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Residues of grape (*Vitis vinifera* L.) seed oil production as a valuable source of phenolic antioxidants

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ABSTRACT

Phenolic compounds of seven grape seed samples originating from mechanical seed oil extraction were identified and quantified by HPLC–DAD before (intact seeds) and after (press residue) the oil recovery process. Total amounts of all identified compounds ranged from 4.81 ('Cabernet Mitos') to 19.12 g/kg ('Schwarzriesling') of defatted dry matter (DM; 'Schwarzriesling') for integral grape seeds, whereas their content in the press residues ranged from 2.80 ('Cabernet Mitos') to 13.76 g/kg of defatted DM ('Spätburgunder'). This is the first study presenting comprehensive data on the contents of individual phenolic compounds comprising all polyphenolic subclasses of press residues from grape seed oil production also covering the determination of the antioxidant activities of each subclass (Folin–Ciocalteu, FRAP and TEAC assays). Additionally, the effects of different solvents on the yields of phenolic compounds were determined. Maximum yields were obtained using methanol/0.1% HCl (*v:v*), water [75 °C] and a mixture of ethanol and water [3:1; *v:v*], respectively, whereas pure ethanol resulted in poor polyphenol extraction. The results of the present study confirm the press residues of grape seed oil production still to be a rich source of polyphenolics with strong antioxidant activity.

1. Introduction

Grapes (*Vitis vinifera* L.) belong to the world's largest fruit crops with a global production of around 69 million tons in 2006 (FAOSTAT, 2007). Since about 80% of the total amount is used in winemaking, some 10 million tons of grape pomace arise within a few weeks of the harvest campaign. The seeds constitute a considerable proportion of the pomace, amounting to 38–52% on a dry matter basis. Their oil is rich in unsaturated fatty acids, in particular linoleic acid (Schieber, Müller, Röhrig, & Carle, 2002). Grape seed oil is mainly produced in Italy, France and Spain; however, the demand for this oil has also increased in the rest of Europe (Kamel, Dawson, & Kakuda, 1985).

Apart from being a rich source of a high-value fatty oil, grape seeds have also been appreciated because of their content of phenolic compounds such as gallic acid, catechin and epicatechin, and a wide variety of procyanidins. The latter are also referred to as condensed tannins. Grape seed extracts and procyanidins have been a matter of intense investigations with respect to their potentially beneficial effects on human health. Recent reports indicate a wide range of biological activities, e.g. antioxidant properties and radioprotective effects (Castillo et al., 2000), prevention of cataract (Yamakoshi, Saito, Kataoka, & Tokutake, 2002), antihyperglycemic effects (Pinent et al., 2004), enhancement of postprandial lipemia (Del Bas et al., 2005), modulation of the expression of antioxidant enzyme systems (Puiggròs et al., 2005), improvement of insulin sensitivity and prevention of hypertriglyceridemia (Al-Awwadi et al., 2005), inhibition of aromatase and suppression of aromatase expression (Kijima, Phung, Hur, Kwok, & Chen, 2006), inhibition of protein kinase activity of the epidermal growth factor receptor, protective effects against oxidative damage in mouse brain cells (Guo et al., 2007), and anti-inflammatory effects (Terra et al., 2007).

While it is well known that grape seed polyphenolics display antioxidant activities, the fate of individual phenolic compounds in the course of seed oil recovery as well as their contribution to the overall antioxidant properties of seed extracts has not yet been investigated. In the present study, grape seed oil was produced from seven grape cultivars grown in southern Germany. The polyphenols were extracted from the press residues, fractionated into phenolic acids and flavonoids, and their contents and antioxidant activities were determined. Furthermore, the effects of different solvents on the yields and phenolic profile of extracts from the residues of the oil recovery process were assessed.

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2. Materials and methods

2.1. Materials

2.1.1. Chemicals

All reagents and solvents were of analytical or HPLC grade and were purchased from VWR (Darmstadt, Germany). C_{18} reversed-phase cartridges (Chromabond®, 1000 mg) were from Macherey-Nagel (Düren, Germany). The following standards were used for identification and quantification purposes with HPLC–mass spectrometry (MS) and HPLC-diode array detection (DAD): (+)-catechin, *p*-coumaric acid (−)-epicatechin, ferulic acid, gallic acid, caffeic acid, protocatechuic acid, quercetin (Roth, Karlsruhe, Germany); quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, procyanidin B1, procyanidin B2 (Extrasynthèse, Lyon, France); epicatechin gallate, *trans*-resveratrol (Sigma, St. Louis, MO, USA); *trans*-resveratrol 3-*O*-glucoside (*trans*-polydatin) (Sequoia Research Products, Oxford, UK).

ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)], trolox [6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid], ABAP [2,2′-azobis(2-amidinopropane) dihydrochloride] and TPTZ-Fe(II) [2,4,6-tri(2-pyridyl)-s-triazine] were used for the determination of the antioxidant activity and the Folin–Ciocalteu assay, respectively. These reagents were purchased from Sigma (St. Louis, MO, USA).

