al., 2002). Gluconeogenesis (GNG) is the universal pathway that generates glucose from non-carbohydrate carbon substrates. Glycolysis and GNG form a futile cycle (Schwender et al., 2004) that results in the hydrolysis of ATP into ADP and heat. Phosphoenolpyruvate carboxykinase, one of the enzymes of GNG, increased in activity during fruit ripening, from an undetectable amount in immature green fruit to a high amount in ripening tomato fruit (Bahrami et al., 2001). ^{14}C malate supplied to pericarp discs was recovered as citric acid and, for a substantial amount, in the soluble sugar fraction. (Halinska and Frenkel, 1991).

Tomato is composed of a number of tissue types such as pericarp and locular gel. The locular gel shows a climacteric rise in ethylene before other tissue types (Atta-Aly et al., 2000) that, in combination with the increasing liquefaction of the locular gel during ripening, might affect taste. The effects of tissue type on taste are hard to investigate due to the usually applied destructive measurement protocols. Preferably, data on sugar and organic acid development over time should be obtained from the same fruit as maturity differences influencing the flavour within tomatoes, are smaller than between tomatoes. Here a medical biopsy needle is introduced that allows separate pericarp and locular gel sampling over time for HPLC measurements from the same tomato.

The aim of this research is to describe sugar and acid levels over time of tomato fruit harvested at varying maturity stages and stored at varying temperatures. This is achieved by generating a set of differential equations based on simplifying the central carbohydrate metabolism in tomato fruits. Describing pathways of the central carbon metabolism is complex due to the many reactions involved and acceptable simplifications are needed to enable quantification with today's enzyme-kinetic modelling technology (Schallau and Junker, 2010). Here an approach is described based on a kinetic model in terms of only substrates. Rontein et al. (2002) measured and calculated 28 fluxes of the central carbon metabolism after steady state labelling with ^{13}C glucose in tomato cell suspensions. Absolute fluxes gradually slowed down with the decrease of glucose influx into the cells. The relative fluxes of glycolysis, the pentose-P pathway, and the TCA cycle remained unchanged at three different stages of the growth cycle. This would indicate that only substrate modelling is required as the involved enzymes are apparently not limiting the conversion of sugars to acids. The universal pathways of glycolysis and the TCA cycle show robust homeostasis likely due to alternative enzymes and pathways for many processes (Plaxton, 1996). Examples are available where enzymes, considered essential in glycolysis, were repressed without significant effect on development (e.g. Gottlob-McHugh et al., 1992). This also indicates that due to pathway flexibility the sugar and acid conversion in tomato might be described by only taking substrates in consideration.

The development and calibration of a sugar-acid model containing only substrates is presented as a function of ripeness, cultivar, tissue type and temperature. Samples for HPLC measurements of tomato pericarp and locular gel tissue were extracted using the medical biopsy needle. The sugar-acid model is calibrated based on HPLC measurements of biopsied tissue from tomatoes of three cultivars varying in size stored at three non-chilling temperatures. The validity of the biopsy method and the significance of the sugar-acid model with regard to taste development are discussed.

2. Materials and methods

2.1. Fruit collecting, labelling and storage

Tomato plants (*Solanum lycopersicum*) of three cultivars: 'Licorossa' (round type), 'Lucino' (cocktail type) and 'Petit Sweet' (cherry type) were grown hydroponically in commercial greenhouses in the southwest of The Netherlands. The number of tomatoes per truss was on average five, eight and fourteen for the round, the cocktail and the cherry type cultivars, respectively.

For the first experiment fifteen trusses per cultivar, with the central tomato in breaker stage, were harvested and transported to the laboratory within three hours on February 16, 2009. From each truss, five tomatoes were selected as to represent different (colour) maturity stages. Tomatoes were detached from the truss and individually labelled opposite to the calyx. Tomatoes from each truss were numbered from 1 (mature green) to 5 (ripe red). On each individually labelled tomato six equally spaced spots on the equator were marked for tissue extraction. Trusses were stored at 12, 19 or 24.5 °C for 19, 16 and 17 days, respectively.

For the second experiment six trusses per cultivar were harvested at the same greenhouse one week later. Maturity assignment, labelling and transport time were similar to those described for the first experiment. Starch and sucrose levels were determined in locular gel and pericarp tissue at harvest.

2.2. Biopsy measurements

A reusable medical biopsy needle (Spirotome SR-10-10, MedInvents.com) with a diameter of 2.588 mm (10 Gauge) and a length of 100 mm was used for tomato tissue extraction. This biopsy needle consists of three parts, a corkscrew needle, a hollow core needle and a spiked needle. For biopsy of pericarp tissue the corkscrew needle was used. The corkscrew was, starting from the tomato skin, rotated into the pericarp and retracted. Tissue was recovered by slowly rotating the corkscrew needle into a 2 × 2 cm piece of aluminium foil to liberate the tissue sample from the needle. For biopsy of the locular gel tissue the same entry point as