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Determination of Anthocyanins in Red Grape Juices Made From Different Varieties by HPLC

İlkay Türkmen Özen[1], Aziz Ekşi[2]

[1]Gumushane University, Faculty of Engineering and Natural Sciences, Baglarbasi, 29100, Gumushane,TURKEY
[2]European University of Lefke, Department of Gastronomy,Gemikonagi –Lefke , KKTC Mersin 10, TURKEY

Abstract*—This study was conducted to determine the anthocyanin profiles of red grape juice. As research material, twelve different red grape varieties which were collected from the main producing regions in Turkey and red grape juice samples made from them were analyzed. The anthocyanins peaks on HPLC-chromatograms in red grapes were identified as cyanidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside and petunidin-3-glucoside. According the results, the pre-dominant anthocyanins of red grape juice was malvidin-3-glucoside which was found between 21.77-277.54 mg/L. It was followed by peonidin-3-glucoside which was found between 3.05-74.26 mg/L and then cyanidin-3-glucoside which was found between 3.02-16.94 mg/L. Delphinidin-3-glucoside and petunidin-3-glucoside were not detected in most red grape juices.*

This work is important to chemical description of local grape varieties and selection of suitable raw material for fruit juice industry.

Keywords*— *cyanidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside, petunidin-3-glucoside.

I. INTRODUCTION

Anthocyanin is a term derived from Greek words "*anthos* (flower)" and "*kyanos* (blue)" (Mazza and Miniati 1994; Castaneda-Ovando 2009).Anthocyanins, which occur in tissues of plants including fruits, flowers, leaves, roots and give those tissues their distinctive coloursat a wide range including pink, red, purple and blue, is a natural water-soluble group of pigments(Gao at al. 1997; Costa et al. 2000; Blando et al. 2004; Cemeroğlu and Karadeniz 2004). Anthocyanins are chemically the glucosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrlium (flavylium cation)(Jackman and Smith 1992) (Fig. 1). They occur in glucoside form in cell cytoplasm and are composed of certain sugars and non-sugar (aglycone) substances. Aglycone part of anthocyanins is called as anthocyanidin(Acar 1998; Koca et al. 2006).

Total anthocyanin amount varies with fruit varieties. Total anthocyanin amount in strawberry is between 450-700 µg/g(Wrolstad et al. 1970), while it is determined as 267-688 mg/L in cherry juice(Erbaş and Cemeroğlu 1992),271-316 mg/L in pomegranate juice(Cemeroğluand Artık 1990) and 285 mg/kg in fresh leaves of Isparta rose(Velioğlu and Mazza 1991).

Anthocyanin distribution reflecting the amount of different fractions such as total anthocyanin amount also varies with fruit varieties. The researches show that dominant anthocyanins are Cy-3-glc and Cy-3-rut in blackberry(Barritt and Torre 1973),Pg-3-glc, Pg-3-gal and Cy-3-glc in strawberry(Belitz and Grosch 1992), Cy-3-glc, Dp-3-glc, Cy-3,5-diglc, Dp-3,5-diglc, Pg-3-glc and Pg-3,5-diglc in pomegranate(Du et al. 1975)andCy-3-rut, Cy-3-glc and Pn-3-rut in cherry (Montmorency)(Dekazos 1970).

One of the main fruits which researches on anthocyanin amount and profile are carried out is black grapes. The reason is that the distinctive and attractive colour of black grapes originates from anthocyanins. In addition, anthocyanins have influence on the taste of black grapes, grapes juice and wine(Mazza and Miniati 1994).

According to Fuleki and Babjak(Fuleki and Babjak 1986), total anthocyanin amount in different grapes varieties varies between 33-603mg/100g, while it is between5.5-105.5 mg/100g according to Lamikanra (1989).

With the implementation of HPLC method, the researches on anthocyanin profile become widespread(Fong et al. 1971; Wulf and Nagel 1978;Pomar et al. 2005). Anthocyanins in grapes are generally in 3-monoglucoside form. The ratio of 3-monoglucoside components including delphinidin,cyanidin, petunidin, peonidinandmalvidinin to total anthocyanins varies between 57.0-84.2% depending on the grapes varieties(Mazza and Miniati 1994). Dominant anthocyanin in grapes is malvidin-3-glucoside.

