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Development and distribution of quality related compounds in apples during growth

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ABSTRACT

Colour and taste are important attributes of apple fruit quality and have therefore been widely studied. Nevertheless, because of the destructive sampling methods commonly used to obtain the data, and of the subsequent traditional analyses, ignoring the effects of biological variation, the knowledge on the kinetic mechanisms of synthesis and degradation of individual quality components during fruit development and growth is still lacking. Spatio-temporal changes of taste components (sugars: fructose, sucrose, glucose, organic acids: malic, citric, shikimic and fumaric acid) and colour aspects (a*) in individual apple fruits were monitored to assess the dynamics and mechanisms of change during development and ripening with respect to location within fruit as a factor and the variation between individual apples. Data were analysed with non-linear indexed regression based on either a logistic or an exponential process oriented model assessing the technical variation simultaneously. The rate constants for colour or taste component were roughly similar between cultivars, suggesting a similar mechanism of development and confirming the generic nature of the model. There was a very large biological variation in individual quality components observed in the raw data (the biological variation), which can be almost exclusively explained by the difference in the maturity stage between individual fruit. The explained parts (R^2_{adj}) were, with one exception, higher than 0.90. The major contribution of this study is the fact that all the herein monitored taste defining components can be analysed and described with the same process-oriented model.

1. Introduction

As apple producers are facing hyper competition at a global level (Axelson and Axelson, 2000), a strong urge exists amongst them to unravel the factors affecting fruit quality, so they could provide the consumers with a constant supply of consistently high quality fruit and keep their share of the market. The main quality aspects determining apple fruit saleability and price are factors that affect fruit appearance and taste (Carew and Smith, 2004). Taste is determined primarily by the balance between total sugars and total organic acids, although the amounts of individual organic acids and sugars may also play a role (Hecke et al., 2006; Kader, 2008; Lobit et al., 2003; Shiratake and Martionia, 2007; Yamaki, 2010). Some studies revealed that consumers are prepared to pay a premium price for a premium taste (Lange et al., 2000). It is mainly due to dissatisfaction with taste, although also partly due to dissatisfaction with certain other quality attributes, that in the middle- to high- income countries, around 15–30% of fresh fruit and vegetables is wasted each year at the consumer (Gustavsson et al., 2011). Lack of taste and quality can be detrimental for the whole industry, as it influences the consumer's decision to re-purchase the product in the future. Batt and Sadler (1998) report that after being disappointed by the quality of apple fruit, around 24% of the consumers chose to buy other fruit types in the future.

Concluding from the above studies, it is obvious that quality is indeed very important in determining the producer's competitiveness on the market. So it is hardly surprising that a plethora of studies were conducted on this issue, collecting a considerable phenomenological knowledge. Knowledge on the kinetic mechanisms of synthesis and degradation of individual quality components (e.g. sugars, organic acids and colour parameters) is, however, rather weak because trends are found to be rather subtle. Traditionally, interpretations of the biochemical data, frequently obtained by destructive sampling techniques, have been based on purely statistical models using mean values of large samples, without taking the effects of biological variation into accountt (Tijskens et al., 2003). Since a huge variation exists in the levels of individual quality compounds, working with mean values completely obscures

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the occurring mechanisms. If one wants to understand quality, its generation during fruit growth needs to be monitored (by non-destructive or semi-non-destructive techniques) at an individual level. When knowledge exists on the type and kinetics of the processes involved, the variation in properties can be described and taken into account by using more fundamental models, built on theoretical considerations. These fundamental models, that have been successfully applied for data analysis of biological materials by several scientists in previous studies (De Ketelaere et al., 2006; Hertog, 2002; Schouten et al., 2002, 2004, 2007, 2009; Tijskens et al., 2003, 2009, 2011; Tijskens and Konopacki, 2003; Unuk et al., 2012), to trace back the source of variation to variations in growing conditions. These techniques should ultimately enable to optimize production, resulting in fruit of highest constant quality irrespective of location, season or weather.

The aim of the study was to monitor the dynamics of certain taste components (sugars, organic acids) and colour as a factor of maturity and cultivar, while taking the variation between individual apples into account. As a prerequisite, the viability and reliability of the novel biopsy sampling method was tested.

