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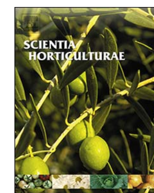

Developmental changes in phenolic compounds, antioxidant capacity and enzymes activity in skin of ‘El-Bayadi’ table grapes

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ABSTRACT

In two successive seasons, changes in phenolic compounds, antioxidant capacity and enzymes activity in skin of ‘El-Bayadi’ table grape skin during development were evaluated. The growth of berries followed a typical double sigmoid curve, and veraison stage corresponded to the inception of the slow growth phase. Total phenols concentration gradually decreased during berry development reaching 0.67 and 1.34 g kg^{-1} at ripening stage for 2014 and 2015 season, respectively. Total flavonoids concentration gradually decreased during growth with a slight increase at ripening stage reaching 0.59 and 0.38 g kg^{-1} for 2014 and 2015 season, respectively. Total phenols concentration showed higher values in 2015 than 2014 season, in contrast to total flavonoids. Both *trans*-resveratrol and *trans*-picied concentrations were highest early in the season, but gradually decreased during growth with an increase during ripening, representing 0.28 and 0.26 mg kg^{-1} for *trans*-resveratrol, and 1.39 and 1.12 mg kg^{-1} for *trans*-picied, for 2014 and 2015 season, respectively. The level of *trans*-picied was much higher than that of *trans*-resveratrol during growth and ripening in both seasons. Antioxidant capacity gradually increased (lower IC_{50} values of DPPH and ABTS) during growth and ripening. Peroxidase (POD) activity gradually increased to a peak at veraison with a slight fluctuation during ripening. While, polyphenoloxidase (PPO) activity initially increased, slightly fluctuated and then decrease during ripening. Polygalacturonase (PG) activity gradually increased to a maximum at 20 days after veraison and, then decrease at ripening. While, xylanase activity was highest early in the season, but sharply decreased during growth and, then increased during veraison and ripening.

1. Introduction

The protective role of fresh grapes and grape processed products consumption against degenerative diseases is generally attributed to their contents of health-promoting polyphenols (Fremont, 2000; Xia et al., 2010; Zhou and Raffoul, 2012). Polyphenols are defined as natural products which contain one or more hydroxyl groups covalently linked to a benzene ring (Croteau et al., 2000). In grapes, polyphenols include two main groups: non-flavonoids (hydroxybenzoic and hydroxycinnamic acids and their derivatives, stilbenes and phenolic alcohols) and flavonoids (anthocyanins, flavanols, flavonols and dihydroflavonols) (Fanzone et al., 2012). In the berry, phenolic compounds are mainly localized in skins and seeds (Jeandet et al., 1995a; Jordao et al., 2012). Both the quantitative and qualitative characteristics of polyphenols determine quality of fresh grapes and grape processed products such as organoleptic and nutritional characteristics (Fanzone et al., 2012; Gonzalez et al., 2015). Resveratrol (3,4′,5-trihydroxystilbene), its 3-glucopyranoside piceid, and their *cis* isomers are non-flavonoids polyphenols, belonging to the class of stilbene phytoalexins (C_6–C_2–C_6) (Fremont, 2000). Resveratrol represent the major active compound of stilbene phytoalexins that mainly occur in grapes, berries, and other dietary constituents and is presumed to be involved in defense system against both plant pathogens and metabolic diseases in human (Adrian et al., 1997; Fremont, 2000; Xia et al., 2010; Zhou and Raffoul, 2012).

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Polyphenols are biosynthesized through the phenylpropanoid pathway that include a combination of precursors from both the shikimate (phenylalanine) and the acetate-malonate (malonyl-CoA) pathways via several enzymatic steps (Staniek et al., 2013). The deamination of L-phenylalanine to *trans*-cinnamic acid by the enzyme phenylalanine ammonia-lyase (PAL) is the first step in the biosynthesis of the different polyphenol classes in plants (Staniek et al., 2013). The phenylpropanoid pathway in grapes, as in other fruit, is genetically, developmentally and environmentally regulated (Awad et al., 2001; Ojeda et al., 2002; Jeandet et al., 1991, 1995a,b; Negri et al., 2008; Ali et al., 2011; Staniek et al., 2013; Garrido et al., 2016). Jordao et al. (2012) reported that in 'Touriga Nacional' and 'Tinta Roriz' grape cultivars, the antioxidant activity in both skins and seeds generally decreased with a slight fluctuation in early stages of maturation, followed by a slight increase during ripening. During veraison and beginning of ripening, a burst in reactive oxygen species (ROS) with a surge in the expression of genes that encode enzymes involved in the generation of antioxidant systems such as POD and PPO in berry skin to enhance resistance against pathogens were reported (Pilati et al., 2007; Negri et al., 2008; Ali et al., 2011; Garrido et al., 2016). PPO activity has attracted much attention due to its action on polyphenols oxidation and the development of browning of grapes and grape processed products (Negri et al., 2008). While cell wall modifying enzymes such as pectin methylesterase (PME), PG and xylanase are involved in berry softening (Nunan et al., 1998; Cabanne and Doneche, 2001; Deytieux-Belleau et al., 2008). The beneficial health-related effects of phenolics in grapes are of importance to consumers, breeders and the grape industry. There is relatively much knowledge on the level of phenolcis, antioxidant capacity as well as antioxidant and degrading enzymes activities during berry ripening and at harvest time. However, relatively little is known about such changes during berry development. 'El-Bayadi' is a mid-season, highly productive, large size white seeded berries with high sensory quality, representing about 90% of total grape production in Taif region (Al-Qurashi and Awad, 2013). Therefore, in this paper, we report the changes that take place in total phenols, flavonoids, *trans*-resveratrol and *trans*-piceid concentrations, antioxidant capacity, as well as some antioxidant and degrading enzymes activities of 'El-Bayadi' table grapes *(Vitis vinifera* L.) grown in Saudi Arabia during development.

