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Screening of bioactive components in grape and apple vinegars: Antioxidant and antimicrobial potential

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Vinegars contain several bioactive compounds that are characterized according to the type of the raw material, such as grape vinegars and apple vinegars. Liquid chromatography coupled to diode array detection and electrospray ionization tandem mass spectrometry was used for identification and quantification of phenolic compounds. Antioxidant properties of vinegars were determined by 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azino-*bis*-3-ethylbenzthiazoline-6-sulphonic acid assays. Antimicrobial activities of vinegars were examined with an agar disc diffusion method with *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Gallic acid and chlorogenic acid were found to be the major phenolic acids accounting for the largest proportion of the total phenolic acid contents in grape vinegars and apple vinegars. Within the flavonols, quercetin-3-*O*-galactoside and quercetin were detected as the major compounds in grape vinegars. Apple vinegars were characterized by phloridzin, phloretin and high chlorogenic acid content. Antimicrobial activity results indicated that grape vinegars exhibited higher antimicrobial activity against tested bacterial strains correlated with their higher antioxidant capacity. **Copyright © 2017 The Institute of Brewing & Distilling**

Keywords: vinegar; phenolic compounds; antioxidant activity; antimicrobial activity

Introduction

Vinegars are fermented food condiments obtained from cereals and fruits *(1)*. They are clustered as wine vinegars and fruit vinegars commercially. The quality of vinegars changes according to the raw material, acidification system and aging procedure *(2,3)*. The consumer demands for natural and high-quality food products have increased the characterization and quality control of these products *(4)*.

The quality and origin of vinegars are related to the chemical composition and sensory properties of vinegars *(4)*. Phenolic and volatile compounds are of interest because of their significant effect on sensory and functional properties of vinegars *(5)*. Cerezo et al. *(4)* investigated the phenolic compounds of red wine vinegars produced with a surface culture system using HPLC (high-performance liquid chromatography) method and found that caftaric acid was the major phenolic acid followed by gallic acid and tyrosol. Nakamura et al. *(5)* determined the major phenolic compounds such as chlorogenic acid, 4-*p*-coumaroylquinic acid and caffeic acid in high brix apple vinegar. Vinegars are rich in antioxidant compounds and the intake of these antioxidant compounds with vinegars could be associated with lower incidences of degenerative disease, such as cardiovascular disease and cancer *(6)*. Sakanaka and Ishihara *(1)* examined the antioxidant activities of two kinds of persimmon vinegars, unpolished rice vinegar, polished rice vinegar and apple vinegar using superoxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activity tests. The results indicated that persimmon vinegar showed high antioxidant activity on fatty tuna homogenates.

Vinegars have also organic acids that have an effect on the flavour of vinegars. Acetic acid is the main organic acid in vinegars. However, other acids (tartaric acid, formic acid, lactic acid and malic acid) are also present in fruit vinegars *(3,7)*. Lin et al. *(8)* have used ion-exclusion liquid chromatography for detection of organic acids and indicated that acetic, ascorbic, citric, malic and malonic acids were the major organic acids in fruit vinegars (apple, apple sparkling, plum, cranberry, and grape). Another study reported that glucose and fructose were the main reducing sugars and acetic acid was the major organic acid in traditional balsamic vinegar as determined by using HPLC method *(9)*. The antimicrobial activities of organic acids could be associated with their inhibitory effect on enzymes, membrane function, nutrient transport and overall metabolic activity of cells *(10)*. Priyadarshini et al. *(11)* investigated the antimicrobial activity of three vinegars prepared with sweet lime peel, sweet lime fruit–peel combined and sweet lime fruit against *Escherichia coli*, *Salmonella typhi* and *Klebsiella* species. Three vinegar combinations showed different antimicrobial activities on these organisms. Chang and Fang *(10)* examined the antimicrobial effect of rice vinegar on lettuce inoculated with *E. coli* O157:H7 and indicated that 5% acetic acid treatment caused 3 log CFU/g reduction on chopped lettuce.

