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Elucidation of Phenolic Profiling of *cv*. Antep Karasi Grapes using LC-DAD-ESI-MS/MS

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Abstract

Phenolic compounds are a large and complex group of chemical constituents found in red grapes and wines, which not only affect their quality, but also contribute to their beneficial health effects. Phenolic composition of *cv*. Antep Karasi grapes (*Vitis vinifera* L.), a native grape variety grown in Turkey, were investigated. Investigation of phenolic compounds were performed by LCDAD-ESI-MS/MS. A total of twelve anthocyanins including, six flavanols, eight phenolic acids and six flavonols were identified and quantified. Among all anthocyanins, malvidin-3-glucoside was the most dominant in Antep karasi grapes, as it accounted for the largest proportion of the total anthocyanin compounds (~50%). Six flavanols; (+)-catechin, (-)-epicatechin, procyanidin B1, procyanidin B2, procyanidin B3 and procyanidin B4 were detected in wines as phenolic compounds. Trans-caftaric acid and trans-coutaric acid were the most dominant phenolic acids in Antep karası grapes, as they accounted for the largest proportion of the total phenolic acids contents. Myricetin-3-Oglucos ide and its aglycone form were the most dominant flavonol in grapes. The phenolic compounds of Antep karasi showed that this variety can be a potential functional; however, further studies should be carried out to evaluate its pharmaceutical efficiencies.

Keywords: Antep karasi, grape phenolics compounds, LC-MS/MS, anthocyanins

1. INTRODUCTION

In recent years, with the increasing importance given to healthy nutrition, called also as nutritionism trend, foods rich in nutritional value and bioactive compounds have gained significant popularity. In this period, along with the changed nutritional habits, the food industry turned to products with high concentration of bioactive compounds, or secondary metabolites, such as phenolic compounds, which have beneficial effects to human health and preventive properties to the serious diseases with their high antioxidant potential. Previous studies have emphasized many times that the higher intake of phenolic rich fruits and vegetables results in lower incident of degenerative diseases such as cardiovascular diseases, several cancers, age-related disorders and some other health problems (Bulotta et al., 2014; Jediyi et al., 2019). Therefore, bioactive compounds, especially plant polyphenols, drawing a great interest owing to their remarkable phenolic potential. Apart from important health effects, these precious compounds highly affect the organoleptic characteristics and so consumer preferences of a food product. Fruits like berries are known to be good source of nutrients and rich bioactive content, responsible for critical functions in human body, therefore play a crucial role in healthy diet. Among berries, grape (*Vitis vinifera* L.) is a prominent and popular fruit actively used in food sector especially in wine and juice production for its unique aroma, taste and ample bioactive compounds (Anastasiadi et al, 2010). Grape is one of the most cultivated and consumed berry fruit as fresh, dried, juice, vinegar, wine and etc. and its production reaches up to 70

million metric tons annually all over the world (Nowshehri et al., 2015). This unique fruit crop contains important vitamins, minerals, lipids, proteins, carbohydrates. Moreover, its seeds, seed oil, flesh and peels possess remarkable amount of polyphenols and can be divided into three main groups, phenolic acids, flavonoids and proanthocyanidins (Peixoto et al., 2018).The skin of the grape possesses a high amount of anthocyanins, in particular malvidin-3-glucoside (Kelebek et al., 2007). Phenolic profile of a grape berry is significantly affected by changes in grape varieties, climatic conditions, soil and canopy management (Downey et al., 2006; Flamini et al., 2013). Over time, apart from the effect of natural conditions on fruit crop phenolics, extensive literature has developed on the methods constituted for the extraction of phenolic compounds from plant materials. Each method used for this purpose showed that their several advantages. For instance, the extraction procedure is essential for the precise identification and quantification of phenolic compounds and to obtain antioxidant capacity accurately as because the structure of phenols are too sensitive in case of high temperatures and other extreme conditions (Naczk and Shahidi, 2006). Besides, with the recent development of supersensitive analytical methods such as LC-DAD-ESI-MS/MS, it became able to perform a comprehensive characterisation of grape phenolics.

