Scientia Horticulturae 168 (2014) 58–63

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Dynamics of total phenolic content in different apple tissues and genotypes: impacts and relevance for breeding programs

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ARTICLE INFO

Article history:
Received 13 October 2013
Received in revised form 10 January 2014
Accepted 15 January 2014

Keywords:
Apple germplasm
enzymatic browning
Malus domestica Borkh
nutraceutical
plant protection
total phenolics

ABSTRACT

The content and quality of phenolic compounds in apple pulp, peel, and leaves constitute important nutraceutical properties of apples in the human diet and are equally important to plant ecology. Moreover, increased understanding of the dynamics of polyphenol synthesis in different apple tissues of different genotypes may assist in apple breeding programs. Thus, the present study aimed to analyze the levels of total phenolics in apple pulp, peel, and leaves from cultivars Aori27, Elstar, Fuji, and Mellow at harvest time. Significant differences in the total phenolic content were found between cultivars for all evaluated tissues, but the differences between cultivars were not consistent among tissues, indicating the potential existence of tissue-specific genetic regulation mechanisms, as well as cultivar-specific dynamics. Additionally, tissues of two cultivars, Fuji and Mellow, were analyzed in the last three months of fruit development, showing a similar overall evolution in the phenolic concentration for all tissues, but some notable cultivar-specific characteristics for apple pulp and peel. These results are relevant for apple breeding when we consider as selection criteria the nutraceutical properties of apple polyphenols, the speed and degree of pulp enzymatic browning, and the physiological and agro-ecological roles of polyphenols in different organs and tissues of apple trees.

1. Introduction

Phenolic compounds are ubiquitous in plants, and their diversity and concentration are influenced by several factors, including plant species, variety, organs and tissues, physiological and phenological stages, soil and climate conditions, and several biotic and abiotic stresses (Michalek et al., 1999; Scalbert & Williamson, 2000).

Phenolics seem to be strongly related to plant-ecosystem interactions, contributing to mechanisms of allelopathy, pollen fertility, attraction of pollinators and seed dispersers, mitigation of photo-oxidative stress, and defense against herbivores and pathogens (Schijlen et al., 2004).

Many of the biological roles of phenolics are attributed to their potential cytotoxicity and capability to act as free-radical scavengers (Pourcel *et al.*, 2007). The oxidation of phenolics has been associated with plant protection mechanisms throughout development and growth, playing important roles in such events as seed desiccation and plant senescence by means of formation of brown pigments in plants. Furthermore, the oxidation of phenolics is also associated with the enzymatic browning of plant-derived foods and beverages (Pourcel *et al.*, 2007).

In the human diet, phenolics are believed to help prevent degenerative diseases by their antioxidant properties that exert scavenging activity toward free radicals (Chinnici *et al.*, 2004; Pourcel *et al.*, 2007). However, the oxidation of phenolics in phenolic-rich foods, such as apples, leads to browning reactions which are usually detrimental to nutritional quality and overall product value (Murata *et* al., 1995; Pourcel *et al.*, 2007; Holderbaum *et al.*, 2010).

Bearing in mind the myriad biological functions of phenolic compounds in apple plants, their importance for the nutraceutical qualities of apples, and their contribution to browning reactions, the present study aimed at analyzing the contents of total phenolics in apple pulp, peel, and apple tree leaves of four cultivars (cvs.) in order to better understand how the dynamics of these compounds in different apple tissues and genotypes might assist apple breeding programs (Fig. 1).

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0304-4238/$ – see front matter © 2014 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.scienta.2014.01.020

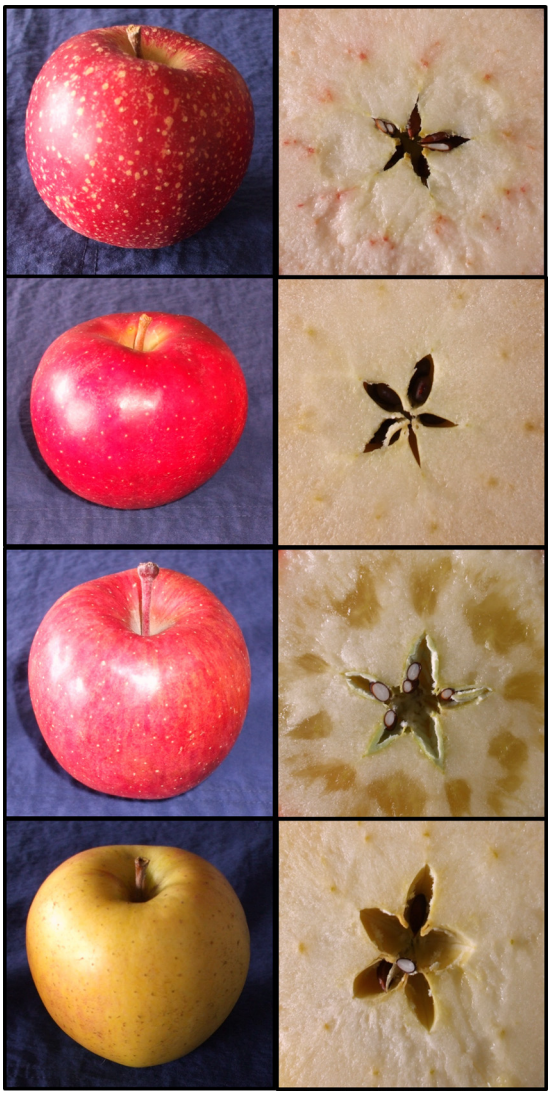

Figure 1. Ripe fruit (left) and details of the pulp in transversally cut fruit (right) of the four apple cultivars analyzed at harvest time. From top to bottom: cvs. Aori 27, Elstar, Fuji, and Mellow.