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To our knowledge, there has been no detailed research on the phenolic composition, antioxidant capacity and antimicrobial activity of commercial apple and grape vinegars. The aims of this study are (a) the characterization and quantification of phenolic compounds accordingly their raw materials and (b) determination and comparison of the antioxidant and antimicrobial activities of commercial apple and grape vinegars.

Materials and methods

Vinegar samples

Three batches of eight apple (AV) and eight grape vinegars (GV) of different brands, produced with biological fermentation, were purchased from a local market in Adana, Turkey. GV were produced from grapes obtained from the Aegean region, while AV were produced from apples obtained from Central Anatolia Region. Bottles of each vinegars were purchased from three different shops on different dates (in 2016) and each sample was individually analysed in triplicate. All commercial vinegars were stored in the laboratory at a constant temperature of 25 ± 1°C prior to analysis.

Chemicals

The HPLC standards gallic acid, vanillic acid, protocatechuic acid, chlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid, syringic acid, tyrosol, catechin, epicatechin, procyanidin B2, rutin, quercetin-3-galactoside, luteolin-3-glucoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, quercetin-3-*O*-rhamnoside, quercetin, luteolin, apigenin and phloridzin were from Sigma Co. (St Louis, MO, USA). Acetonitrile, formic acid, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), potassium persulphate and DPPH were purchased from Merck (Gernsheim, Germany). All chemicals and solvents were analytical or HPLC grade.

LC-DAD-ESI-MS/MS analysis of phenolic compounds

Prior to injection, filtration of samples was performed with a 0.45-μm pore size polyvinylidene fluoride membrane filter. An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) was used with a diode array detector (DAD). The system consisted of a binary pump, degasser and autosampler. The column used was a Phenomenex Luna reversed-phase C_{18} column (4.6 × 250 mm, 5 μm; Torrance, CA, USA). 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 compounds were eluted according to the method described by Kelebek et al. *(12)*. The characterization and detection of each compound were done by comparison of their retention times and UV spectra with authentic standards and LC–MS/MS analysis. An Agilent 6430 LC–MS/MS spectrometer with electrospray ionization mass spectrometry was performed in negative ion mode *(12–16)*. The quantification of individual compounds was calculated with a calibration curve of the standard compound purchased. The limits of detection (LOD) and quantification (LOQ) were calculated at signal-to-noise ratios (S/N) of about 3 and 10, respectively.

Antioxidant assays

DPPH assay. The DPPH assay was performed as described by Brand-Williams et al. *(15)*. Amounts of 0.1 mL of diluted samples were added to 3.9 mL of DPPH solution in methanol (6×10^{-5} M DPPH). The solution was mixed vigorously and kept at room temperature for 30 min. The absorbance of the solution was then measured at 515 nm by a Cary 60 UV–vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

ABTS assay. The ABTS assay was performed according to the method of Re et al. *(16)*. The ABTS radical cation was obtained by the reaction of 7 mM ABTS with 2.54 mM potassium persulphate, incubated at room temperature for 12–16 h. ABTS solution was diluted using ethanol to an absorbance of 0.70 ± 0.02 at 734 nm before analysis. A 3.9 mL aliquot of the diluted ABTS solution was added to 0.1 mL of each sample. The reaction mixture was kept at room temperature during 30 min and the absorbance at 734 nm was recorded.

Antimicrobial assay with disc diffusion method

In this study, *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as test organisms. These microorganisms were inoculated into 9 mL tryptic soy broth and incubated at 35°C for 18 h. After incubation, the turbidity of bacterial suspension was adjusted to 0.5 McFarland units (corresponding to 10^8 CFU/mL) with sterile saline solution. These bacterial cultures were inoculated at 1% level into Mueller Hinton agar cooled to 45–50°C. The agar containing bacteria was mixed and poured into plates *(17)*.