As the phenolic compounds are important contributors for human health, especially their anti-cancer, anti-inflammatory, cytotoxic and anti-viral effects in human body. Previous studies have almost exclusively focused on phenolic profiling and antioxidant capacity of common varieties and their health benefits (Dani et al., 2010). However, phenolic composition combined with other bioactive components and so the health benefits of a grape berry may alter significantly with respect to grape variety. Turkey is one of the spearheading countries for the table grape and raisin production. Among some other important local grape varieties in Turkey, cv. Antep Karasi is one of the most cultivated and consumed variety all over the country. The phenolic composition and antioxidant capacities of *cv*. Antep Karasi and *cv*. Besni Karasi (*Vitis vinifera* L.) sun dried raisins was successfully established as described by Kelebek et al., 2013. Researchers were found that the raisins of cv. Antep Karasi possessed a high amount of flavan-3-ols, phenolic acids and flavonols.

To our knowledge, no prior studies have examined the phenolic profile of fresh grapes of *cv*. Antep Karasi (*Vitis vinifera* L.) by means of LC-DAD-ESI-MS/MS. Therefore, the present study was aimed to elucidate the phenolics of cv. Antep Karasi grapes by means of LC-DAD-ESI-MS/MS.

2. MATERIALS AND METHODS

2.1. Materials

Sound grapes from Antep Karasi cultivars were manually harvested (10 kg) at optimum maturity in the 2019 vintage in Adıyaman province and transported to the Food Engineering Department, Nevsehir Haci Bektas Veli University located in Nevsehir province, Turkey.

2.2. Chemicals

Solvents like methanol, acetonitrile and formic acid HPLC-grade were bought from Riedel–deHaen (Switzerland). All of ther reagents used in the study were of analytical grade. Ultrapure water generated by the MilliQ system (Millipore, Bedford, Massachusetts, USA) was used. All of the standard phenolic compounds were purchased from Sigma-Aldrich (Steinheim, Germany).

2.3. Extraction and analysis of phenolics by LC-DAD-ESI-MS/MS

The extraction methodology was slightly modified from an earlier study of Kelebek et al. (2013). Freshly squeezed juices of Antep Karasi grapes were centrifuged at 4000 rpm in a centrifuge (Eppendorf 3810 R, Hamburg, Germany) for 20 min, the supernatant were then subjected to 0.45 μm membrane filters and were kept at -18 °C prior to the analysis.

Table 1. Retention Times (Rt), family, wavelength for detection (DAD), and mass spectral data for analyses of phenolic compounds in grapes using LC-DAD-ESI-MSn detection

No	Anthocyanins	RT (min)	UV-Vis (nm)	[M⁺]	Mass loss [M⁺]-MS_2	[MS2]	Content (mg/L)
1	Delphinidin-3-*O*-glucoside	15.86	277, 298(sh), 346, 440(sh), 524	465	- 162	303	9,81±0,21
2	Cyanidin-3-glucoside	17.45	280, 292(sh), 325(sh), 380(sh), 440(sh), 517	449	- 162	287	1,75±0,04
3	Petunidin-3-glucoside	19.09	276, 298(sh), 348, 440(sh), 527	479	- 162	317	10,95±0,24
4	Peonidin-3-glucoside	20.74	280, 292(sh), 325(sh), 380(sh), 440(sh), 518	463	- 162	301	9,46±0,20
5	Malvidin-3-glucoside	21.58	276, 298(sh), 348, 440(sh), 528	493	- 162	331	77,18±1,66
6	Delphinidin-3-*O*-acetylglucoside	27.12	280, 298(sh), 346, 440(sh), 526	507	- 204	303	2,27±0,05
7	Cyanidin-3-*O*-acetylglucoside	31.28	283, 313, 440(sh), 522	491	- 204	287	1,24±0,03
8	Petunidin-3-*O*-acetylglucoside	33.19	269, 298(sh), 348, 440(sh), 528	521	- 204	317	1,92±0,04
9	Peonidin-3-*O*-acetylglucoside	37.58	280, 292(sh), 325(sh), 380(sh), 440(sh), 529	505	- 204	301	1,53±0,03
10	Malvidin-3-*O*-acetylglucoside	38.59	277, 298(sh), 348, 440(sh), 529	535	- 204	331	10,89±0,23
11	Delphinidin-3-*O*-*p*-coumaroylglucoside	46.41	282, 298(sh), 316(sh), 440(sh), 530	611	- 308	303	1,46±0,03
12	Petunidin-3-*O*-*p*-coumaroylglucoside	49.47	282, 298(sh), 316(sh), 440(sh), 531	625	- 308	317	0,83±0,02
13	Peonidin-3-*O*-*p*-coumaroylglucoside	50.60	283, 313, 440 (sh), 521	609	- 308	301	0,95±0,02
14	Malvidin-3-*O*-*p*-coumaroylglucoside	50.71	283, 298(sh), 316(sh), 440(sh), 532	639	- 308	331	6,06±0,13
						Total	136,30±2,94