Many researches on anthocyanins in grapes and food products processed from grapes(Pomar et al. 2005; Bub et al. 2001;Garcia-Beneytez 2002;Bitsch et al. 2004;Revilla et al. 2001)as well as the impacts of anthocyanins on human health (Tsuda et al. 1994;Tamura and Yamagami 1994;Karaivanova et al. 1990;Kamei et al. 1995;Bridle and Timberlake 1997)have been carried out up to date. However, comprehensive anthocyanin profile of black grapes grown in Turkey has not been determined yet. The aim of this research is to determine anthocyanin distribution of grapes juice produced from different black

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grapes varieties in Turkey and to make a contribution to the understanding of the importance of grapes juice in diet.

II. MATERIAL AND METHOD

2.1. Materials

Research material is composed of twelve different grapes varieties. Grapes varieties, regions where they are grown and their processing dates are given in Table 1.

Each grapes varieties is first washed and then grape berries are separated from the stems. Later on, mash is produced by smashing grape berries by hand under laboratory conditions. Mash is first heated to 60 ºC in steam jacketed heater for enzymation application, then it is rapidly cooled to 55 ºC which is the optimum working temperature of mash enzyme and finally a dose of150 mL/ton mashenzyme (Pectinex Ultra Color) is added. Samples to which enzymation is applied are pressed in a laboratory-type press after 1 hour and grapes juice is produced.

2.2. Method

For anthocyanin profile analysis, the HPLC method defined by DrustandWrolstad (2001) is modified and applied.

2.3. Chemicals

Acetonitrile HPLC gradient (Sigma-Aldrich), o-fosforic acid (Sigma-Aldrich), methanol HPLC gradient (Sigma-Aldrich), ciyanidin-3-glucoside, peonidin-3-glucoside, delphinidin-3-glucocide, malvidin-3-glucoside, petunidin-3-glucoside (Sigma-Aldrich), HCl

2.4. Preparation of anthocyanin standards

First of all, 1000 ppm stock solution for each anthocyanin standard is prepared with ultra pure water including 0.1% HCl. For plotting anthocyanin standard curves, solutions at different concentrations are prepared from each stock solution by using 4% phosphoric acid and injected into HPLC device.

2.5. Extraction

50 grams from each grained grapes varieties are taken, homogenised with 100 mL methanol/HCl (98:2) in blender (WARING marka)for 1 minute and retained in the dark under 4 ºC for 24 hours. Samples are then centrifuged at 3500xg for 20 minutes. After liquid phase is separated, 100 mL methanol/HCl (98:2) is added to the residue, homogenised for 1 minute and centrifuged again. This process is repeated till a colourless residue is obtained. All liquid extracts are put together and the final volume is completed to appropriate volume with methanol/HCl (98:2). Sample at certain volume is taken from the extracts and metanol/HCl is evaporated in a rotating vacuum evaporator under 30 ºC. Remaining part is diluted with 4% phosphoric acid.

Samples to be used in analysis are filtered through 0.45 µm membrane filters and 20 µL filtrate is injected into HPLC device.

2.6. Chromatographic conditions

Solvent A : %4 Fosforic acide

Solvent B : %100 Acetonitrile

Flow rate : 1.0 mL/dk

Wavelenght : 520 nm

Linear gradient flow(Table 2):

Coloumn : Reverse phase C_{18} coloumn (250 x 4.6 mm, 5um)

Temperature : 30 ºC

Analysistime : 72 min.

2.7. Identification and Calculation

Acquired chromatograms are evaluated by means of Agilent Chemstationsoftware.

Primary peaks detected in chromatograms (Fig. 2) are identified by comparing them with the incidence time of standard substance of each anthocyanin. Anthocyanin amounts are calculated quantitatively by using equations derived from standard substance curves.

