Scientia Horticulturae 160 (2013) 115–122

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

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

Caprification modifies polyphenols but not cell wall concentrations in ripe figs

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ARTICLE INFO

Article history:
Received 29 January 2013
Received in revised form 21 May 2013
Accepted 28 May 2013

Keywords:
Figs *Ficus carica*
Pectin
Anthocyanins
Caprification
Textural properties
Nutritional composition

ABSTRACT

Figs, when ripe, move to their final colour and lose radically their firmness, and caprification (artificial pollination) leads to increased fruit softening during maturity. To identify the effects of caprification on cell wall amounts and phenolics composition, cell wall material and polyphenols were investigated in five Tunisian figs. Cell walls were isolated as alcohol insoluble solids (AIS) content. AIS yield was not affected by caprification in all cultivars. Pectin polymers decreased as the fruits fulfil their development after being caprified. The pectin in figs exhibited high degree of methylation (>50%) which increased more with caprification. Major neutral sugars components of the AIS were glucose, from cellulose, followed by arabinose. Caprification had no effect on neutral sugars composition of the cell wall. Methanol extraction followed by HPLC–DAD analysis revealed two anthocyanins, three flavonols and one hydroxycinnamic acid. For all varieties the total polyphenols were less in the flesh (up to 1.27 mg/kg FW) than in the peel (up to 12.64 mg/kg FW). Anthocyanins were the major compounds described in figs representing more than 98% of total polyphenols in both dark and white coloured fruit. Textural components and properties of figs are moderately affected by caprification, while anthocyanins biosynthesis appears to be stimulated by pollination with higher concentrations of total polyphenols in caprified fig fruits.

1. Introduction

Ficus carica is one of the oldest known fruit crop and used for fruit production (Beck and Lord, 1988; Kislev et al., 2006). Beside olive tree, fig species is considered a biomarker of Mediterranean ecosystem. Tunisia, a cradle for common fig tree, exhibits an attractive biodiversity and provides a large array of genotypes (Baraket et al., 2009). Figs have been used for human consumption for centuries and recently their laxative and nutritive values have been investigated (Çaliskan and Polat, 2011; Trad et al., 2013). Fig cultivars are several and well adapted to local agrosystems. Some are of the *Common* type that produces figs without pollination. Many others are of *Smyrna* type that need caprification (Mars et al., 1998). The caprification is quite a common practice in all regions and was cited as an important factor affecting the quality of the fruit (Mars et al., 2009). The general practice of caprification is to distribute the *profichis* (male syconia) at intervals of few days over a period of about three weeks corresponding really to the receptivity of female figs (Rahemi and Jafari, 2008). In Tunisia, the general tendency is to connect two to six caprifigs with a wire or a stick passed through their neck and hung onto branches of female tree. It is important for fig growers and consumers to understand the incidence of caprification on quality of figs at maturity. Analysis of fig quality in response to caprification is limited to physical and chemical aspects (Condit, 1947; Gaaliche et al., 2011) or aromatic profile released by the fruit at full ripeness (Trad et al., 2012). Caprified figs are usually larger, greener and more prone to splitting, and have a darker interior pulp colour (Oukabli et al., 2003; Michailides et al., 2008). Increased risk of splitting, i.e. a textural modification, could be linked to the cell wall compositions and/or of the relative ratios of peel, the resistant tissue, to pulp. Little is known about fig cell walls, though dried fruits are known to be rich in dietary fibres (Marlett and Vollendorf, 1994; Vinson, 1999). Modifications in colour could be related in terms of chemical composition to the polyphenols composition. The main polyphenols of figs are anthocyanins, flavonols and phenolic acids which have been identified and quantified in several varieties of figs with different colour (black, red, yellow and green) (Solomon et al., 2006; Del Caro and Piga, 2008; Veberic

Abbreviations: BHL, Bouhouli; ZD, Zidi; THG, Thgagli; BD, Bidhi; KHD, Khedri; AIS, alcohol insoluble solids; HPLC–DAD, high performance liquid chromatography–diode array detector; MHDP, *m*-hydroxydiphenyl; GC–MS, gas chromatography–mass spectrometry; GC–FID, gas chromatography–flame ionisation detector; PTFE, polytetrafluoroethylene; SPSS, statistical package for the social sciences; GLM, general linear model; DM, degree of methylation; PG, polygalacturonase; AUA, anhydro-uronic acid.

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http://dx.doi.org/10.1016/j.scienta.2013.05.037

Table 1
General description of the five Tunisian figs *Ficus carica*.

Cultivar	Label	Botanical type	Origin	Shape[a]	Skin colour[a]	Pulp colour[a]
Bouhouli	BHL	San Pedro	Thibar	Oblate	Purple green	Amber
Zidi	ZD	Smyrna	Thibar	Oblong	Purple black	Red
Thgagli	THG	Smyrna	Thibar	Oblate	Yellow green	Amber
Bidhi	BD	Smyrna	Bekalta	Round	Yellow green	Pink
Khedri	KHD	Smyrna	Bekalta	Round	Yellow green	Pink

[a] Descriptors for figs (IPGRI and CIHEAM, 2003).

et al., 2008). Duenas et al. (2008) identified the main anthocyanin in figs as cyanidin-3,5-diglucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside and pelargonidin-3-rutinoside. Crisosto et al. (2010) studied the influence of fruit variety and harvest season on the phenolic compound content. Some others have been made on the health-promoting potential of figs due to the phenolics they contain (Harnly et al., 2006; Duenas et al., 2008; Veberic et al., 2008).

To understand the difference in texture and colour between caprified and non caprified fig fruit, we have studied the impact of caprification on morphology, texture and tissular repartition, on cell wall contents and composition, and on phenolics in fig syconia of five Tunisian (dark and light) fruit cultivars.