The antimicrobial activities of different types of vinegars on the selected test organisms were determined with standard disc diffusion technique. Vinegar samples were filter sterilized by using membrane filter with 0.22 μm. A 20 μL aliquot of vinegar was added to the sterile paper discs. Discs inoculated with sterile distilled water were used as negative controls. The discs were placed on inoculated agar plates and incubated at 35°C for 18–24 h. Each experiment was carried out in duplicate. After incubation, the inhibition zones were measured three times from different parts of the discs and the average was calculated.

Statistical analysis

One way analysis of variance (ANOVA) was applied to indicate the differences among the samples combined with the Fisher's least significant difference test at $p < 0.05$ significance level. Principal component analysis (PCA) was performed to analyse all data. The data matrix consisted of vinegar samples as observations and antimicrobial and antioxidant activity, and phenolic composition of vinegar samples as variables. PCA score and loading plots were constructed for visual interpretation of the results. Additionally, Pearson's correlation coefficient, *r*, was calculated to find the correlation among these parameters. All results were analysed using Minitab® 17 programme (Minitab Inc., State College, USA).

Results and discussion

Identification of phenolic compounds

In total, 27 compounds were identified and quantified in GV and AV based on the analysis and comparison of retention times and spectra of the individual peaks of GV and AV, on the basis of

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spectral data (liquid chromatography coupled to diode array detection and electrospray ionization tandem mass spectrometry, LC-DAD-ESI-MS/MS) and by comparison with standard compounds and literature data *(13,16)*. These compounds belonged to various family types that included phenolic acids, flavan-3-ols and flavonols (Table 1).

Phenolic acids. Twelve phenolic acids were identified and quantified for GV, while the AV had six compounds. Vanillic, gallic, protocatechuic, chlorogenic acid, caffeic and *p*-coumaric acid were identified and quantified in AV. In addition to these compounds, caftaric, coutaric, fertaric, ferulic and syringic acids were also determined in GV. The spectra generated for phenolic acids showed the deprotonated molecule $[M - H]^-$ and some fragments. A typical loss of CO_2 was observed for gallic, protocatechuic, caffeic and ferulic acids, giving $[M - H - 44]^-$ as a characteristic ion; loss of a methyl group [M – H – 15] was observed for ferulic acid. Vanillic acid is a methoxyhydroxybenzoic acid and the mass spectrum of vanillic acid displayed ions at *m/z* 167 $[M - H]^-$, 152 $[M - H - CH_3]^-$, 123 $[M - H - CO_2]^-$ and 108 $[M - H - CH_3 - CO_2]^-$. The *m/z* 122 ion was not observed in the mass spectrum of the compound eluting at retention time (R_t) 15.04 min, thus it is unlikely to be vanillic acid. The presence of *m/z* 107 ion possibly arises from a 59 amu loss ($-COOCH_3$) from the *m/z* 167 ion, suggesting that the parent compound could be an ester. Gallic acid lost H_2O in addition to the loss of the carboxylic acid group and of the 28 amu moiety. The caftaric, coutaric and fertaric acids were identified by their $[M - H]^-$ ions at *m/z* 311 (Fig. 1A), 295 (Fig. 1B) and 325, respectively. MS^2 experiments yielded the corresponding $[M - H]^-$ ions of caffeic, coumaric and ferulic acids by neutral loss of tartaric acid moiety (132 mass units) *(17)*. Chlorogenic acid showed the $[M - H]^-$ deprotonated molecule (*m/z* 353) and the ion corresponding to the deprotonated quinic acid (*m/z* 191), which was consistent with a previous report *(15)*. According to phenolic acid profile, the GV and AV were characterized by different phenolic acids. GV were characterized by high content of phenolic acids including gallic, protocatechuic, caffeic and syringic acids. AV were characterized by protocatechuic and chlorogenic acid. *p*-Coumaric acid showed the $[M - H]^-$ deprotonated molecule (*m/z* 163) and the ion corresponding to the decarboxylated coumaric acid (*m/z* 119).