2.1.2. Plant material

Grape pomace was obtained from a commercial winery (Felsengartenkellerei Besigheim, Hessigheim, Germany). Seeds from the pomace of five different red grape cultivars (vintage 2006) were used for polyphenol analysis and oil production, respectively ('Cabernet Mitos', 'Lemberger', 'Samtrot', 'Spätburgunder'). The red wines were produced using high-temperature short-time treatment of the mash and subsequent enzymatic degradation of grape pectins. Musts were obtained using a screw extrusion press. Additionally, seeds of 'Schwarzriesling' (rosé wine production) and two pomace samples from white wine production ('Kerner', 'Müller-Thurgau'; vintage 2006) originating from the same process but without mash heating were included in the study. Pomace samples were collected after pressing the mash, sealed in polyethylene bags and kept at −20 °C.

2.2. Methods

2.2.1. Grape seed oil production

Frozen grape pomace was manually separated into skins and seeds using a sieve (mesh size 5.6 mm). The seeds were sealed in polyethylene bags *in vacuo* and kept at −20 °C until analysed. Prior to oil production, the seeds were dried in a cabinet drier for 8 h (60 °C) and pressed using a screw extrusion press (KOMET S 87 G, IBG Monforts, Mönchengladbach, Germany). Grape seed pressing was performed without heating, however, temperature increased to 60–68 °C due to dissipation of mechanical energy. The resulting press residues were cooled, sealed in polyethylene bags *in vacuo* and kept at −20 °C until analysed.

2.2.2. Determination of total lipid contents

Oil contents of the seed samples and press residues were determined after acid hydrolysis of matrix components and Soxhlet extraction with petroleum ether ('Weibull-Stoldt'; Matissek, Schnepel, & Steiner, 1992) using a Soxtherm 2000 automated extraction equipment (Gerhardt, Bonn, Germany; Matthäus & Brühl, 2001).

2.2.3. Extraction of phenolic compounds

For the extraction of phenolic compounds, the integral seeds and the press residues originating from oil production were lyophilised and finely ground using an S 1/2 ball mill (Retsch, Haan, Germany). Aliquots of 5 g of the pulverised samples were extracted and individual phenolic compounds were determined according to a previously published method (Kammerer, Claus, Carle, & Schieber, 2004). Before the identification and quantification of phenolic compounds by HPLC and the determination of total phenolic contents (Folin–Ciocalteu assay) and of the antioxidant activity using TEAC and FRAP assays (see below), the crude extracts were fractionated using RP18 Sep-Pak cartridges. Briefly, 5 mL of the polyphenolic crude extracts were adjusted to pH 7 and applied to the preconditioned cartridges. Phenolic acids were eluted with 10 mL of deionized water and 10 mL of 0.01% HCl (*v*/*v*). Subsequently, flavonoids were recovered with 20 mL of ethyl acetate (Kammerer et al., 2004).

For assessing the effects of the solvent composition on extraction yields and the phenolic profile of the extracts, the press residue of 'Lemberger' seeds was extracted using ethanol, a mixture of ethanol and water (3:1; *v:v*), bidistilled water (75 °C), and methanol/0.1% HCl (*v:v*), respectively. Aliquots of 5 g of the pulverised samples were weighed into Erlenmeyer flasks and extracted with 100 mL of the aforementioned solvents for 2 h under stirring after flushing with nitrogen in order to prevent oxidation. The extracts were centrifuged (10 min, 5366*g*), and solids were re-extracted with 100 mL of the respective solvent (60 min).

2.2.4. Determination of individual phenolic compounds by HPLC

2.2.4.1. HPLC–DAD system. The determination of phenolic compounds was performed using an Agilent HPLC series 1100 (Agilent, Waldbronn, Germany), equipped with Chemstation software, a model G1322A degasser, a model G1312A binary gradient pump, a model G1329/G1330A thermoautosampler, a model G1316A column oven, and a model G1315A diode array detector. The separation was carried out with a Phenomenex (Torrance, CA, USA) Aqua C18 column (250 × 4.6 mm i.d.; 5 μm particle size) with a C18 ODS guard column (4.0 × 3.0 mm i.d.) operated at 25 °C. UV–Vis spectra were recorded in the range of 200–600 nm at a spectral acquisition rate of 1.25 scans/s (peak width 0.2 min). A mobile phase consisting of water, acetic acid, and acetonitrile was employed for the determination of phenolic acids and flavonoids as previously described (Kammerer et al., 2004). The injection volume for all samples was 10 μL. Simultaneous monitoring was performed at 280 nm (hydroxybenzoic acids, flavanols), 320 nm (hydroxycinnamic acids) and 370 nm (flavonols), respectively, at a flow rate of 1.0 mL/min.

2.2.4.2. HPLC–MS^n system. For peak assignment, polyphenols were analysed with the HPLC system described above coupled on-line to a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an electrospray ionisation (ESI) source. Data acquisition and processing were performed using Esquire Control software. Mass spectra were recorded in the negative ion mode. Mass spectrometric conditions were applied as previously reported (Kammerer et al., 2004).

2.2.4.3. Quantification of individual phenolic compounds in the seeds and press residues. Individual polyphenols were quantified using a calibration curve of the corresponding standard compound. In case of lacking reference components, the calibration of structurally related substances was used including a molecular weight correction factor (Chandra, Rana, & Li, 2001). The yields of the target compounds were calculated based on total amounts of the respective compounds in grape pomace, which were determined after extraction with methanol/0.1% HCl (*v:v*; Kammerer et al., 2004).

2.2.4.4. Quantification of individual phenolic compounds in grape seed oil. The phenolic content of 'Lemberger' grape seed oil, produced as described under 2.2.1, was determined according to Pour Nikfardjam (2001). Aliquots of 20 g of the grape seed oil were weighed