2. Materials and methods

2.1. Fruit sampling and preparation

Figs from five cultivars (Bouhouli 'BHL' and Zidi 'ZD': dark coloured figs; Thgagli 'THG', Bidhi 'BD' and Khedri 'KHD': white coloured figs) were sampled and prepared for AIS and polyphenols determination. Fruits were harvested from two regions well-known by fig tree growing: 'Thibar' (governorate of '*Béjà*') in the North West and 'Bekalta' (governorate of '*Monastir*') representing the Central East of Tunisia. Samples of thirty homogenous fruits (three replicates of 10 fruits each) were picked for each variety and from caprified and non-caprified trees (distant about 300 m from the rest of the orchard). Fruits were selected fully ripe from the main crop at the last of July in 'Bekalta' for 'BD' and 'KHD' cultivars and the last of august in 'Thibar' for 'BHL', 'ZD' and 'KHD' during the two cropping season 2009 and 2010. General aspects of the five fig cultivars are given in Table 1. Fruit weight, flesh weight, fruit size, skin thickness, firmness and dry matter content were measured to disclose caprification impact on morphological aspects of quality in figs. Firmness was determined using durofel (Duro10, SETOP GIRAUD Technology, Cavaillon, France). Four grams of fresh powder of the fruit were dried (70 °C – 96 h) and removed to be weighed again for dry matter determination.

Samples were stored at −20 °C then thawed for peel/flesh separation. Cell wall and phenolics' characterization was carried out in the two separate tissues (peel and flesh). Peel samples, meticulously removed using a scalpel, were ground after being soaked in liquid nitrogen. Fresh powder obtained was conserved at −80 °C until analysis. Flesh samples were ground in liquid nitrogen using an IKA®A11 basic analytical mill (Ika Labortechnik, Staufen, Germany) and the powder was sieved to remove seeds and other debris mainly composed by the ostiole scales. Samples intended for polyphenols determination were lyophilized and the freeze-dried powder was finally stored at −20 °C.

2.2. Cell wall isolation and analysis

Alcohol insoluble solids (AIS) were prepared according to Renard (2005). Uronic acids were measured spectrophotometrically by the *m*-hydroxydiphenyl (MHDP) assay as described by Blumenkrantz and Asboe-Hansen (1973) with galacturonic acid as external standard and expressed as anhydro-uronic acids (AUA). Sample preparation for this analysis consists in sulphuric acid hydrolysis according to Saeman et al. (1954) as described below. Methanol was determined by Headspace-GC–MS after saponification as described by Renard and Ginies (2009). Neutral sugars were analysed as alditol acetates after acid hydrolysis with 2 options. For cell walls including cellulose analysis samples (c.a. 10 mg of AIS) were submitted to prehydrolysis with 250 μl 72% sulphuric acid for 1 h at room temperature (Saeman et al., 1954) and then diluted to 1 M sulphuric acid by addition of water and internal standard (inositol). For extracted polysaccharides no prehydrolysis was carried out, samples were dissolved in 1 M sulphuric acid with internal standard. All samples were placed in oven at 100 °C for 3 h for hydrolysis. After hydrolysis they were derivatised to volatile alditol acetates (Englyst et al., 1982). They were injected on a GC–FID HP 5890 Series II (Agilent, Inc., Palo Alto, USA) with capillary column of 30 m × 0.25 mm i.d. coated with DB225 MS, 0.25 μm film thickness (J&W Scientific, Agilent, Inc., Palo Alto, USA). The conditions were: temperature of injection 250 °C in split mode (ratio 1:25); hydrogen as carrier gas at 45 cm/s (at 215 °C), column flow was 1.3 ml/min and the oven temperature was isothermal at 215 °C.

2.3. Polyphenols in figs

Polyphenols were extracted by suspension of the freeze-dried fig powder (circa 200 mg) in 1200 μL acidic methanol (1% acetic acid, v/v) and 15 min sonication in a melting ice bath as described by Guyot et al. (2001), followed by filtration (PTFE, 0.45 μm) before HPLC–DAD analysis. Polyphenols were measured by HPLC as described by Guyot et al. (2001). Phenolic compounds were separated in an Agilent 1050 separation system (Agilent Technologies, Santa Clara, CA, USA) including a quaternary pump coupled to a diode array detector and controlled by Chemstation A.10.02 software. Separations were achieved using a (250 mm × 4 mm i.d.) Licrospher PR-18 5 μm column (Merck, Darmstadt, Germany) with a guard column (Licrospher PR-18 5 μm column, Merck, Darmstadt, Germany) operated at 30 °C. The mobile phase consisted of water/formic acid (98:2, v/v) (eluent A) and acetonitrile (eluent B). The flow rate was 1 ml/min. The elution programme was as follows: 3–9% B (0–5 min); 9–16% B (5–20 min); 16–50% B (20–45 min); 50–100% B (45–50 min); 100–100% B (50–52 min); 100–3% B (52–55 min). Triplicate samples were injected at a level of 20 μl. The column effluent was monitored at 280, 320, 350 and 520 nm. Quantification was achieved by injection of standard solutions of known concentrations. Standard of 5-cafeoylquinic acid was ordered from Sigma–Aldrich (Deisenhofen, Germany). Quercetin-3-rutinoside, quercetin-3-glucoside, cyanidin-3-glucoside and cyanidin-3-rutinoside were obtained from Extrasynthese (Lyon, France).

2.4. Statistics

Data analysis was established using SPSS package software (version 13.0; SPSS Inc.). The results presented are means ($N = 3$) ± standard deviations (SD) from samples harvested over two