NUTRITIONAL VALUE, PHENOLIC, VITAMIN AND MINERAL CONTENTS OF A FERMENTED BEVERAGE FROM GRAPES OF 'MERLOT' AND ' CABERNET SAUVIGNON'

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In this study, Hardaliye, the non-alcoholic fermented beverage from 'Merlot' and 'Cabernet Sauvignon' grapes, was successfully examined for its nutritional value, phenolic compounds, water-soluble vitamin and mineral contents, and was chemically characterized using different analytical methods. Twenty-one phenolic compounds and eight water-soluble vitamins were quantified using ultra-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) as well as ten minerals using inductively coupled plasma-mass spectrometry (ICP-MS) in Merlot and Cabernet Hardaliye beverages, respectively. Catechin hydrate (171.28 ± 0.24 and 182.71 ± 0.11 mg/l), ascorbic acid (893.72 ± 5.09 and 976.43 ± 7.40 µg/l) and potassium (2050.77 ± 6.71 and 1908.51 ± 5.64 mg/l) were found as the constituents being present in the highest concentrations in the Merlot and Cabernet Hardaliye samples, respectively. Due to its beneficial contents, this beverage can be consumed by humans of all ages and it can be recommended for adolescents and athletes.
Keywords: phenolic profile, water-soluble vitamins, non-alcoholic fermented beverage, Hardaliye, chemical composition, UPLC-ESI-MS/MS, ICP-MS

Nährwert, Phenol-, Vitamin- und Mineralstoffgehalte eines fermentierten Getränks (Hardaliye) aus Trauben der Sorten 'Merlot' und 'Cabernet Sauvignon'. In dieser Studie wurde Hardaliye, das alkoholfreie, fermentierte Getränk aus Merlot- oder Cabernet Sauvignon-Trauben auf seinen Nährwert und die Gehalte an phenolischen Verbindungen, wasserlöslichen Vitaminen und Mineralstoffen untersucht und mittels verschiedener analytischer Methoden chemisch charakterisiert. In Merlot- und Cabernet-Hardaliye wurden einundzwanzig phenolische Verbindungen und acht wasserlösliche Vitamine (mittels UPLC-ESI-MS/MS) sowie zehn Mineralstoffe (mittels ICP-MS) quantifiziert. Catechin-Hydrat (171,28 ± 0,24 und 182,71 ± 0,11 mg/l), Ascorbinsäure (893,72 ± 5,09 und 976,43 ± 7,40 µg/l) und Kalium (2050,77 ± 6,71 und 1908,51 ± 5,64 mg/l) lagen in den höchsten Konzentrationen in den Merlot- bzw. Cabernet-Hardaliye-Proben vor. Aufgrund seiner förderlichen Inhaltsstoffe kann dieses Getränk von Menschen aller Altersstufen konsumiert und für heranwachsende Kinder und Sportler empfohlen werden.
Schlagwörter: Phenolprofil, wasserlösliche Vitamine, alkoholfreies fermentiertes Getränk, Hardaliye, chemische Zusammensetzung, UPLC-ESI-MS/MS, ICP-MS

Natural foods of high nutritional quality play an important role in maintaining human health (Toaldo et al., 2015). Grape (*Vitis* sp.) is one of the most commonly eaten and cultivated fruit worldwide as a natural food. More than 80 % of viticulture is used for the production of juice and wine (Schieber et al., 2001). Grape and its products also contain several bioactive ingredients including flavonoids, polyphenols, anthocyanins and the stilbene derivative resveratrol. Currently, people are prioritizing a more healthy diet and there are increasing knowledge and concern regarding food quality, food safety and environmental protection (Cardoso et al., 2011; Toaldo et al., 2015). Phenolic compounds are secondary plant metabolites that play a key role in the sensory and nutritional quality of fruits, vegetables and other plants (Ignat et al., 2011). Phenolic acids and flavanols contents of organic juice are quite high. Previous studies have reported that grape and grape products have biological and therapeutic effects such as antioxidative, anticarcinogenic, antimicrobial, antiviral, antiaging, antiinflammatory, antidiabetic activities as well as having cardioprotective, hepatoprotective and neuroprotective effects. Due to these results grapes are widely accepted as beneficial for human health to treat and prevent from diseases (Çetin and Sağdiç, 2009). Health promotion effects based on a reduction in oxidative stress have also been reported for non-alcoholic grape juice, the consumption of which has been related to reduced ageing and a decrease in atherosclerosis, Parkinson's disease, cancer and cataracts (Dani et al., 2009; Moreno-Montoro et al., 2015; Park et al., 2009). Besides, the positive change in oxidative status caused by the juice may also favour a reduction in cardiovascular disease risk by exerting a preventive effect against LDL-c oxidation, endothelial function, atherothrombotic events, inflammatory cascade (Castilla et al., 2006) and hypertension (Mosele et al., 2012). Grape juice has also been reported to improve cognitive and motor function (Joseph et al., 2009; Krikorian et al., 2012). Some of these effects are related to protection against oxidative stress (Yuan et al., 2011). The antioxidant activity of grape juice may be an indicator of the relative level of health benefit they offer (Moreno-Montoro et al., 2015). The consumption of lactic acid fermented foods as a natural beverage is highly prevalent and consumed widely in Mediterranean countries. These products display several benefits. They are important in digestibility and have nutritive values compared to their unfermented counterparts. Fermentation enhances the organoleptic properties of products. Vegetable blends and fermented vegetable juices from cabbage, carrots, celery, tomatoes, red beets and turnips are currently available on the market. Besides, lactic acid fermented grape juice, fermented cherry and medlar, are common in some Middle Eastern countries (Arici and Coskun, 2001). On the other hand, Hardaliye beverage is one of the fermented drinks, and it can be prepared by traditional methods. The process used in Hardaliye production is as follows: Washed red grapes are pressed and mixed with crushed cherry leaf and raw mustard seeds, and then the mixture is placed in a plastic barrel. It will be ready after 25 to 30 days depending on the ambient temperature. In addition, sodium sorbate is added into the mixture as a preservative against mould growth. The mixture will be filtered for usage.

