Food Chemistry 190 (2016) 879–888

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Metabolic profiling of a range of peach fruit varieties reveals high metabolic diversity and commonalities and differences during ripening

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ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form 10 June 2015
Accepted 16 June 2015
Available online 16 June 2015

Keywords:
Prunus persica
Fruit
Metabolic profiling
Metabolic diversity
Ripening
Varieties

ABSTRACT

Peach (*Prunus persica*) fruits from different varieties display differential organoleptic and nutritional properties, characteristics related to their chemical composition. Here, chemical biodiversity of peach fruits from fifteen varieties, at harvest and after post-harvest ripening, was explored by gas chromatography–mass spectrometry. Metabolic profiling revealed that metabolites involved in organoleptic properties (sugars, organic and amino acids), stress tolerance (raffinose, galactinol, maltitol), and with nutritional properties (amino, caffeoylquinic and dehydroascorbic acids) displayed variety-dependent levels. Peach varieties clustered into four groups: two groups of early-harvest varieties with higher amino acid levels; two groups of mid- and late-harvest varieties with higher maltose levels. Further separation was mostly dependent on organic acids/raffinose levels. Variety-dependent and independent metabolic changes associated with ripening were detected; which contribute to chemical diversity or can be used as ripening markers, respectively. The great variety-dependent diversity in the content of metabolites that define fruit quality reinforces metabolomics usage as a tool to assist fruit quality improvement in peach.

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1. Introduction

Peach (*Prunus persica* (L) Batsch), with a production of nearly 20 millions tonnes of fruits per year (FAOSTAT, http://faostat.fao.org/), is one of the most economically important fruit crop in the Rosaceae family, mainly because of its broad climate range and relatively high yield. In addition, peach has become one of the reference species for *Prunus* due to its diploid compact and small genome (227.3 Mb), taxonomic proximity to other important species and availability of homozygous doubled haploids (Aranzana, Abbassi, Howad, & Arús, 2010; Shulaev et al., 2008). Important genes related to production and fruit quality have been described and mapped in peach, revealing that many morphological and quality traits have a simple genetic basis (Horn et al., 2005). Furthermore, a peach reference genome sequence, based on a doubled-haploid of the Lovell cultivar, has recently been obtained (The International Peach Genome et al., 2013), which shows high correspondence to the previously obtained physical maps for peach (Dirlewanger et al., 2006; Zhebentyayeva et al., 2008).

There are several hundreds of peach varieties distributed around the world displaying a wide phenotypic variability and producing fruits that offer the consumers a great mixture of flavours, textures, and sweetness/acidity ratios (Okie, 1998; Okie, Bacon, & Bassi, 2008). This high phenotypic diversity is the result of several peach breeding programmes in different countries that focus on the development of new varieties to satisfy diverse demands. Among others, these demands include a higher yield, the expansion of their production zones, disease resistance and the need for a better post-harvest quality of the fruit, this is especially critical given its short post-harvest lifespan (Byrne, 2005; Sansavini, Gamberini, & Bassi, 2006). The wide phenotypic variability of peach contrasts with a restricted genetic diversity, probably as a consequence of self-compatibility (Font i Forcada, Oraguzie,

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http://dx.doi.org/10.1016/j.foodchem.2015.06.043
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Igartua, Moreno, & Gogorcena, 2012; Fresnedo-Ramírez, Martínez-García, Partiff, Crisosto, & Gradziel, 2013; The International Peach Genome, 2013). Although traits affecting yield and disease resistance remain essential, plant breeding efforts should additionally address the needs of consumers, which include fruit flavour, quality, nutritional improvement, and enhanced health-promoting properties. All these characteristics are in close connection with the final chemical composition of the fruit at the time when they reach the consumers. Thus, in order to realign breeding programs to satisfy these demands, it is essential to assess the metabolic diversity of peaches from different varieties. In this sense, all the available high-throughput technologies based on genome, transcriptome, proteome, and metabolome analysis offer new possibilities for *Prunus* breeders in the post-genomic era (Martínez-Gómez, Sánchez-Pérez, & Rubio, 2012).

The fruit is one of the most metabolite-rich plant organs and as such contains a massive range in its metabolic complement, where metabolites involved in taste and flavour, with nutritional or pharmaceutical properties, or even with plant defence properties against biotic and abiotic stress can be found. Moreover, the metabolome represents the ultimate phenotype of the cells, and can also influence gene expression and protein function. Thus, metabolic profiling of fruits is a key tool to identify biomarkers related to quality and could be employed to improve breeding strategies and post-harvest storage of fresh products and optimise processing methods (Hu & Xu, 2013; Oms-Oliu, Odriozola-Serrano, & Martín-Belloso, 2013). Metabolic profiling study of primary metabolites during Dixiland peach development, using gas chromatography–mass spectrometry (GC–MS), revealed the metabolic dynamics during peach development at high resolution, and highlighted the key metabolic processes during the different peach developmental stages (Lombardo et al., 2011). More recently, metabolomics studies of Dixiland peach fruits during post-harvest treatments used to prevent fruit decay, allowed the identification of crucial metabolites, which prime the fruit to cope with different stress situations and, thus, which may be involved in better quality properties for consumers (Lauxmann et al., 2014). In the present work, in order to explore the metabolic biodiversity of peach, we performed a profiling study of primary metabolites, which are essential for survival and modulate secondary metabolism. For this purpose, we selected fifteen different varieties with diverse origins and phenotypic properties. One of the goals of this study was to assess how metabolically different the peach varieties are and to find out if there is any particular metabolic profile which could be associated with given phenotypic properties of the fruit. On the other hand, by evaluating the metabolomic pattern at both harvest and after post-harvest ripening of the fifteen varieties, we explored the common and distinct metabolic processes related to ripening across the different peach varieties. In summary, this study explores a part of the enormous chemical potential available in the biodiversity of peach fruit, which aids in the future construction of a catalogue of metabolites that may correlate to different phenotypic, organoleptic and quality properties of the fruit.