2. Material and methods

2.1. Plant material and orchard conditions

Field trials were performed in 2011 at the University Agricultural Centre Pohorski Dvor (46,5 latitude;15,6 longitude, 313 m.a.s.l.) in Pivola, Slovenia on fruit of 11 yrs-old trees (cv. 'Gala') and 5 years-old trees (cv. 'Pinova'). The trees were of a uniform vigour and crop loads (10% ± sd), grafted on M9 EMLA rootstocks, trained as super-spindle, grown in a single row system (3.2 m × 0.7 m) under a black hail net, drip irrigated/fertilized and managed according to the rules of integrated fruit production practices. The soil in both orchards has a sandy loamy texture (15% clay, 30% sand, and 46% silt). According to the results of soil analysis in the orchard of cv. 'Pinova', the levels of plant available P and K at a depth of 0–20 cm were optimal for P (19 mg/100 g dry soil) and low for K (6.9 mg/100 g dry soil), whereas at the depth of 20–40 cm, the levels of both were low (2.1 mg/100 g dry soil and 4 mg/100 g dry soil, respectively). At both depths, the soil was weakly acidic (pH 6.39 to 6.55) thus suitable for apple production. The results of soil analysis in the orchard of cv. 'Gala', reveal moderate levels of plant available P (8.2 mg/100 g dry soil), optimal levels of plant available K (20.9 mg/100 g dry soil), and a lower than optimal value of pH (4.92) at a depth of 0–40 cm.

2.2. Fruit selection and labelling

For each cultivar, 55 uniform fruit with similar light exposure from the east side of the canopy between 1.3 m and 1.8 m about the ground were selected and labelled to be monitored repeatedly over time. Colour measurements were performed on each fruit starting at designated spot in the transition area between the ground and the blush colour i.e. "base location". This location was located at the SW position on the equator of the fruit, and was set at 300° in cv. 'Gala', and 315° in cv. 'Pinova'. The blush side of the fruit was for both cultivars located at around 270° i.e. at the southeast side of the fruit. The part that received the least radiation from the sun was thus located at around 90° i.e. at north-west exposition. Depending on cultivar, on each of these fruit six to eight locations, positioned around the apple in regular intervals at 75° above the equator, were labelled and numbered. These were referred to as the "biopsy locations", although both colour and subsequent biopsy samples were taken at these spots.

All samplings were conducted in weekly intervals from approx. 40 and 54 days before, to approximately 16 and 32 days after the determined optimal maturity in cv. 'Gala' and cv. 'Pinova', respectively. Because metabolites follow a pronounced diurnal rhythm with the maximum amount during the light period (Tausz et al., 2003), samplings were always done on clear days between 11:00 and 14:00 solar time.

2.3. Colour measurements

Colour of the skin of individual apple fruit was measured with a commercial colorimeter Minolta CR400, and expressed according to CIE (Commission Internationale d'Eclairage) in chromaticity coordinates L*, a*, and b*, as was done in the study of Unuk et al. (2012).

At each sampling date, the measurements were taken from the "base location", located at the transition between blush (i.e. 270°) and opposite (i.e. 90°) side of the fruit, and from the respective "biopsy location" per date. On the first measuring date the "biopsy location" where the colour was determined was positioned at a distance of 90° from the blush in the NW direction, on the second measuring date at a distance of 135° and 150° in the NW direction for cv. Gala and Pinova, respectively etc.

2.4. Biopsy sampling for biochemical analyses

To obtain samples for biochemical analyses, a novel sampling technique, modified after the one used by Bingqing et al. (2010) on stored tomatoes, was used. This modified technique enables to take small biopsy samples of fruit tissue without damaging the remainder of the fruit. To the best of our knowledge, this sampling technique, although also deemed appropriate for biochemical studies on stored cassava roots (Garcia et al., 2013), and tomatoes (Schouten et al., 2016) has never before been used for taking samples from growing and developing fruit, nor has it ever been used on apple fruit. Biopsy samples were taken from only one biopsy position per individual fruit per date. The sampling was done as is described in the subsection "colour measurements – at the first sampling date the samples were taken from a position located at 90° from the blush position in the NW direction and at each successive measuring date, samples were taken from consecutive positions, distanced 60° and 45° apart in cv. Gala and cv. Pinova, respectively. In order to prevent injury to the apple core, a probe with a diameter of 4/3 mm, 45° angle and a sampling length of 2.5 cm was constructed. The weight of the taken sample was 150–250 mg. Biopsy sampling was always carried out upon the completion of colorimetric measurement. Immediately after taking them, samples were stored in criovials and frozen in liquid nitrogen to prevent oxidation. Wounds resulting from biopsying were filled with vaseline mixed with fungicide (Dithane, 50 mg/L), which prevented rotting and allowed fruit to remain vital thus enabled repeated sampling over time. Later these *in vivo* samples were biochemically analysed to determine the amounts of the monitored taste components.

2.5. Biochemical analyses

Individual sugars (fructose, sucrose, and glucose) were analysed according to the modified high-performance liquid chromatographic (HPLC) method of Dolenc and Štampar (1997) under isocratic conditions using a Waters Alliance 2695 HPLC system with Waters 410 Differential Refractometer (Refractometer temperature: 35° C). HPLC conditions: Column: Rezex RCM-monosaccharide column (300 mm × 7.8 mm, Phenomenex). Column temperature: 75° C. Solvent: bidestilled water. Run time: 30 min. Flow rate: 0.5 mL min^{-1}.