Although different techniques, such as the most commonly used high performance liquid chromatography (HPLC), were used for the separation, determination and quantification of phenolic compounds (Fontana et al., 2013), the advantages of the ultrahigh performance liquid chromatography (UPLC), which displays high resolution and sensitivity and short retention times, put forward the use of this technique in the present study. Besides, several in-vitro methods such as spectrophotometric methods (ABTS, FRAP, DPPH and ORAC assays) were used widely in the measurements of the antioxidant activity (Floegel et al., 2011; Jara-Palacios et al., 2014). In this study, various analysis techniques such as UPLC-ESI-MS/MS (for phenolic compounds and water-soluble vitamins), HPLC-RID (for sugars), ICP-MS (for minerals) were used to characterize Merlot Hardaliye and Cabernet Hardaliye samples in detail, and to the best of our knowledge, these techniques have not been used to study Hardaliye beverage before. In this sense, the aim of this work was to elucidate nutritional composition, individual phenolic compounds, elemental content and water-soluble vitamin constituents of Merlot Hardaliye and Cabarnet Hardaliye samples.

MATERIAL AND METHODS

GRAPE SAMPLES AND HARDALIYE DRINKS

Samples of Merlot and Cabernet grapes for the production of Hardaliye were used in this study. Samples of Hardaliye of two different grape varieties, 'Merlot' and 'Cabernet Sauvignon', were used in the study. Hardaliye samples prepared by traditional methods were supplied from the Thrace region of Turkey.

STANDARDS AND REAGENTS

Acetonitrile 99.9 %, n-hexane 98 %, diethyl ether and ethyl acetate 99.8 % of analytical grade purity were supplied by Merck (Darmstadt, Germany). Standards (phenolic compounds, vitamins, and sugars) were supplied from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Water was HPLC grade (18.2 MΩ/cm), purified by a Milipore Milli-Q (Molsheim, France) system that includes reverse osmosis, ion exchange and filtration steps. All other chemicals and solvents were of analytical grade and purchased from usual suppliers.

NUTRITIONAL VALUE

Moisture, proteins, fat, carbohydrates and ash were analysed using the Association of Analytical Communities procedures (Aoac, 2007). The crude protein content (N × 6.25) of the samples was estimated by the micro-Kjeldahl method (Aoac, 2007; method 12.960.52). The crude fat was determined by extracting a known weight sample with petroleum ether, using a Soxhlet method (Aoac, 2007, method 996.01). The ash amount was determined by burning at 650 ± 15 °C. Total carbohydrates were calculated by difference. Energy was calculated according to Heleno et al. (2013) to Eq. (1).

Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g fat) Eq. (1)

WATER-SOLUBLE VITAMIN ANALYSIS BY UPLC-ESI-MS/MS

Analysis of vitamins was carried out using a Waters (Milford, MA, USA) Acquity Ultra Performance LC with a Waters binary system manager coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer with ESI probe (UPLC-ESI-MS/MS). The samples (20 ml) were extracted with separation funnel by three portions of 50 ml of hexane, and then the water phase was thoroughly freeze-dried at -75 °C, and the residue was redissolved with methanol, and then it was filtered using Macherey-Nagel Chromafil Xtra PTFE-20/25 0.20 µm, and injected to UPLC-ESI-MS/MS. Chromatographic separation was done with an analytical column, Acquity UPLC BEH C18 (1.7 µm 2.1 x 100 mm) using mobile phase A, 1 % acetic acid in water; and mobile phase B, 1 % acetic acid in acetonitrile with a linear gradient mode, 0 to 2 min 99 % A, 2 to 3 min 45 % A, 3 to 6 min 99 % A, 6 to 12 min 99 % A at 0.500 ml/min flow rate.