The HPLC chromatograms of anthocyanins in grape juices from grape varieties (only Köhnü, Öküzgözü and Papazkarası varities are given) are shown in Fig. 3 to 5.

III. RESULTS AND DISCUSSION

Anthocyanin profile of grapes juice samples and descriptive values for these data are given in Table 3 and Table 4 respectively.

According to the findings; dominant anthocyanin in grapes juice samples is determined as malvidin-3-glucoside with an average of 114.15±23.21 mg/L. It is also determined that the richest grapes juices in terms of malvidin-3-glucoside amount are produced from Syrah, Cabernet Sauvignon, CiminandÖküzgözüvarieties (277.54 mg/L, 208.32 mg/L, 196.27 mg/L and 170.81 mg/L respectively).

Following malvidin-3-glucoside, the second dominant anthocyanin fraction in grapes juice is determined as peonidin-3-glucoside with 25.63±6.86 mg/L. It is found that the richest grapes juices in terms of peonidin-3-glucoside amount are produced from Merlot, Syrah, Köhnü and Alicante varieties (74.26 mg/L, 66.54 mg/L, 36.08 mg/L and 30.05 mg/L respectively).

Average cyanidin-3-glucoside amount in all varietiesis determined as8.03±1.93 mg/L. Grapes juices produced from Cimin, Köhnüand Merlotvarieties are the richest juices in terms ofcyanidin-3-glucoside (16.94 mg/L, 15.82 mg/L and 9.04 mg/L respectively).

Delphinidin-3-glucoside and petunidin-3-glucoside are not detected in the samples.

Several research (Wulf and Nagel, 1978;Piergiovanni and Volonterio, 1981;Roggero et al., 1984)previously conducted also show that the dominant anthocyanin in grapes is malvidin-3-glucoside.

In accordance with the findings of this research, Nunez et al. (2004) reports in their research carried out for

Graciano, Tempranillo and Cabernet Sauvignon varieties that the dominant anthocyanin is malvidin-3-glucoside and the second dominant anthocyanin in Graciano grapes is peonidin-3-glucoside. Furthermore, according to the research carried out byHmamouchi et.al. (1995), it is determined that malvidin glucosides are the dominant anthocyanins in Alicante Bouschet, Cinsault, Grenache Noir andCarignanevarieties and the amounts ofdelphinidin, cyanidinandpetunidinglucosides are comparatively low.

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Table.1: The growth regions and processing dates of the red grape cultivars

Grape Variety	Growth Region	Processing date
Kalecik karası	Ankara	07.09.2012
Cabernet Sauvignon	İzmir	21.09.2012
Syrah	İzmir	21.09.2012
Alicante	İzmir	21.09.2012
Papazkarası	Tekirdağ	22.09.2012
Isabella	Ordu	25.09.2012
Horozkarası	Kilis	28.09.2012
Köhnü	Ankara	03.10.2012
Öküzgözü	Elazığ	8.10.2012
Boğazkere	Diyarbakır	05.10.2012
Merlot	İzmir	12.10.2012
Cimin	Erzincan	15.10.2012

Table.2: Linear gradiet flow of solvent A and B

Time (min.)	Solvent A (%)	Solvent B (%)
0	94	6
55	80	20
57	30	70
60	5	95
60.1	94	6
70	94	6

Table.3: Anthocyanin profiles of grape juices (mg/L)

Variety	Siyanidin-3-glucoside	Delphinidin-3-glucoside	Peonidin-3-glucoside	Malvidin-3-glucoside	Petunidin-3-glucoside
KalecikKarası	-	-	9.05	65.19	-
Cabernet Sauvignon	-	-	16.01	208.32	-
Syrah	6.17	-	66.54	277.54	-
Alicante	3.02	-	30.05	45.16	-
Papazkarası	-	-	5.52	63.57	-
Isabella	3.86	-	4.48	21.77	-
Horozkarası	-	-	8.17	40.98	-
Köhnü	15.82	-	36.08	110.72	-
Öküzgözü	4.29	-	29.17	170.81	-
Boğazkere	5.10	-	3.05	96.28	-
Merlot	9.04	-	74.26	73.22	-
Cimin	16.94	-	25.15	196.27	-

*calculationswere done accordingto15.9Brix.

