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Phenolic composition of grape pomace skin of four grape cultivars

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Abstract
Grape skins are a potential source of phenolics that can be used in the food, cosmetic and pharmaceutical industries. The phenolic composition of grape pomace skin, a by-product of winemaking, was investigated. The air-dried pomace skin of four grape cultivars, Carignan, Merlot, Cabernet Sauvignon, and Syrah, was studied. Carignan skin had the highest concentrations of caftaric acid (11.96 ± 3.99 μg/g DW), rutin (9.23 ± 1.15 μg/g DW), quercetin 3-glucoside (97.76 ± 10.00 μg/g DW), kaempferol 3-glucoside (82.23 ± 33.22 μg/g DW) and resveratrol (4.83 ± 2.02 μg/g DW). Syrah skin had the highest concentration of gallic acid (36.33 ± 1.75 μg/g DW) while Cabernet Sauvignon skin had the highest concentration of catechin (12.83 ± 2.20 μg/g DW).

Keywords: Grape skins, Pomace, Phenolics, Carignan, merlot, cabernet sauvignon, syrah

Introduction
Grapes are the world's largest fruit crop with an annual production of 63.85 million metric tons in 2014 (Europe – 29.19, Asia – 16.63, Americas – 12.84, Africa – 3.54, Oceania – 1.65; www.statista.com). About 80% of the grapes produced are used in winemaking [1, 2]. Grape pomace, a by-product of winemaking, consists of pressed skins, seeds and stems which account for about 20% of the weight of the grape [1, 3]. Therefore, approximately 10 million tons of pomace are available annually from wineries. Disposal of winery by-products can have adverse environmental effects due to the presence of relatively high levels of phenolic compounds [4]. Phenolic compounds can inhibit germination when used as soil conditioners or fertilizers and can cause detrimental effects on plants and animals due to the increased biochemical and chemical oxygen demands. However, phenolic compounds have potentially positive effects on human health such as inhibition of the oxidation of low-density lipoprotein *in vitro* [5, 6]. Grape and wine phenolics have anti-inflammatory [7], anti-ischemic [8], anti-obesity [9, 10], and anti-platelet aggregating effects and other potentially disease preventing cellular actions [11, 12, 13]. Phenolic compounds have also received attention for their potential use as food antioxidants [14].
Recovery of the phenolic compounds as value-added byproducts would be a possible solution to the environmental problems posed by large quantities of grape pomace generated annually. Extraction of phenolics during winemaking is an incomplete process, typically only reaching about 30 to 40%, depending on the grape variety, vineyard location and winemaking parameters such as destemming, temperature, use of enzymes, crushing, maceration and pressing [15, 16, 17]. Cabernet Sauvignon and Merlot are among the world's most widely recognized red grape cultivars. Though some phenolics are lost through transfer from skin to wine during the winemaking process and the possible loss of some phenolics by oxidation during the air-drying process, skins are still a potential source of phenolics.
The objective of this study was to elucidate the phenolic composition of grape pomace skins from the four red wine cultivars, Carignan, Merlot, Cabernet Sauvignon, and Syrah, obtained as by-products of winemaking.

2. Materials and methods
2.1 Materials
HPLC grade acetonitrile and formic acid (88% ACS reagent grade) was purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A). Purified water was obtained from a Milli-Q Plus water purification system (Millipore, now MilliporeSigma, Burlington, MA, U.S.A.). Standards used for HPLC including gallic acid, caftaric acid, (+)-catechin, (-)-epicatechin, rutin hydrate, quercetin 3-glucoside, kaempferol 3-glucoside and resveratrol were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.).

2.2 Grape skin powder
Grape pomace skin powders were processed in 2015 from grape cultivars: Carignan, Merlot, Cabernet Sauvignon and Syrah of *Vitis vinifera* from northern Tunisia (Grombalia: forty kilometers south-east of Tunis). Waste winemaking pomace was collected from Cave Viticole Bou-Argoub cooperative winery (Bou-Argoub). Skin was obtained by manually removing seeds. Skin was then air dried and ground with a coffee grinder (FP 3121 Moulinex, Groupe SEB (Société d'Emboutissage de Bourgogne), Écully, France) until a fine powder was obtained.

2.3 Grape pomace skin powder extraction
Five hundred mg of air-dried grape pomace skin powder (four cultivars: Carignan, Merlot, Cabernet Sauvignon and Syrah) was placed in a screw test tube. Three mL of 0.01% HCl in MeOH/EtOH (8:2 v/v) was added and the mixture was

sonicated for 10 min in a Branson 8200 ultrasonic cleaner (Branson Ultrasonics Corp., Danbury, CT, U.S.A.). After 20 min., the mixture was sonicated for another additional 10 min. The sample was stored in a refrigerator overnight. The mixture was centrifuged and the sample was extracted two times with 3 mL of the same solvent (total of three extractions). The combined extract was evaporated under a stream of nitrogen. The residue was dissolved in 2 mL of 8:2 MeOH/water (v/v) and stored in the refrigerator until HPLC analysis.