UPLC-ESI-MS/MS ANALYSIS FOR INDIVIDUAL PHENOLICS

The phenolic compounds of Hardaliye extracts were determined with UPLC using the modified method of Cadahía et al. (2009). The Hardaliye samples (50 ml) were extracted with ethyl acetate (100 ml) by using a shaker for 3 h and the extracts were poured into separation funnels for phase separation for 6 h, then ethyl acetate fractions were filtered using Whatman No. 4. The residue was then extracted with three additional 100 ml portions of ethyl acetate, as described above. The solvent in the combined extracts was evaporated at 40 °C and redissolved in methanol and filtered through a PTFE-20/25 LC filter disk for LC analysis. The separation was done with a Waters analytical C18 column, Acquity UPLC BEH C18 (1.7 µm 2.1 x100 mm) at 40 °C column oven temperature and 2 µl injection volume with the two mobile phases (mobile phase A, 0.5 % (v/v) acetic acid in ultrapure water and mobile phase B, 0.5 % (v/v) acetic acid in acetonitrile) with a linear gradient mode, 0

to 1 min 99 % A, 1 to 10 min 70 % A, 10 to 12 min 5 % A, 12 to 13 min 99 % A at 0.650 ml/min flow rate. The chromatographic and mass spectrometry conditions were used as previously given elsewhere (Kivrak et al., 2013; Kivrak, 2015). Detection was carried out with a tandem mass spectrometry (MS/MS) with electrospray ionization (ESI), using multiple reaction monitoring (MRM) mode. The selected phenolics were measured using a Waters UPLC-ESI-MS/MS (Acquity Ultra Performance LC, Xevo TQ-S MS-MS, Waters Co., Milford, MA, USA).

EXTRACTION AND DETECTION OF SUGARS BY HPLC-RID

The analysis of sugars was performed using the modified method of Melgarejo et al. (2000). The sugar analyses were done with the standards of fructose and glucose of fruit juice. The samples were analysed using a high performance liquid chromatography with a refractive index detector (HPLC-RID) (Agilent 1260, Agilent Technologies, Santa Clara, USA), separated by Zorbax NH_2 Analytical column 4.6 x 250 mm, 5µm (Agilent Technologies, Santa Clara, USA). The mobile phase was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1.25 ml/min. Sugars were determined by comparing the relative retention times of sample peaks with standards. Data of the instrument were analysed using ChemStation Software (Agilent), and quantification was based on the RI signal response of each standard using calibration curves, plotted by standards of each compound. The results were given in g per 100 ml of beverage weight.

DETERMINATION OF METAL CONTENTS USING ICP-MS

Samples (0.2 g) were weighed into falcon tube, and mixed with nitric acid (3 ml), hydrochloric acid (0.5 ml) and hydrogen peroxide (0.5 ml), then extracted with microwave (Cem Mars 5, Matthews, NC, USA) at 1200 W power. After the extract solution was cooled down, it was added to flask (20 ml) by diluting ultrapure water. Hardaliye samples were analysed for the assessment of metal content using an Agilent ICP-MS (Agilent 7700x, Tokyo, Japan). Operation parameters for the analysis were as follows: plasma power, 1550 W; plasma mode, normal; plasma gas flow rate, 15.0 l/min; auxiliary gas flow rate, 1.0 l/min; carrier gas flow rate, 0.89 l/min; dilution gas flow rate, 0.15 l/min; sample depth, 8.0 mm; spray chamber temperature, 2 °C; kinetic energy discrimination, 3V; helium gas flow rate, 4.5 ml/min.

MEASUREMENT OF IN-VITRO ANTIOXIDANT CAPACITY

INHIBITION OF β-CAROTENE BLEACHING ASSAY

The total antioxidant activity was evaluated using β-carotene-linoleic acid test system with slight modifications (Kivrak et al., 2009). This method is based on bleaching of β-carotene. β-carotene (0.5 mg), dissolved in 1 ml of chloroform, was mixed with linoleic acid (25 µl) and Tween 40 emulsifier (200 mg). Chloroform was evaporated under vacuum, 50 ml of distilled water saturated with oxygen was added by vigorous shaking. Aliquots (1.60 µl) of this emulsion were added to 40 µl of the extract solutions at different concentrations. As soon as the emulsion was added to each tube, the zero time absorbance was initially measured at 470 nm, and then the absorbance measurements were done for every 30 min until 120 min. The results were given as 50 % inhibition concentration (IC_{50}).

DPPH FREE RADICAL SCAVENGING ACTIVITY

The free radical scavenging activity of extract was determined using DPPH free radical according to Kivrak (2015). The extract solutions with different concentrations (40 µl) and ethanolic solution (120 µl) containing DPPH radicals (0.4 mM) were incubated at room temperature in darkness for 30 min. Absorbance was measured at 517 nm.