2. Materials and methods

2.1. Plant materials and experimental procedure

During 2014 and 2015 seasons, uniform vines were selected in a commercial drip irrigated vineyard of 'El-Bayadi' table grape in Taif region (21.4333°N, 40.3500°E), Saudi Arabia. The vines were growing on their own roots and cane trained on arbors. The soil texture is sandy loam. The mean precipitation was 69.1 and 53.3 mm in 2014 and 2015, respectively and mean air temperature was 27.5C during the growing period from the beginning of April (flowering) to the end of July (harvesting) (Alrashdi et al., 2017). The experimental design was a completely randomized design with three replicates/time and each replicate contained five vines. During development and ripening, berry samples (30 berries/replicate) were randomly collected from different clusters, to avoid possible thinning effects, of each replicate/time starting at the pea stage (berry diameter 4–5 mm, 30 days after setting (DAS)), and then every 20 days with the last one at commercial maturation (13–14 Brix% and 0.6–0.7% titratable acidity). The collected samples were kept in perforated carton and directly transferred to the horticulture laboratory at King Abdulaziz University, Jeddah for biochemical determinations.

2.2. Berry fresh weight

Berry fresh weight (g) was calculated by tacking the mean values of 30 berries randomly collected from several clusters per replicate/time.

2.3. Extraction and quantification of trans-resveratrol and its glycoside trans-piceid

Extraction and quantification of *trans*-resveratrol and *trans*-piceid were carried out according to Romero-Perez et al. (2001) with modifications. Two grams of frozen berry skin (randomly collected from 30 berries/replicate) were homogenized with 25 ml of ethanol/water (80:20 v/v) using a homogenizer and maintained at 60 °C for 30 min. The extract was filtered through a Whatman inor-ganic 15 μm and concentrated to 3 ml by rotary evaporation (in vacuo) at room temperature (20 °C ± 2). The concentrated extracts were filtered through CA Syringe filters 0.2 μm and injected into a high-performance liquid chromatography (Shimadzu HPLC Class VP series, Japan) coupled with ultraviolet-visible diode array detector (HPLC-UV-VIS-DAD) for *trans*-resveratrol and *trans*-piceid quantification. The system was equipped with a Tracer Agilent ZORBX Eclipse plus C18 Analytical column (4.6 × 150 mm), 5 μm particle size. The column temperature was kept at 30 °C. The mobile phase consisted of A and B where solvent A was glacial acetic acid in water mixture (0.1 glacial acetic acid:70 water v:v) and solvent B 29.9 aceto- nitrile/acetic acid, with a flow rate of 1.0 ml/min. Injection volume was 20 μl. Detection was performed at a 310 nm wavelength and run time was 15 min. Retention time was about 2 and 4.5 min for *trans*-piceid and *trans*-resveratrol, respectively. Quantification was based on the peak area. The chromatogram peaks of individual compounds were identified by comparing their retention times with the retention times of pure standards. trans-resveratrol standard was purchased from Baoji Guokang Bio-Technology Co., Ltd (Baoji, China). *trans*-piceid standard was purchased from Sigma Chemical Co., St. Louis, MO. (USA). Integrated peaks were calculated by comparison with standard solutions of known concentration and the results expressed as mg kg^{-1} on a fresh weight (FW) basis.

2.4. Preparation of the methanol extract for total phenols, flavonoids and antioxidant activity determinations

Two grams of berries skin tissue (randomly collected from 30 berries/replicate) were extracted by shaking at 150 rpm for 12 h with 20 ml methanol (80%) and filtered through filter paper No. 1. The filtrate designated as methanol extract that was used for total phenols and flavonoids and antioxidant activity estimations.

2.4.1. Estimation of total phenols by the Folin-Ciocalteu test

Total phenols concentration was measured according to Hoff and Singleton (1977). Fifty μl of the methanol extract was mixed with 100 μl Folin-Ciocalteu reagent, 850 μl of methanol and allowed to stand for 5 min at ambient temperature. A 500 μl of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured at 750 nm using a UV–vis Spectrophotometer (PU8625 Series, Philips, UK). Total phenols was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid and the results expressed as g kg^{-1} FW gallic acid equivalent.

2.4.2. Estimation of total flavonoids

Total flavonoids concentration was determined using a modified colorimetric method described previously by Zhishen et al. (1999). Methanol extract or standard solution (250 μl) was mixed with distilled water (1.25 ml) and 5% $NaNO_2$ solution (75 μl). After standing for 6 min, the mixture was combined with 10% $AlCl_3$ solution (150 μl), 1 M NaOH (0.5 ml) and distilled water (275 μl) were added to the mixture 5 min later. The absorbance of the solutions at 510 nm was then measured. Total flavonoids was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of catechin and the results expressed as g kg^{-1} FW catechin equivalent.

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