2.4 HPLC analysis

HPLC characterization of phenolic compounds was performed using a Hewlett-Packard 1100 Series HPLC system consisting of an 1100 Series quaternary pump, an 1100 Series vacuum degasser, a manual injector (model 7725i, Rheodyne, now IDEX Health & Science LLC, Rohnert Park, CA, U.S.A.), equipped with a 20 µL sample loop, an 1100 Series thermostated column compartment, and an 1100 Series diode array detector. The instrument was controlled and data were processed by an HP ChemStation for LC 3D (Rev. A.08.03 [847]). Analyses were conducted at 30 °C using a 250 x 4.6 mm i.d. Gemini C18, 110 Å (Phenomenex, Torrance, CA, U.S.A.) column (5 µm particle size) fitted with a C18 guard column. Elution was performed using mobile phase A (5% formic acid in water) and mobile phase B (5% formic acid in acetonitrile). The gradient system was 0-3 min, 5% B; 3-15 min, 9% B; 15-22 min, 13.5% B; 22-42 min, 18.5% B; 42-48 min, 18.5% B; 48-51 min, 22.5% B; 51-55 min, 22.5% B; 55-56 min, 30% B; 56-57 min, 40% B; 57-58 min, 5% B. The flow rate was 1 mL/min, and the detection wavelength was set at 280, 306, 320 and 370 nm. The injection volume was 20 µL. Quantification of phenolic compounds was performed by constructing standard curves of individual phenolic standards. Values were reported as µg/g dry weight (DW) ± SD and represent the average of three independent analyses.

3. Results and Discussion

The identification of phenolic compounds in grape pomace skin powders was determined by retention time, UV spectra, and spiking extracts with phenolic standards. Quantification of the phenolic compounds was performed by constructing standard curves of the phenolic compound standards: gallic acid, caftaric acid, (+)-catechin, (-)-epicatechin, rutin hydrate, quercetin 3-glucoside, kaempferol 3-glucoside and resveratrol. Quercetin and kaempferol glucosides and glucuronides, gallic acid and its glucosides, resveratrol, caftaric and coutaric acids are typical monomeric phenols reported in grape skins [18, 19]. The concentration of gallic acid ranged from 10.16 ± 1.04 µg/g DW (Merlot) to 36.33 ± 2.45 µg/g DW (Syrah). Obreque-Slier *et al.* [20] reported a concentration of 3.1 ± 0.2 µg/g FW of gallic acid was in Cabernet Sauvignon skins at harvest time. A previous study found 30 µg/g dm of gallic acid in Merlot skins [21]. The concentration of caftaric acid ranged from not detectable (Merlot) to 11.96 ± 3.99 µg/g DW (Carignan). Previous studies found caftaric acid concentrations in Cabernet Sauvignon skins ranging from 0.5 µg/g FW (at harvest time [21]) to 9.5 µg/g FW in Cabernet Sauvignon skins [22]. Rodríguez Montealegre and co-workers [22] observed higher concentrations of *trans*-caftaric acid in white grape skins compared to red grape skins. Substantially higher concentrations of caftaric acid (ranging from 153. 5 µg/g DW to 598.7 µg/g DW) were reported in sixteen white raisin grape cultivars and selections [23]. The concentration of phenolics can vary by location, climate, agricultural practices, stages of ripeness, postharvest handling and extraction methods. Cabernet Sauvignon skin had the highest concentration of catechin (12.83 ± 2.02 µg/g) though epicatechin was not detected. Catechin concentrations ranging from 8 µg/g to 33 µg/g DW and epicatechin concentrations ranging from 3 µg/g to 13 µg/g DW were reported in Cabernet Sauvignon skins [24]. Catechin and epicatechin were not detected in Merlot skin. Our results are in contrast to those of Yilmaz and Toledo [21] who reported catechin and epicatechin in Merlot skin at concentrations of 16 mg/100 g dm) and 13 mg/100 g dm, respectively, and Lorrain *et al.* [24] who found concentrations of catechin ranging from 17 µg/g to 53 µg/g DW and epicatechin ranging from 8 µg/g to 55 µg/g DW in Merlot skin from seven Bordeaux vineyards. Much higher levels of catechin and epicatechin ranging from 400 to 600 µg/g DW and 200 to 300 µg/g DW, respectively, were reported in Syrah pomace skin [25]. High concentrations of catechin (400 µg/g DW) and epicatechin (200 µg/g DW) were found in Carignan Noir pomace skin [25]. The low levels of catechin and non-detection of epicatechin in our samples may be the result of losses during the vinification process. Ky *et al.* [25] found that vinification process removed more than 65% of the monomeric flavan-3-ols from grape skin with catechin levels being especially affected. The same researchers also reported that Syrah and Carignan Noir skins were the most extracted varieties (as a result of vinification), retaining less than 10% of monomers and dimers. Experimental data suggest that catechins might prevent chronic diseases in humans. Teissedre and co-workers [6] reported that catechin and epicatechin are powerful inhibitors of *in vitro* human LDL oxidation. A strong inverse association between intake of (+)-catechin and (-)-epicatechin and death resulting from coronary heart disease has been shown [26]. Another study concluded that catechins, whether from tea or other sources, might reduce the risk of ischemic heart disease mortality [27]. Rutin was only detected in Carignan skins (9.23 ± 1.15 µg/g DW). Supplementation of female humans with rutin for 6 weeks led to significantly elevated plasma levels of quercetin, kaempferol and isorhamnetin [28]. This was probably due to the deglycosylation of rutin and further metabolism of quercetin. Quercetin 3-glucoside concentrations ranged from 14.16 ± 2.17 µg/g DW; Cabernet Sauvignon) to 97.76 ± 10.00 µg/g DW; Carignan). Quercetin glucoside concentrations of 48 ± 20.4 µg/g FW and 31 ± 13.5 µg/g FW in were reported in Cabernet Sauvignon skins and Merlot skins, respectively [22]. Obreque-Siler and co-workers [20] found a quercetin 3-glucoside concentration of 13.4 ± 3.8 µg/g FW in ripe Cabernet Sauvignon skins. Kaempferol 3-glucoside was only detected in Carignan skins (82.23 ± 33.52 µg/g DW). Rodríguez Montealegre *et al.* [22] found kaempferol glucoside concentrations of 13 ± 5.4 µg/g FW and 8.0 ± 2.93 µg/g FW in Cabernet Sauvignon skins and Merlot skins, respectively. Kaempferol 3-glucoside (0.9 ± 0.5 µg/g FW) had been