ABTS CATION RADICAL DECOLORIZATION ASSAY

ABTS cation radical decolorization assay was analysed according to Thaipong et al. (2006). The ABTS (7 mM) in water and potassium persulfate (2.45 mM) reacted to give ABTS•+, stored in the dark at room temperature for 12 h, and oxidation of ABTS appeared immediately; however, the stability of absorbance was gained after 6 h. Then, the sample solution (40 µl) in ethanol at different concentrations was mixed with ABTS•+ solution (160 µl), giving the absorbance at 734 nm.

EVALUATION OF THE ANTICHOLINESTERASE ACTIVITY

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were measured by Ellman et al. (1961). Briefly, sodium phosphate buffer (150 µl, 100 mM at pH 8.0), sample solution (10 µl) dissolved in ethanol at different concentrations and AChE (5.32 × 10-3 U) or BChE (6.85 × 10-3 U) solution (20 µl) were mixed and incubated for 15 min at room temperature and then (5,5′-dithio-bis(2-nitrobenzoic) acid) (DTNB) (10 µl, 0.5 mM) was added. Then, the reaction was initiated by the addition of acetylthiocholine iodide (10 µl, 0.71 mM) or butyrylthiocholine chloride (10 µl, 0.2 mM). The hydrolysis of these substrates was monitored spectrophotometrically at a wavelength of 412 nm.

STATISTICAL ANALYSIS

The results were reported as mean and standard deviation of the mean. Data were subjected to analysis of variance and the LSD (least significant difference) value, to identify pairs of means that are significantly different, was calculated using the STATISTICA for Windows release 5.0.

RESULTS AND DISCUSSION

NUTRITIONAL COMPOSITION

Nutritional composition of the fermented grape beverage "Hardaliye" showed significant difference ($p < 0.05$) between the varieties of grape studied for moisture, ash, total carbohydrate, fat, protein, energy, fructose, and glucose. The Hardaliye from Merlot grapes had higher contents of total carbohydrates, fat, energy, fructose, and glucose than those of Hardaliye from Cabernet Sauvignon grapes. All showed higher levels of moisture and ash content. However, protein was not detected in both samples.

The moisture content of Cabernet and Merlot Hardaliye beverages was 86.73 % and 85.30 %, respectively, and ash contents were 0.733 % and 0.677 %, respectively. Total carbohydrate content, fat, and energy were 20.08 %, 0.233 %, and 344.59 kJ (82.36 kcal) for the Merlot Hardaliye beverage and it was 19.76 %, 0.206 %, and 340.12 kJ (81.29 kcal) for the Cabernet Hardaliye beverage, respectively. In addition, fructose and glucose were 10.73 % and 10.62 % for Merlot Hardaliye, whereas fructose and glucose of Cabernet Hardaliye values were found to be 10.52 % and 10.40 %.

PHENOLIC COMPOUNDS

The individual phenolic compounds' values for each Hardaliye type are presented in Table 1. ANOVA of the data showed that the effects of grape varieties were statistically significant ($p < 0.05$) in the content of all detected phenolics. All phenolic compounds were detected by UPLC-ESI-MS/MS. Our results identified nine phenolic acids and derivatives in both Hardaliye beverages of the tested grape varieties. The nine detected compounds were caffeic, gallic, protocatechuic, p-hydroxy benzoic, chlorogenic, vanillic, syringic, p-coumaric, and ferulic acid. Results showed that Merlot Hardaliye contained more caffeic, gallic, protocatechuic, chlorogenic, and p-coumaric acid than those of Cabernet Hardaliye. On the other hand, Cabernet Hardaliye had more p-hydroxy benzoic (1.59 mg/l), vanillic (50.84 mg/l), syringic (8.91 mg/l) and ferulic acid (7.01 mg/l) than Merlot Hardaliye. Vanillic acid was the main phenolic acid in Merlot Hardaliye and Cabernet Hardaliye. Merlot Hardaliye had high levels of vanillic acid (44.41 mg/l) but less than Cabernet Hardaliye (50.84 mg/l). Moreover, caffeic acid (25.18 and 21.86 mg/l) and p-coumaric acid (17.96 and 12.45 mg/l) were found in Merlot and Cabernet Hardaliye, respectively. Results showed that p-hydroxy benzoic acid was the lowest phenolic acid which was found at 1.27 mg/l in Merlot and at 1.59 mg/l in Cabernet Hardaliye. Additionally, Merlot Hardaliye had 13.37 mg/l gallic, 8.24 mg/l protocatechuic, 4.78 mg/l chlorogenic, 7.14 mg/l syringic, and 6.78 mg/l ferulic acids. The total of phenolic acids and their derivatives were determined as 129.13 mg/l in Merlot Hardaliye and as 125.41 mg/l in Cabernet Hardaliye.