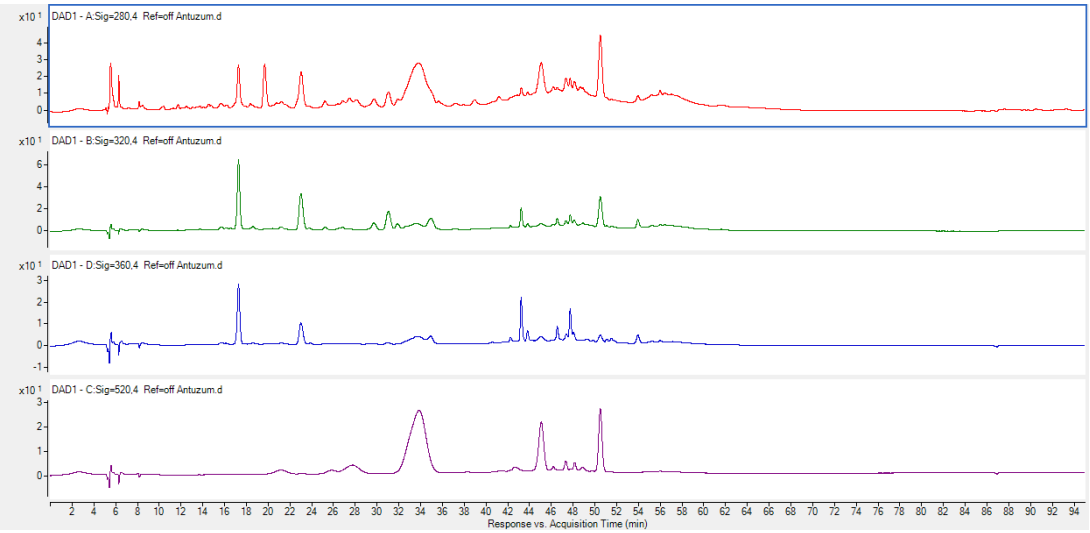
Eluted extracts then injected on an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto CA-USA), equipped with a quaternary pump, a diode-array detector, an automatic injector, and the ChemStation software. The column used for the phenol analysis was a reversed-phase Phenomenex C-18 column (4.6 mm x 250 mm, 3.5 m; Torrance, CA). The mobile phase consisted of two solvents: solvent A, water/formic acid (99:1; v/v) and solvent B, acetonitrile/solvent A (60:40; v/v). Phenolic com-pounds were eluted under the following conditions: 0.5 mL min-1flow rate and the temperature was set at 25°C, isocratic conditions from 0 to 10 min with 0% B, gradient conditions from 0% to 5% B in 30 min, from 5% to 15% B in 18 min, from 15% to 25% B in 14 min, from 25% to 50% B in 31 min, from 50% to 100%Bin three minutes, followed by washing and reconditioning the column. The DAD was set at 280, 320, and 360 nm for real-time monitoring of the peak intensity, and full spectra (190–650 nm) were continuously recorded for the identification of the components. The identification and assignation of each compounds was performed by comparing their UV spectra to authentic standards, retention times and also approved by an LC-MS/MS spectrometer (Agilent 6430) conducted with an electrospray ionization source. The electrospray ionization mass spectrometry detection was per-formed in negative mode with the following optimized parameters: capillary temperature 400°C; drying gas; nebulizer pressure, 45 psi. Data acquisition was performed using multiple reaction monitoring (MRM) that only monitors specific mass transitions during pre-set retention times. The quantification of individual compounds was calculated with a calibration curve of the standard compound (Kelebek et al., 2011)