Flavan-3-ols. Three flavan-3-ols including catechin, epicatechin and procyanidin B2 were determined for GV and AV. Catechin and epicatechin yielded the deprotonated molecule $[M - H]^-$ (*m/z* 289) and a loss of a $-CH_2CHOH$ group (*m/z* 245), as described by Kelebek *(12)*. The cleavage of the native procyanidin B2 ($[M - H]^-$, *m/z* 577) led to the formation of two fragments with *m/z* 289 and *m/z* 245.

Table 1. Retention times (R_t), family, wavelength for detection (DAD) and mass spectral data for analyses of phenolic compounds in vinegars using LC-DAD-ESI-MS^n detection

Code	Phenolic compounds	R_t (min)	Family	λ_{max} (nm)	MS, *m/z* M^-	MS/MS ions *m/z*	GV	AV
1	Gallic acid[a]	15.26	Phenolic acids	272	169	125	+	+
2	Protocatechuic acid[a]	22.42	Phenolic acids	294, 256	153	109	+	+
3	Caftaric acid	25.43	Phenolic acids	328	311	179, 149	+	–
4	Chlorogenic acid[a]	28.67	Phenolic acids	326	353	191	+	+
5	Coutaric acid	31.16	Phenolic acids	310	295	163	+	–
6	Caffeic acid[a]	34.28	Phenolic acids	328	179	135	+	+
7	Ferulic acid[a]	35.9	Phenolic acids	293, 323	193	149, 178, 134	+	–
8	Fertaric acid	36.07	Phenolic acids	322	325	193, 149	+	–
9	Vanillic acid[a]	39.54	Phenolic acids	260, 290	167	152, 123, 108	+	+
10	*p*-Coumaric acid[a]	43.28	Phenolic acids	310	163	119	+	+
11	Syringic acid[a]	42.31	Phenolic acids	272	197	182, 167, 153	+	–
12	Tyrosol[a]	18.41	Phenolic acids	275	137	93	+	–
13	Procyanidin B2[a]	30.19	Flavan-3-ols	265	577	289, 245	+	+
14	Catechin[a]	28.59	Flavan-3-ols	277	289	245, 205, 179	+	+
15	Epicatechin[a]	34.78	Flavan-3-ols	277	289	245, 205, 179	+	+
16	Rutin (quercetin-3-*O*-rutinoside)[a]	41.12	Flavonols	255, 352	609	301, 273, 257, 179, 151	+	+
17	Quercetin-3-*O*-galactoside[a]	42.48	Flavonols	350	463	301	+	–
18	Luteolin-*O*-glucoside[a]	44.89	Flavones	255, 262, 347	447	285, 267, 251, 243	–	+
19	Isorhamnetin-3-*O*-rutinoside[a]	54.95	Flavonols	348	623	315	–	+
20	Isorhamnetin-3-*O*-glucoside[a]	56.09	Flavonols	354	477	315	+	+
21	Kaempferol-3-*O*-rutinoside[a]	56.61	Flavonols	346	593	285	+	+
22	Quercetin-3-*O*-rhamnoside[a]	46.41	Flavonols	348	447	301	–	+
23	Quercetin[a]	57.67	Flavonols	360	301	151	+	+
24	Luteolin[a]	64.705	Flavones	259, 348	285	217, 241, 175	–	+
25	Apigenin[a]	70.96	Flavones	234, 338	269	117	–	+
26	Phloretin	48.85	Flavones	284	273	167, 123	–	+
27	Phloridzin[a]	48.31	Flavones	284	435	273, 167	–	+

GV, Grape vinegars; AV, apple vinegars.
[a]Identification confirmed by comparison with standards.