Four flavonols were determined in Hardaliye samples. The content of rutin, myricetin, quercetin, and kaempferol in Hardaliye beverages were significantly ($p < 0.05$) affected by grape variety. Merlot Hardaliye had the highest content of quercetin which was 16.57 mg/l. Cabernet Hardaliye contained more rutin and kaempferol (9.11 and 4.48 mg/l) than Merlot Hardaliye. Cabernet

Table 1: Identified compounds for phenolic contents of Merlot and Cabernet Hardaliye

Phenolic compounds	Merlot Hardaliye (mg/l)	Cabernet Hardaliye (mg/l)	p*
Phenolic acids and their derivatives			
Caffeic acid	25.18 [a] ± 0.10	21.86 [b] ± 0.14	< 0.001
Gallic acid	13.37 [a] ± 0.08	11.57 [b] ± 0.14	< 0.001
Protocatechuic acid	8.24 [a] ± 0.04	8.07 [b] ± 0.02	< 0.001
p-hydroxy benzoic acid	1.27 [b] ± 0.06	1.59 [a] ± 0.04	< 0.001
Chlorogenic acid	4.78 [a] ± 0.06	3.11 [b] ± 0.10	< 0.001
Vanillic acid	44.41 [b] ± 0.12	50.84 [a] ± 0.19	< 0.001
Syringic acid	7.14 [b] ± 0.12	8.91 [a] ± 0.13	< 0.001
p-coumaric acid	17.96 [a] ± 0.05	12.45 [b] ± 0.06	< 0.001
Ferulic acid	6.78 [b] ± 0.03	7.01 [a] ± 0.04	0.001
Total phenolic acids and their derivatives	129.13 [a] ± 0.62	125.41 [b] ± 1.20	0.021
Flavonols			
Rutin	7.49 [b] ± 0.07	9.11 [a] ± 0.09	< 0.001
Myricetin	8.39 [a] ± 0.23	7.42 [b] ± 0.09	< 0.001
Quercetin	16.57 [a] ± 0.07	7.42 [b] ± 0.08	< 0.001
Kaempferol	3.18 [b] ± 0.07	4.48 [a] ± 0.05	< 0.001
Total flavonols	35.63 [a] ± 1.12	28.43 [b] ± 0.86	< 0.001
Flavanols (Flavan-3-ols)			
Catechin hydrate	171.28 [b] ± 0.24	182.71 [a] ± 0.11	< 0.001
Epicatechin	49.78 [a] ± 0.12	47.03 [b] ± 0.09	< 0.001
Catechin gallate	12.28 [a] ± 0.03	10.96 [b] ± 0.04	< 0.001
Flavanones			
Hesperetin	2.46 [a] ± 0.04	1.71 [b] ± 0.03	< 0.001
Naringenin	1.04 [b] ± 0.01	1.28 [a] ± 0.03	< 0.001
Flavones			
Apigenin	1.90 [a] ± 0.03	1.03 [b] ± 0.03	< 0.001
Benzenediols			
Pyrocatechol	0.87 [b] ± 0.03	0.99 [a] ± 0.04	0.01
Stilbenoid			
Resveratrol	5.19 [a] ± 0.05	4.77 [b] ± 0.04	< 0.001

Results expressed as mean ± standard deviation. Means in the same line followed by different letters are significantly different ($p < 0.05$).
* Probability values obtained by one-way ANOVA

Hardaliye showed the same levels of myricetin and quercetin (7.42 mg/l).

Total flavonols content of Hardaliye samples were found to be 35.63 and 28.43 mg/l in Merlot and Cabernet Hardaliye, respectively. Catechin hydrate, epicatechin, and catechin gallate were obtained from Hardaliye beverages as flavan-3-ols. Catechin hydrate, which is in flavan-3-ol structure, is a secondary plant metabolite. Cabernet Hardaliye contained 182.71 mg/l catechin hydrate which was the highest flavan-3-ols among all results. Also Merlot Hardaliye had 171.28 mg/l catechin hydrate, 49.78 mg/l epicatechin and 12.28 mg/l catechin gallate. Flavanones, flavones, benzenediols, and stilbenoid were extracted from Hardaliye beverages, in addition to phenolic acids, flavonols, and flavan-3-ols. Hesperetin varied between 1.71 and 2.46 mg/l in Cabernet and Merlot Hardaliye, respectively. Merlot Hardaliye had 1.04 mg/l naringenin, 1.90 mg/l apigenin, and 0.87 mg/l pyrocatechol. Cabernet Hardaliye showed higher naringenin (1.28 mg/l) and pyrocatechol (0.99 mg/l) contents than Merlot Hardaliye. One of the most important phenolic compounds in grape, resveratrol, was also determined in Merlot and Cabernet Hardaliye at 5.19 and 4.77 mg/l, respectively.