previously reported in ripe Cabernet Sauvignon skins [20]. With flavonoids, generally, the more OH substitutions, the stronger the $ORAC_{ROO^{\bullet}}$ activity [29]. Kaempferol and quercetin which have four and five OH substitutions, respectively, have $ORAC_{ROO^{\bullet}}$ absorbing activities of 2.7 and 3.3 (μM Trolox equivalents/μM sample), respectively [30]. Quercetin has shown to possess antioxidant, anti-inflammatory, anti-proliferative, and gene expression changing capacities *in vitro* [31]. A study with human ileostomy volunteers showed that quercetin glycosides are more efficiently absorbed than quercetin in the small intestine (52% vs. 24%, respectively [32]). The authors concluded that humans absorb appreciable amounts of quercetin and that absorption is enhanced by glucosylation. They also found quercetin glycosides are absorbed moderately rapidly in humans and are eliminated slowly throughout the day (elimination half-life of 17 h [33]. The researchers concluded that quercetin could contribute significantly to the antioxidant defenses present in blood plasma. In a study assessing the individual flavonoid intake with chronic disease, high kaempferol intake was significantly correlated to a reduced risk of cerebrovascular disease (relative risk 0.7, $P = 0.003$ [34]). DuPont *et al.* [35] observed that kaempferol is adsorbed more efficiently than quercetin in humans even at low oral dose. The concentration of resveratrol ranged from not detectable (Cabernet Sauvignon) to 4.83 ± 2.02 μg/g DW (Carignan). Resveratrol has been reported in Cabernet Sauvignon skins in concentrations ranging from 19.35 to 39.38 μg/g DW [36]. Resveratrol has diverse and physiological actions, including anti-inflammatory, anti-oxidant, anti-proliferation and promotion of differentiation, and chemopreventative effects [37]. Based on a review of human clinical trials, Smoliga *et al.* [38] stated that resveratrol has considerable potential to improve health and prevent chronic disease in humans.

Table 1: Content of phenolic compounds (μg/g DW) in freeze-dried grape skin powders.

Sample	gallic, acid	callakic., acid		wiratechin	aktia	quercetin	bempfersa 3-glub
Carignan	21.43 ± 8.71	11.96 ± 3.99	9.36 ± 0.32	nd	9.23 ± 1.15	97.76 ± 10.00	82.23 ± 33.52
Nlerlot	10.16= 1.04	nd	nd	nd	nd	66.44 ± 26.42	2.70 ± 0.72
Cabernet Sauvignon	33.90 ± 2.45	7.83±0.05	12.83 ± 2.02	nd	nd	14.16 ± 2.17	as
Syrah	36.33 = 1.75	7.83 ± 0.95	8.93 ± 0.05	nd	nd	16.36 ± 1.72	ad,

4. Conclusions

The skins of four grape varieties, Carignan, Merlot, Cabernet Sauvignon, and Syrah produced during winemaking were studied for their phenolic composition. Knowledge of the identity and individual concentrations of phenolic constituents is essential for the utilization of grape pomace by pharmaceutical, cosmetic and food industries. Carignan skins had the highest concentrations of caftaric acid, rutin, quercetin 3-glucoside, kaempferol 3-glucoside and resveratrol and appeared to be the most promising source of phenolics in the study. The use of Carignan pomace as a source of phenolic constituents merits further investigation.

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