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Changes in polyphenol content during production of grape juice concentrate

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ABSTRACT

The production of grape juice concentrate on an industrial scale was evaluated and samples from the main steps of processing have been collected and analyzed. The sampling steps included the selection and washing of grapes (Nevsehir Patlak variety), pressing in order to obtain the juice separate from the seed and the skin fraction, pasteurization, clarification, filtration, evaporation, and filling-packing at 27 °C with a Brix of 45°. Samples from each of the processing steps were analyzed by a number of spectrophotometric analyses. A series of anthocyanin compounds was identified using HPLC-MS, and the fate of anthocyanins, quercetin rutinoside and procyanidins was followed using HPLC. The results indicate that the removal of seed and fruit skin removes most of the procyanidins and anthocyanins, while subsequent clarification and filtration treatments further reduce the anthocyanin content.

1. Introduction

It has been reported that there is an inverse association between the consumption of some fruits and vegetables and mortality from age-related diseases. This could be partly attributed to the presence of antioxidants, especially phenolic compounds, in the diet (Dudonne, Vitrac, Coutiere, Woillez, & Merillon, 2009). Grapes (*Vitis vinifera*) are among the most widely consumed fruits, and the demand for grapes and grape products is increasing, partly because of their associated health benefits (Ghafoor, Choi, Jeon, & Jo, 2009).

Grapes can be consumed as fresh fruit, but they may also be dried, processed into wine, vinegar, fruit juice concentrate (and then processed into fruit juice), *pekmez* (syrup obtained by condensing juices of the fruit must), *pestil* (a dried form of marmalade), jam, and marmalades (Liyana-Pathirana, Shahidi, & Alasalvar, 2006). Grape and its products contain a wide range of polyphenolic constituents that have been reported to show anticancer, and anti-inflammatory effects *in vitro*, as well as the ability to block cellular events predisposing atherosclerosis and coronary heart disease (Castilla et al., 2006; Gurbuz et al., 2007; Keevil, Osman, Reed, & Folts, 2000). The compounds present in grape and its products which are presumed to provide positive health effects are mainly flavonols, procyanidins, anthocyanins, and phenolic acids (Andrade, Mendes, Falco, Valentao, & Seabra, 2001; Chedea, Braicu, & Socaciu, 2010).

Changes in antioxidants during wine processing (Netzel et al., 2003; Puertolas, Saldana, Alvarez, & Raso, 2010) and storage (Musingo, Sims, Bates, O'Keefe, & Lamikanra, 2001; Zafrilla et al., 2003) were investigated in several studies, and factors influencing antioxidants in grapes and wines were reviewed by Lachman, Sulc, Faitova, and Pivec (2009). However, to the best of our knowledge there are only a few studies which have investigated the effect of processing on grape juice or concentrate antioxidants (Cabrera et al., 2009; Fuleki & Ricardo-Da-Silva, 2003; Iyer, Sacks, & Padilla-Zakour, 2010; Spanos & Wrolstad, 1990), and not every step of processing was included in these studies. The aim of this study was therefore to evaluate the changes in the antioxidants of grape berries during its processing into grape juice concentrate and to pinpoint those steps that mostly influence the antioxidant composition of this major grape product.

2. Materials and methods

2.1. Grape processing material

Fresh grape samples (Nevsehir Patlak variety), intermediate by-products and final grape concentrate products were collected in three replicate and independent processing events in 2007, from a fruit juice factory in Turkey. The main processing steps in the grape juice concentrate production are: selection of grapes;

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washing; removal of stalks; pre-heating at 40–50 °C; transferring the sample to the mash tank where it is kept for 30–45 min; pressing; pasteurization at 100–107 °C; clarification; filtration; evaporation; filling-packing at 27 °C, and finally frozen storage (Fig. 1). Juice clarification involves a number of steps including centrifugation to remove suspended particles in the juice, depectinization of juice by the addition of enzymes (pectinase and amylase), and a fining treatment to remove cloudiness. The clarified juice is filtered by using diatomaceous earth to remove the fining agents. Evaporation is performed to concentrate the grape juice to a Brix value of 70°–72°. Finally, the concentrated grape juice is packed in 10 L polyethylene containers. The pH and titratable acidity of the final grape concentrate were 3.8–4.0 and 0.8–1.0% (w/w) (expressed as tartaric acid), respectively.

Samples from several steps in the entire production chain, i.e. fresh grape, press cake waste, steps post pasteurization, clarification, filtration and end-product grape concentrate (Fig. 1), were collected from three different grape processing batches and analyzed. All samples were snap-frozen in liquid nitrogen and ground to a fine powder using a precooled grinder. Samples were then stored at −80 °C until further analysis. All collected samples were analyzed in three replicates.

2.2. Moisture content analysis

Moisture content of the samples was analyzed according to "Association Official of Analytical Chemists" (AOAC, 1990) method 925.10.