WATER-SOLUBLE VITAMINS

In the present study, we used UPLC-ESI-MS/MS to determine the content of water-soluble vitamins in the Hardaliye beverages produced from 'Merlot' or 'Cabernet Sauvignon' grapes. The results are shown in Table 2. Only biotine (B7) was not detected in any of Hardaliye samples. The eight water-soluble vitamins were identified for both Hardaliye samples. Total ion chromatograms of water-soluble vitamins are displayed in Figure 1. The effects of the grape variety on the contents of water-soluble vitamins of Hardaliye beverages were statistically significant ($p < 0.05$).

Results showed that, Merlot Hardaliye contained more riboflavin (B2), pantothenic acid (B5), nicotinamide (B3), nicotinic acid (B3) and folic acid (B9) than Cabernet Hardaliye. On the other hand, Cabernet Hardaliye samples had more thiamine (B1), pyridoxine (B6), and ascorbic acid (vitamin C) than those of Merlot Hardaliye. The results revealed that ascorbic acid is the predominant water-soluble vitamin in the Hardaliye beverages followed by nicotinic acid in both Hardaliye types, contents being 327.03 and 298.82 µg/l for Merlot Hardaliye and Cabernet Sauvignon Hardaliye, respectively. The other form of vitamin B3, nicotinamide, was found in contents of 191.45 µg/l in Merlot Hardaliye and 121.88 µg/l in Cabernet Hardaliye.

The content of riboflavin, pantothenic acid, and pyridoxine was 17.61, 92.49, and 69.32 µg/l for Merlot Hardaliye and the corresponding results were 9.80, 81.26 and 74.22 µg/l in the Cabernet Hardaliye. Results also indicated that thiamine and folic acid were the two water-soluble vitamins that showed the lowest contents in both Hardaliye types (8.17 and 4.13 µg/l for Merlot Hardaliye and 8.77 and 3.81 µg/l for Cabernet Hardaliye). The ascorbic acid contents were found to be 976.43 and 893.72 µg/l in Cabernet Hadarliye and Merlot Hadarliye, respectively. Total contents of water-soluble vitamins varied between 1574.99 and 1603.92 µg/l with significant differences ($p < 0.05$) between the varieties, respectively.

MINERALS

The minerals, determined in both Hardaliye types, were analysed by ICP-MS and the results are shown in Table 3. For each grape variety the five macro-elements (Na, Ca, Mg, P, and K) and the five micro-elements (Se, Cu, Zn, Mn, and Fe) were determined in all samples. Generally, the macro-elements potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) were the most abundant minerals in all juices. All mineral contents were significantly affected ($p < 0.05$) by the grape variety. Merlot Hardaliye had higher total mineral contents than Cabernet Hardaliye. Total mineral contents were found to be 2690.85 mg/l for Merlot Hardaliye and 2650.66 mg/l for Cabernet Hardaliye. The total macro-elements contents were determined as 2658.77 mg/l for Merlot

Table 2: Water soluble vitamin contents of Merlot and Cabernet Hardaliye by UPLC-ESI-MS/MS

Vitamin compounds	Merlot Hardaliye (µg/l)	Cabernet Hardaliye (µg/l)	p*
Thiamine (Vitamin B_1)	$8.17^{b} \pm 0.15$	$8.77^{a} \pm 0.11$	< 0.001
Riboflavin (Vitamin B_2)	$17.61^{a} \pm 0.72$	$9.80^{b} \pm 0.57$	< 0.001
Nicotinamide (Vitamin B_3)	$191.45^{a} \pm 3.52$	$121.88^{b} \pm 1.99$	< 0.001
Nicotinic acid (Vitamin B_3)	$327.03^{a} \pm 7.45$	$298.82^{b} \pm 3.80$	< 0.001
Pantothenic acid (Vitamin B_5)	$92.49^{a} \pm 5.17$	$81.26^{b} \pm 3.47$	0,011
Pyridoxine (Vitamin B_6)	$69.32^{b} \pm 1.86$	$74.22^{a} \pm 2.47$	0,019
Folic acid (Vitamin B_9)	$4.13^{a} \pm 0.11$	$3.81^{b} \pm 0.06$	0,002
Ascorbic acid (Vitamin C)	$893.72^{b} \pm 5.09$	$976.43^{a} \pm 7.40$	< 0.001
Total water soluble vitamins	$1603.92^{a} \pm 4.98$	$1574.99^{b} \pm 4.13$	< 0.001

Results expressed as mean ± standard deviation. Means in the same line followed by different letters are significantly different ($p < 0.05$).
* Probability values obtained by one-way ANOVA