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Table.4: Descriptivevaluesoftheanthocyaninfraction ofgrapejuice

Anthocyanin fraction (mg/L)	Minimum	Maximum	Mean	SE[b]	CV[c] (%)
Cyanidin-3-glucoside (N=8)[a]	3.02	16.94	8.03	1.93	68.15
Peonidin-3-glucoside (N=12)	3.05	74.26	25.63	6.86	92.74
Malvidin-3-glucoside (N=12)	21.77	277.54	114.15	23.21	70.44

[a]number of samples
[b]standard error of mean
[c]coefficient of variance

R^1, R^2, R^3 : H, OH, OCH_3
R : saccharine

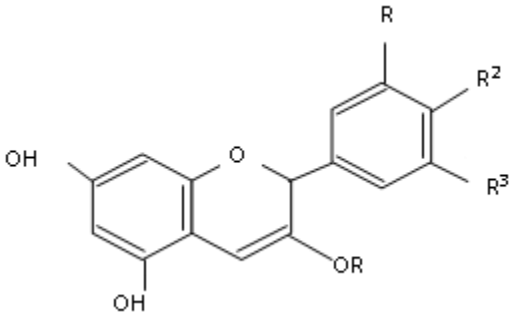

Fig. 1: Chemical structure of flavylium cation

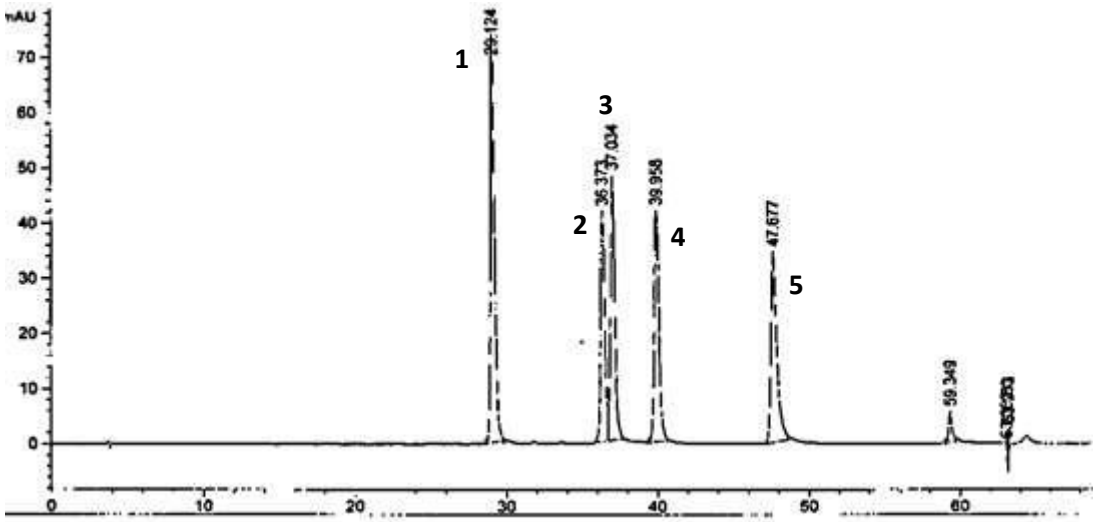

Fig. 2: HPLC chromatogram of standardsubstances of anthocyanin (1: cyanidin-3-glucoside, 2: delphinidin-3-glucoside, 3: peonidin-3-glicoside, 4: malvidin-3-glucoside, 5: petunidin-3-glucoside)