3. RESULTS AND DISCUSSION

LC-DAD-ESI-MS/MS data including retention time, molecular ion, main fragment ions and tentative compound identification are shown in Tables 1 and 2 while Figure 1 displays the HPLC-DAD chromatograms of the phenolic compounds identified in Antep karasi grape samples. In the present study fourteen anthocyanins were

detected and quantified by means of liquid chromatography equipped with MS/MS configuration. As shown in Table 1, malvidin-3-glucoside was the major antocyanin that comprised about 56% of total phenolic composition of Antep karasi grape juice which is in agreement with the general perception that malvidin-3-glucoside is the main anthocyanin in different grape cultivars (Benmeziane et al., 2016). Grape anthocyanins are mainly composed of five anthocyanidins, called malvidin, delphinidin, cyanidin, peonidin and petunidin. The variety has a great effect on phenolic profiles of grapes. For instance, non-vinifera species contain significant amounts of of 3,5-diglucosides while *Vitis vinifera* are mostly contain 3-monoglucosides. Malvidin is a well-known anthocyanidin that is delphinidin carrying methyl substituents at positions 3' and 5'. It has a number of different roles as a biological pigment or a metabolite. Apart from this precious compound, delphinidin-3-Oglucoside, petunidin-3-O-glucoside, peonidin-3-Oglucoside and malvidin-3-O-acetylglucoside were other anthocyanin compounds that were present in high amounts. Cyanidin-O-glucoside is among the minör anthocyanins in the Antep karasi cultivar. The amount of this compound was 1.75 mg/L. It is well known that cyanidin is a precursor to the other anthocyanidins and is converted into peonidin by the action of 3´-O methyltransferase or into delphinidin by the action of 3´-hydroxylase (Boss et al., 1996; Benmeziene et al., 2016). Distribution of these anthocyanin compounds have been associated with the alteration of grape phenolic composition during ripening stages of a grape berry. In the literature these compounds were mainly found in the grape skin and its concentration altered between varieties (Romero-Cascales et al. 2005). Total concentration of phenolic compounds were quantified as 163.7 mg/L. The six phenolic acids identified in the studied raisins were protocatechuic acid, cis-caftaric acid, trans-caftaric acid, cis-coutaric acid, transcoutaric acid, caffeic acid, ferulic acid, fertaric acid, and *p*-coumaric acid. With regard to the individual phenolic acids, it was noted that trans-caftaric acid, a well-known phenolic acid of grapes derives from the tartaric acid, was the major phenolic acid and it accounted for the largest proportion of the total phenolic acid contents. Breksa et al. (2010) examined 16 commercially important raisins and reported that trans-caftaric acid concentrations in the raisins ranged from 153 (A95-15) to 598 (Fiesta) mg/kg dry weight. With regard to flavanols, catechin, epicatechin and procyanidin B2 were found to be the major compound in Antep karasi grapes. Meng et al.(2011) reported that (+)-catechin was the major flavanol in raisins, varying from 17.5 to 544 μg/g of dry weight.

Figure 1. Total ion chromatogram of phenolic compounds



Breksa et al. (2010) reported that the (+)-catechin concentration in the raisins ranged

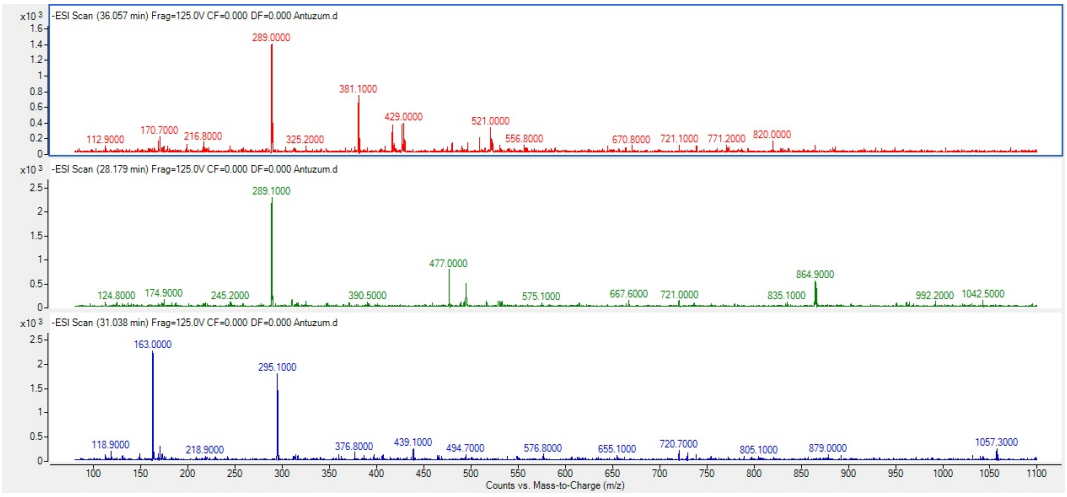
Table 2. Retention Times (Rt), family, wavelength for detection (DAD), and mass spectral data for analyses of phenolic compounds in vinegars using LC-DAD-ESI-MSn detection