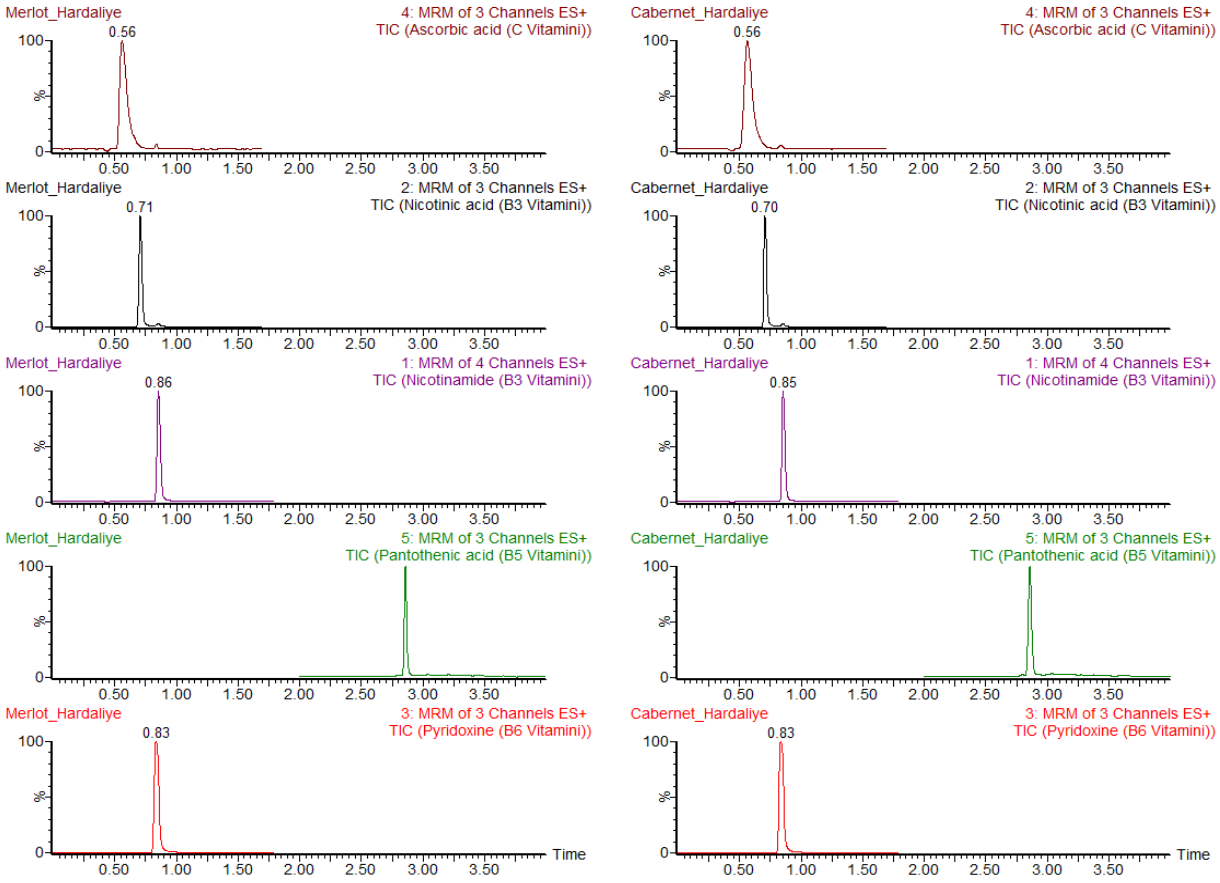

Fig. 1: Total ion chromatograms of water-soluble vitamins (major compounds)

Table 3: Mineral contents of Merlot and Cabernet Hardaliye by ICP-MS

Minerals	Merlot Hardaliye (mg/l)	Cabernet Hardaliye (mg/l)	p*
Micro elements			
Selenium (Se)	1.05 ^{b} ± 0.08	1.20 ^{a} ± 0.03	0.017
Copper (Cu)	2.82 ^{b} ± 0.06	4.61 ^{a} ± 0.13	< 0.001
Zinc (Zn)	5.80 ^{a} ± 0.12	4.83 ^{b} ± 0.15	< 0.001
Manganese (Mn)	7.30 ^{b} ± 0.15	8.15 ^{a} ± 0.06	< 0.001
Iron (Fe)	15.11 ^{b} ± 0.04	17.79 ^{a} ± 0.09	< 0.001
Total micro elements	32.08 ^{b} ± 0.17	36.58 ^{a} ± 0.23	< 0.001
Macro elements			
Sodium (Na)	6.35 ^{a} ± 0.03	5.64 ^{b} ± 0.07	< 0.001
Calcium (Ca)	97.62 ^{b} ± 1.33	111.85 ^{a} ± 1.04	< 0.001
Magnesium (Mg)	165.78 ^{b} ± 1.60	194.28 ^{a} ± 2.83	< 0.001
Phosphor (P)	338.25 ^{b} ± 5.66	393.80 ^{a} ± 5.57	< 0.001
Potassium (K)	2050.77 ^{a} ± 6.71	1908.51 ^{b} ± 5.64	< 0.001
Total macro elements	2658.77 ^{a} ± 4.05	2614.08 ^{b} ± 8.11	< 0.001
Total mineral content	2690.85 ^{a} ± 3.93	2650.66 ^{b} ± 6.13	< 0.001