2.3. Extract preparation

Extracts for spectrophotometric analyses were prepared by adding 3 ml of 75% methanol to 200 ± 1 mg of freeze-dried samples and sonicated for 15 min. After centrifugation at 2500 rpm (1200*g*) for 10 min, the upper layer was separated and 2 ml of 75% methanol were added to the residue. The upper layers were collected and this solution was used as the extract for total phenolics, total flavonoids, and antioxidant capacity assays.

For analyses by HPLC-PDA and LC-MS, 25 (±0.05) mg freeze-dried sample was extracted with 2.0 ml 75% methanol and 0.1% formic acid in HPLC grade water (Capanoglu, Beekwilder, Boyacioglu, Hall, & De Vos, 2008).

2.4. Spectrophotometric assays

The total phenolic content was estimated using the Folin–Ciocalteu reagent (Spanos & Wrolstad, 1990), using 100 µl extract, 900 µl pure water and 5 ml reagent. For preparation of a standard curve, 0.10–0.50 mg/ml gallic acid was used and data were expressed in mg gallic acid equivalents (GAE) per 100 g dry weight (DW).

Total flavonoid content was determined according to Dewanto, Wu, Adom, and Liu (2002), using aluminium chloride and sodium nitrite as reagents. Absorbance of the samples was measured at 510 nm against a reagent blank. Catechin at concentrations of 0.01–0.25 mg/ml was used to create a calibration curve and data were expressed as mg catechin equivalents (CE) per 100 g DW.

Total antioxidant activities were evaluated by the ABTS (2,2-azinobis 3-ethylbenzothiazoline-6-sulphonic acid diammonium salt) method, the FRAP (ferric reducing antioxidant power) method, the CUPRAC (copper reducing antioxidant capacity) method, and the DPPH (1,1-diphenyl-2- picrylhydrazyl) method, as described previously (Capanoglu et al., 2008). In all assays, trolox was used as a reference compound and results were expressed in terms of µmol trolox equivalent antioxidant capacity (TEAC) per 100 g DW.

2.5. Identification of anthocyanins using LC-MS

An Accela HPLC-PDA (Thermo, Bannockburn, IL, USA) coupled to a LTQ Ion Trap-Orbitrap FTMS hybrid mass spectrometer (Thermo, Bannockburn, IL, USA) system was used to identify the major anthocyanins present in the processing samples. The same gradient conditions as used for quantitative HPLC (described below) were applied, while using a LUNA 3 µ C18 (2) 150 × 2.00 mm microbore column (Phenomenex, Torrance, CA, USA) with a corresponding flow rate of 0.19 ml/min. MS instrument calibration and settings were as described in Van der Hooft, Vervoort, Bino, Beekwilder, and de Vos (2011), using positive ionization mode, a mass resolution of 60,000 HWHM and a mass range of *m*/*z* 100–1200. Identification was based on accurate masses of parent and their fragment ions, in combination with their photodiode array (PDA) absorbance spectra (recorded at 240–600 nm). Elemental formulae were compared to those of known anthocyanins in *Vitis vinifera* (http://kanaya.naist.jp and Xu et al., 2011) using a threshold of 3 ppm mass accuracy.

2.6. Quantitative HPLC analysis

The system used to determine the content of individual anthocyanins was composed of a Waters 600 control unit, Waters 996 PDA detector and column incubator at 40 °C. The column used for flavonoid analysis was a LUNA 3 µ C18 (2) 150 × 4.60 mm (Phenomenex, Torrance, CA, USA). Solvent systems were A (MQ water with 0.1% formic acid) and B (acetonitrile) for flavonoids with a linear acetonitrile gradient from 5% to 50%. The flow rate was 1 ml/min. Eluting compounds were detected by a PDA (Waters 996) detector at 512 nm for anthocyanins and 360 nm for rutin. Rutin and cyanidin-3-glucoside were identified by comparing to authen-

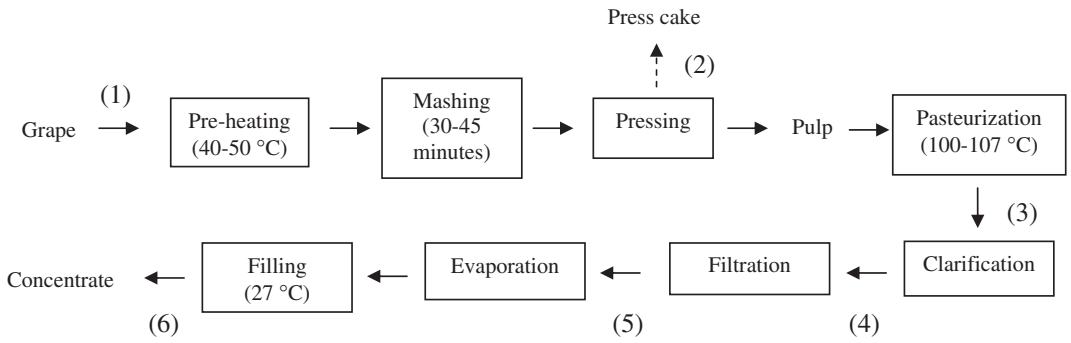

Fig. 1. Flow diagram of industrial grape concentrate production. Numbers in brackets represent the sampling steps.