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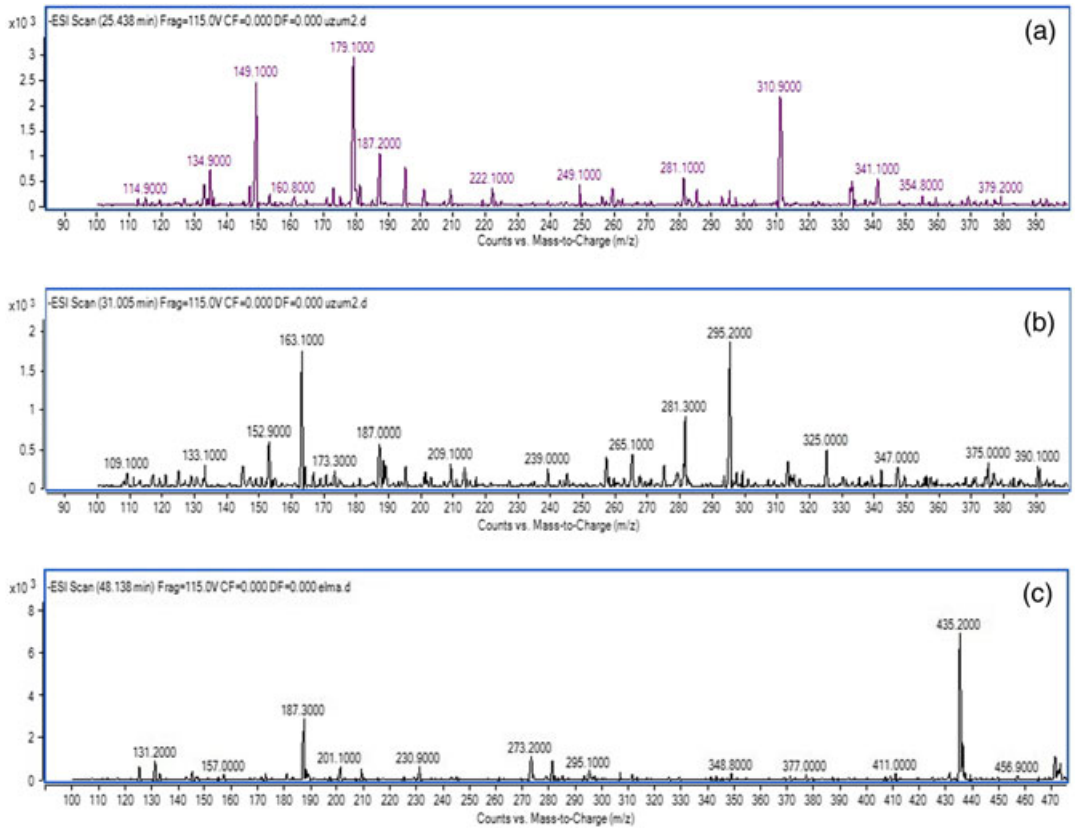

Figure 1. (a) Full mass spectrum of caftaric acid (peak 3). (b) Full mass spectrum of coutaric acid (peak 5). (c) Full mass spectrum of phloridzin (peak 27). [Colour figure can be viewed at wileyonlinelibrary.com]

Flavonols. Five flavonols were identified and quantified for GV (quercetin-3-*O*-galactoside, kaempferol-3-*O*-rutinoside, rutin, isorhamnetin 3-glucoside, quercetin), while the AV had 11 compounds (luteolin-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, quercetin-3-*O*-rhamnoside, quercetin, rutin, luteolin, apigenin, phloretin and phloridzin).