Results expressed as mean ± standard deviation. Means in the same line followed by different letters are significantly different ($p < 0.05$).
* Probability values obtained by one-way ANOVA

Hadarliye and 2614.08 mg/l for Cabernet Hadarliye. Merlot Hardaliye had lower total micro-element contents than Cabernet Hardaliye was 32.08 and 36.58 mg/l for Merlot Hadarliye and Cabernet Hadarliye, respectively. The most abundant macro-element in all samples was potassium which was detected being 2050.77 mg/l in Merlot Hadarliye and 1908.51 mg/l in Cabernet Hadarliye. Phosphor and magnesium were second and third in both Hardaliye types. Phosphor and magnesium contents were 338.25 and 165.78 mg/l in Merlot Hadarliye and 393.80 and 194.28 mg/l in Cabernet Hardaliye. Among the macro-elements, calcium showed the highest contents in Cabernet Hardaliye ranging significantly ($p < 0.05$) from 97.62 to 111.85 mg/l. Sodium showed the lowest contents of all macro-elements (6.35 mg/l in Merlot Hadarliye and 5.64 mg/l in Cabernet Hardaliye). Iron was the most abundant micro-element mineral in both Hardaliye types (15.11 mg/l in Merlot Hadarliye and 17.79 mg/l in Cabernet Hardaliye). Cabernet Hardaliye had micro-elements more than Merlot Hardaliye except for zinc. The contents of manganese, zinc, and copper were 7.30, 5.80, and 2.82 mg/l for Merlot Hardaliye and corresponding values were 8.15, 4.83, and 4.61 mg/l for Cabernet Hardaliye. Selenium showed the lowest contents of all micro-elements more in both Hardaliye types, ranging from 1.05 to 1.20 mg/l.

ANTIOXIDANT ACTIVITY OF MERLOT AND CABERNET GRAPES

The antioxidant activity of Merlot and Cabernet Sauvignon grape samples was measured in the extracts of hexane, ethyl acetate, methanol and water. In the case of radical scavenging activity, the DPPH assay of grape samples displayed a good radical scavenging inhibition, better than BHA and BHT standards, as half maximal inhibitory concentration IC_{50} = 61.33 and 57.73 µg/ml in ethyl acetate extracts of Merlot and Cabernet grapes, respectively. In the ABTS cation radical scavenging activity, ethyl acetate extracts of Merlot and Cabernet grape samples showed moderate activity as IC_{50} = 19.28 and 17.34 µg/ml, respectively, but still lower than standards. Both of the assays displayed less scavenging inhibition compared to standards in the extracts of hexane, methanol and water. However, in terms of lipid peroxidation inhibition, the β-carotene-linoleic acid assay of hexane extracts of Merlot and Cabernet grape samples (IC_{50} =14.71 and 16.13 µg/ml, respectively) revealed activity close to standards. The extracts of ethyl acetate, methanol and water exhibited limited activity, compared to BHA, BHT and α-tocopherol standards. Thus, results of antioxidant activity of analysis of studied grapes performed a good correlation between antioxidant activity and phenolic content.

ANTICHOLINESTERASE ACTIVITY OF MERLOT AND CABERNET GRAPES

The butyrylcholinesterase activity of the studied grapes of ethyl acetate extract exhibited higher activity, compared with galanthamine, as IC_{50} = 37.24 and 41.50 µg/ml, respectively. On the other hand, all four extracts of Merlot and Cabernet grape samples showed almost no activity against acetylcholinesterase.

CONCLUSION

The fermented grape beverage Hardaliye is a very valuable non-alcoholic beverage, having distinctive taste and smell. In this study, nutritional value, phenolic compounds, water-soluble vitamins, sugar profiles and mineral contents of Hardaliye beverages from Merlot and Cabernet Sauvignon grapes were extensively investigated and chemically characterized using different analytical methods and instruments; UPLC-ESI-MS/MS, ICP-MS, HPLC-RID. The most abundant ingredients in all samples were catechin hydrate as phenolic compound, potassium as macro-element, ascorbic acid and nicotinic acid as water-soluble vitamin. In addition, the ethyl acetate extracts of the grape samples performed the highest radical scavenging activity, whereas the hexane extracts displayed highest lipid peroxidation inhibitory activity. Finally, with respect to all beneficial features, this non-alcoholic drink can be consumed by humans of all ages and recommended to adolescents and athletes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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