2. Materials and methods

2.1. Plant material

Assays were conducted with peach fruit (*P. persica* L. Batsch) of fifteen different varieties grown in the Estación Experimental Agropecuaria INTA, San Pedro, Argentina (Borsani et al., 2009; Budde, Polenta, Lucangeli, & Murray, 2006). The varieties selected were: Flordaking (FD), Don Agustín (DA), Spring Lady (SL), Goldprince (GP), Don Carlos INTA (DC), Red Globe (RG), Elegant Lady (EL), Fred (FR), 95 ED 1 (9S), María Anna (MA), Texprince (TX), María Delizia (MD), Limón Marelli (LM), Rojo 2 (R2), and 55 RA 15 (SS) (Okie, 1998; Okie et al., 2008). All the peach varieties used were grafted on Cuaresmillo rootstock (*P. persica*). Trees from the different varieties were trained in a similar way, typically to a vase shape with three to five main branches and three sub-branches each. Three adult trees (of nearly 10 years each) of each variety were used for fruit collection. Relevant agronomic characteristics of each variety are listed in Table 1 and Table S1. Fruits were collected at commercial maturity. Depending on the variety, the flesh firmness of the fruits at harvest was between 55 and 70 N (Fig. 1). Immediately after harvest, fruits were manually selected for uniformity of colour, size and firmness and kept in a chamber at 20 °C and 90% relative humidity from 3 to 8 days depending on the variety and until the fruits reached firmness and organoleptic characteristics suitable for consumption (Fig. 1). Representative mesocarp tissue was collected from at least 20 fruits from the different varieties at harvest (H) or post-harvest ripened stage (R), immediately frozen in liquid nitrogen and stored at −80 °C for further experiments (Lauxmann et al., 2014; Lombardo et al., 2011). Five separate pools, each one composed of three different fruits of each variety at harvest (H) and ripened stage (R), were used for further metabolic analysis. The results shown in the present work correspond to fruits collected during the 2009/2010 season, although similar results were obtained for some peach varieties grown during 2011/2012 (not shown in the present work).

2.2. Fruit quality traits determination

Flesh firmness was evaluated with a penetrometer (Effegi 327, Alfonsine, Italy) with a 7.9-mm tip and expressed in newton (N). Measurements were carried out on two opposite sides of each individual fruit after peel removal. Ground colour was measured on the greenest portion of the peel free of red blush and flesh colour was determined on the equatorial zone of a longitudinal section with a chromameter (Minolta CR 300; Budde et al., 2006). Twenty to thirty fruits of each variety at harvest (H) and post-harvest ripened stage (R) were used for each quality trait determination.

2.3. Metabolite measurements

Metabolite analysis by gas chromatography–mass spectrometry (GC–MS) was carried out essentially as described by Roessner-Tunali et al. (2003). Representative mesocarp tissue of peach fruits from the different varieties (250 mg) at harvest (H) or ripened stage (R) were ground using ceramic mortar and pestle pre-cooled with liquid nitrogen and extracted in 3 mL of methanol. Internal standard (180 μL, 0.2 mg ribitol mL^{-1} water MilliQ) was subsequently added for quantification purposes. The mixture was extracted for 15 min at 70 °C (vortexing every 3 min) and mixed vigorously with pre-cooled water MilliQ (1.5 mL). After centrifugation at 2,200 *g*, an aliquot of the supernatant (50 μL) was transferred to a reaction tube (1.5 mL) and vacuum dried. Tubes were filled with argon gas and stored at −80 °C. Samples were derivatised using methoxyamine hydrochloride in pyridine followed by *N*-methyl-*N*-[trimethylsilyl]trifluoroacetamide treatment. Derivatisation and GC–MS were performed as described by Roessner-Tunali et al. (2003). Mass spectra were cross-referenced with those in the Golm Metabolome Database (Kopka et al., 2005). Five independent determinations composed of three fruits each, and each one repeated three times for methodological replica, were performed for each variety at harvest (H) and ripened stage (R). Metabolite quantification was based on the relative peak response area of each chromatogram and expressed relative to the internal standard (ribitol; Table S2). The relative values were also expressed as log2 using the MultiExperiment Viewer software with a colour

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