2. Material and Methods

2.1. Plant material

All plant material used for analyses was obtained from scions grafted on M26 EMLA rootstock, cultivated under the same growing conditions at an experimental orchard of the Aomori Apple Institute, in Kuroishi, Aomori, Japan. Three ripe apples and three samples of mature leaves (eight leaves per sample) were randomly collected from cvs. Aori 27 (Kinsei × Mahe7 [Indo × Golden Delicious] × [Redgold]), Elstar (Ingrid Marie × Golden Delicious), Fuji (Ralls Janet × Delicious), and Mellow (Golden Delicious × Indo) at harvest time. Additionally, three apples and leaf samples of Fuji and Mellow, which have the same cycle length, were collected 125, 152, and 173 days after full bloom (DAFB), the latter being the harvest time collection. After collection, apple and leaf samples were bagged and kept at 2 °C in the dark for a maximum period of 3 days, until use for extraction of total phenolic compounds.

2.2. Extraction of phenolic compounds

2.2.1. Fruit pulp

Three radial longitudinal slices were cut per fruit. Slices were peeled and chopped after removal of the ovary portion and the peel. Samples of pulp (10g fresh weight – FW) were put together with 30ml 100% MeOH (Wako Pure Chemical Industries, Osaka, Japan) in a 200ml Erlenmeyer flask, and the mixture was treated at 90 °C under reflux in an Eyela Digital Water Bath SB-1000 (Tokyo Rikakikai Co., Tokyo, Japan) for 10min. Subsequently, the heated mixture was ground in a mortar with a pestle, and the macerate was filtered in cheesecloth. The retained macerated pulp went through two more extraction steps, using 20mL of cold 80% MeOH and horizontal agitation (120 times per minute) for 10min, followed by new maceration and filtering. The combined extract was filtered in ADVANTEC N°2 filter paper (Toyo Roshi Kaisha, Tokyo, Japan) and completed to 100mL with distilled/deionized water in a volumetric flask, rendering a final polyphenol extract with 62% MeOH that was stored at 2 °C in the dark until analysis.

2.2.2. Fruit peel and leaves

Fruit peel and leaf blades (excluding the central rib) were chopped, and 5g FW of tissue were mixed together with 30ml 100% MeOH in a 200ml Erlenmeyer flask. The mixture was then treated at 90 °C under reflux in an Eyela Digital Water Bath SB-1000 (Tokyo Rikakikai Co., Tokyo, Japan) for 10min. Next, the heated mixture was homogenized in a process homogenizer at 10,000rpm for 5min under ice cooling. The homogenate was centrifuged at 16,099*g* for 20min at 4 °C and then filtered in ADVANTEC N°2 filter paper. The pellet went through two more extraction steps, using 20mL of cold 80% MeOH and horizontal agitation (120 times per minute) for 10min, followed by new centrifugation and filtering. The combined extract was completed to 100mL with distilled/deionized water in a volumetric flask, rendering a final polyphenol extract with 62% MeOH that was stored at 2 °C in the dark until analysis.

2.3. Quantification of total phenolic compounds

Total polyphenols were quantified by the Folin–Denis method (Waterman & Mole, 1994). All reagents were acquired from Wako Pure Chemical Industries, Osaka, Japan. Serial dilutions of catechin (98%) (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) were employed to produce standard curves for the polyphenol assay. All standard concentrations and biological samples were read in three technical replicates, measuring absorbance at 760nm with a UV-Visible UV-1600 spectrophotometer (Shimadzu, Kyoto, Japan), 1h after addition of the last reagent in the Folin–Denis assay (sodium carbonate solution). Total phenolic concentration was estimated based on the nominal concentrations (mg $100mL^{-1}$) and respective absorbance readings of catechin standard curves built by means of polynomial regression. Total phenolic concentration in different apple tissues was calculated and reported as mg of catechin equivalents (CE) by 100g of tissue fresh weight.

2.4. Statistical analysis

Data from total phenolic content (TPC) in different tissues from cvs. Aori27, Elstar, Fuji, and Mellow at harvest time were analyzed by ANOVA with a completely randomized design and a factorial scheme with two factors: *Cultivar* and *Tissue*. When significant ($P<0.05$) main effects or interaction were detected, treatment means were compared by the Student–Newman–Keuls test (SNK). The evolution of total phenolic content in each tissue of 'Fuji' and 'Mellow', observed monthly in the last three months of fruit development, was analyzed by least-squares multiple regression, having as explanatory variables *Cultivar*, *Time* (days after full bloom), the