No	Phenolic compounds	Rt (min)	Family	λ_{max} (nm)	MS, m/z M^-	MS/MS ions m/z	Content (mg/L)
1	Protocatechuic acid*	22,42	Phenolic acids	294, 256	153	109	0.23±0.02
2	*cis*- Caftaric acid	26,77	Phenolic acids	328	311	179, 149	3.21±0.32
3	*trans*- Caftaric acid	27,13	Phenolic acids	328	311	179, 149	56.70±2.32
4	*cis*- Coutaric acid	32,56	Phenolic acids	310	295	163	1.22±0.16
5	*trans*-Coutaric acid	33,16	Phenolic acids	310	295	163	15.46±0.55
6	Caffeic acid*	34,28	Phenolic acids	328	179	135	1.52±0.11
7	Ferulic acid*	35,9	Phenolic acids	293, 323	193	149,178,134	0.51±0.01
8	Fertaric acid	36,07	Phenolic acids	322	325	193,149	1.32±0.07
9	*p*-Coumaric acid*	43,28	Phenolic acids	310	163	119	0.47±0.04
10	Procyanidin B2*	30,19	Flavan-3-ols	265	577	289,245	35.27±1.08
11	Catechin*	28,59	Flavan-3-ols	277	289	245, 205, 179	28.29±1.62
12	Epicatechin*	34,78	Flavan-3-ols	277	289	245, 205, 179	11.26±1.80
13	Rutin (quercetin-3-rutinoside) *	41,12	Flavonols	255, 352	609	301,179,151	0.56±0.26
14	Quercetin-3-*O*-galactoside*	42,48	Flavonols	355	463	301	2.34±0.21
15	Luteolin-*O*-glucoside*	44,89	Flavones	255, 262, 347	447	285, 267, 251, 243	0.56±0.08
16	Isorhamnetin-3-*O*-rutinoside*	54,95	Flavonols	348	623	315	0.21±0.42
17	Isorhamnetin-3-*O*-glucoside*	56,09	Flavonols	354	477	315	3.76±0.12
18	Quercetin-3-*O*-rhamnoside*	46,41	Flavonols	348	447	301	0.82±0.34
						Total	163.71±9.52

*Identification confirmed by comparison with standards.

from 1.8 (Dovine) to 209 (A95-27) mg/kg. Procyanidin B2, a proanthocyanidin composed of two (-)-epicatechin molecules conjoint by a bond between positions 4 and 8' in a beta-configuration, was found as the second most abundant phenolic with the concentration of 35.3 mg/L. Catechin was another abundant phenolic compound of Antep Karasi grape juice extract with the concentration of 28.3 mg/L. *trans*-Coutaric acid and epicatechin were other remarkable compounds found in the sample with the concentrations of 15.5 and 11.3 mg/L.

Figure 2. Fragmentations of some phenolic compounds in cv. Antep karasi



4. CONCLUSIONS

Anthocyanins and phenolic compounds of the Antep Karasi grape (*Vitis vinifera* L.) have been

investigated by means of LC-DAD-ESI-MS/MS. According to the data revealed from this study, it is observed that the Antep Karasi grape variety has a remarkable potential when compared to some other red table grape varieties. The dominant anthocyanin compound was malvidin-3-glucoside and the most abundant phenolic compounds were the member of phenolic acids group. As the grape is a famous phenol-rich fruit, there are a number of papers focused on the phenolic characterisation of grapes in different aspects. However, in order to perform a comprehensive characterisation of grape phenolics, LC-DAD-ESI-MS/MS and nuclear magnetic resonance (NMR) technology can be used to have a complementary data. For the future studies, together with the complementary technologies, multidisciplinary works also required to monitorize the changes of anthocyanins and phenolic compounds during berry development and their transition into wine.

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