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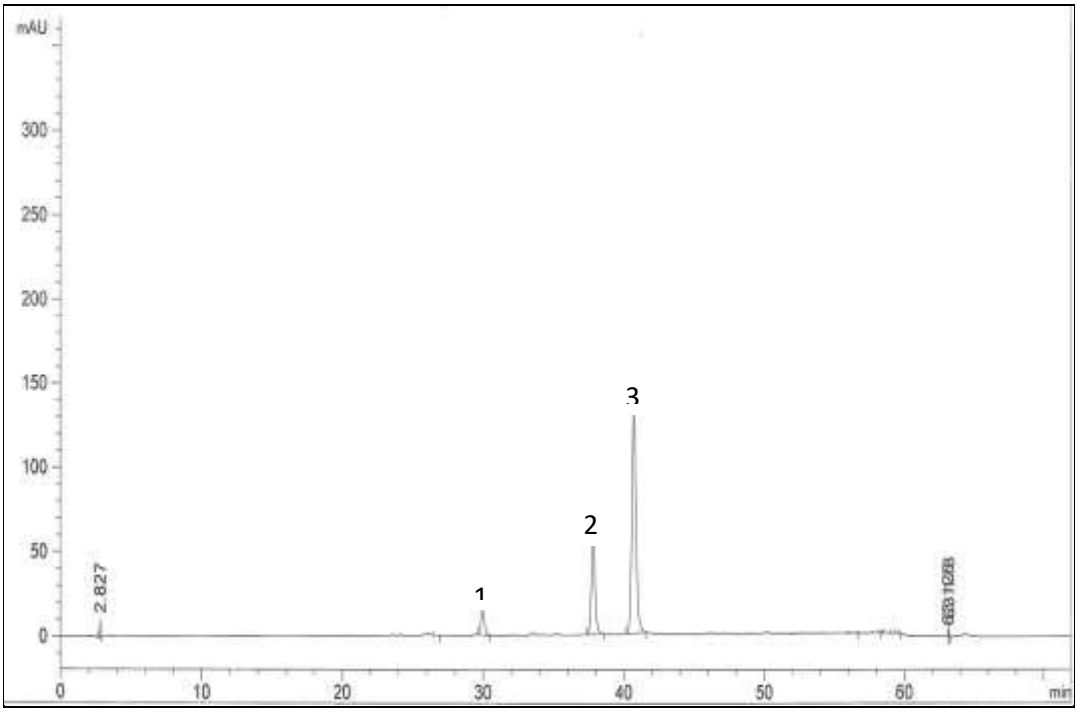
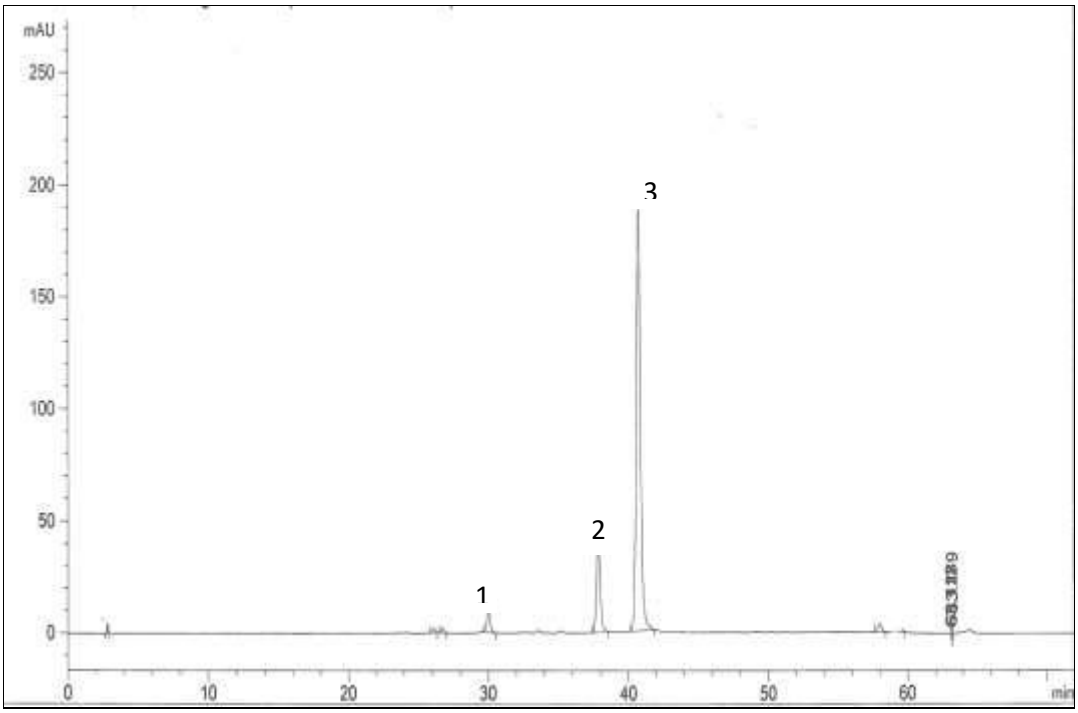


(1) Cyanidin-3-glucoside, (2) Peonidin-3-glucoside, (3) Malvidin-3-glucoside
Fig. 3: Anthocyanin chromatograph of grape juice from Köhnü variety



(1) Cyanidin-3-glucoside, (2) Peonidin-3-glucoside, (3) Malvidin-3-glucoside,
Fig. 4: Anthocyanin chromatograph of grape juice from Öküzgözü variety

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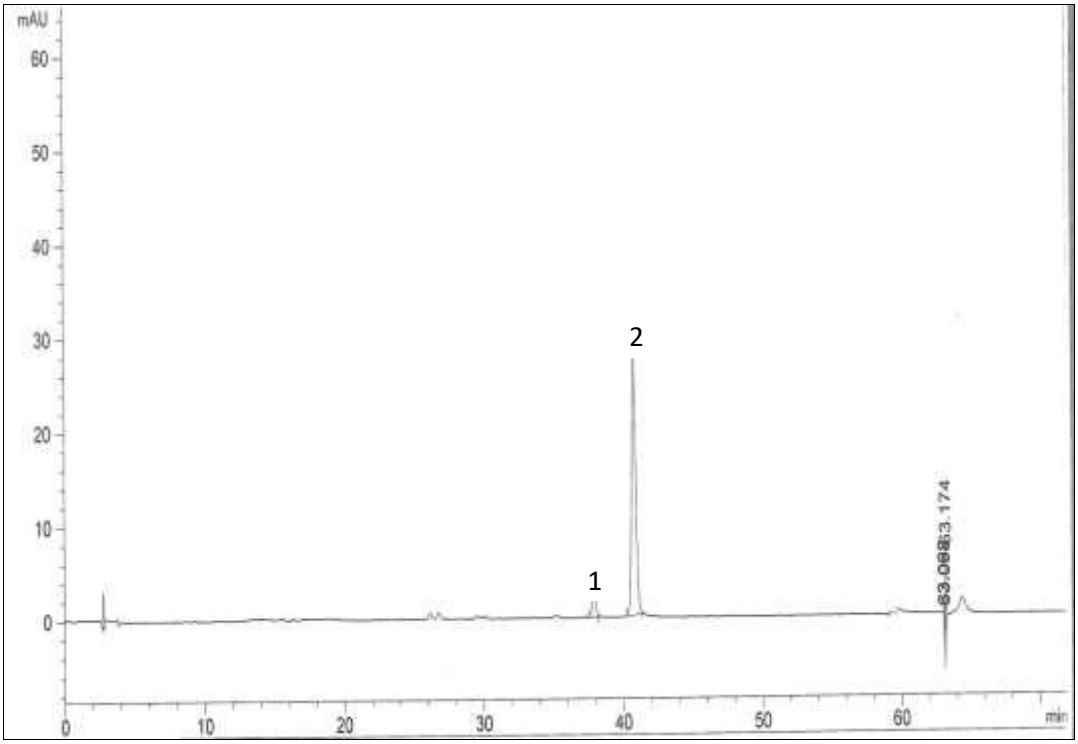

(1) Peonidin-3-glucoside, (2) Malvidin-3-glucoside

Fig. 5: Anthocyanin chromatograph of grape juice from Papazkarası variety