Four peaks were identified as quercetin derivatives according to their UV spectra and MS fragmentation leading to the quercetin aglycone at *m/z* 301 in negative mode. The flavonols identified in this manner were quercetin-3-rutinoside (peak 16, $[M-H]^-$ detected at *m/z* 609), quercetin-3-rhamnoside (peak 22, $[M-H]^-$ detected at *m/z* 447) and quercetin (peak 23, $[M-H]^-$ detected at *m/z* 301). Two peaks were identified as isorhamnetin derivatives according to their UV spectra and MS fragmentation leading to the isorhamnetin aglycone at *m/z* 315 in negative mode. The flavonols identified in this manner were isorhamnetin-3-*O*-rutinoside (peak 19, $[M-H]^-$ detected at *m/z* 623) and isorhamnetin-3-*O*-glucoside (peak 20, $[M-H]^-$ detected at *m/z* 477). Furthermore, luteolin-*O*-glucoside (peak 18) and luteolin (peak 24) were also detected. The $[M-H]^-$ ion at *m/z* 447 was detected and the fragmentation yielded the aglycone luteolin at *m/z* 285, by the neutral loss of 162 mass units. Phloretin is a characteristic flavonoid found exclusively in apples and in apple-derived products where it is present as the glucosidic form, namely, phloridzin (phloretin 2′-*O*-glucose). LC–MS/MS displayed molecular ion peak $[M-H]^-$ at *m/z* 435 for phloridzin, and a fragment of *m/z* 273 for the phloretin aglycon (Fig. 1C).

Phenolic composition of grape vinegars

Phenolic acids (35.52–70.77 mg/L) followed by flavanols (5.59–45.09 mg/L) were the most abundant compounds of the GV (Table 2). Andlauer et al. *(18)* reported a decrease in total phenol content by the acidification process which was highest for cider vinegars (40%) and lower for red (13%) and white wine vinegars (8%). In general, antioxidant activities and phenolic compounds in drinking vinegars were higher than those in seasoning vinegars.

Phenolic acids. Hydroxycinnamic acids (caftaric, coutaric, caffeic, ferulic, *p*-coumaric acids) comprise the most important group of non-flavonoid phenols in grape vinegars (Table 2). With respect to the composition of phenolic acids, it was noticed that gallic acid was the main phenolic acid with the highest percentage of the total phenolic acid contents. The concentration of gallic acid ranged from 7.45 to 21.84 mg/L in GV5 and GV2, respectively (Table 2). The gallic acid content in GV depends on the origin of the wine and on the enological techniques to which it has been subjected. According to the literature, the content in this phenolic compound increases with aging in wood. This fact is explained by the hydrolysis of gallic tannins during aging *(19)*. The levels of gallic acids are in the same range as the data reported previously. Parrilla et al. *(20)* examined 92 commercial GV produced from different wines from the south of Spain and reported that gallic acid concentrations in the GV ranged from 6.00 to 36.66 mg/L. Tyrosol was the second most common phenolic acid (11.54–17.68 mg/L), followed by protocatechuic acid (7.21–11.05 mg/L) and caftaric acid (1.76–15.83 mg/L) and syringic acid (1.24–9.24 mg/L) in GV.

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Table 2. Phenolic compounds of grape vinegars (mg/L ± standard deviation)

Phenolic compounds	GV1	GV2	GV3	GV4	GV5	GV6	GV7	GV8	Minimum	Maximum	Average
Gallic acid	13.25 ± 0.79	21.84 ± 1.47	12.33 ± 0.83	12.44 ± 0.84	7.45 ± 0.21	11.89 ± 0.49	15.81 ± 0.12	13.53 ± 0.91	*7.45*	*21.84*	*13.57*
Tyrosol	14.86 ± 0.84	15.96 ± 1.07	14.08 ± 0.95	11.54 ± 0.78	15.27 ± 0.42	14.63 ± 0.60	15.42 ± 0.69	17.68 ± 1.19	*11.54*	*17.68*	*14.93*
Protocatechuic acid	9.29 ± 0.52	9.98 ± 0.67	8.80 ± 0.59	7.21 ± 0.49	9.54 ± 0.26	9.14 ± 0.38	9.64 ± 0.43	11.05 ± 0.74	*7.21*	*11.05*	*9.33*
Caftaric acid	6.83 ± 0.41	4.14 ± 0.14	11.55 ± 0.78	2.93 ± 0.20	1.76 ± 0.05	4.78 ± 0.20	9.48 ± 1.45	15.83 ± 1.07	*1.76*	*15.83*	*7.16*
Chlorogenic acid	0.72 ± 0.05	0.41 ± 0.03	1.51 ± 0.10	1.77 ± 0.12	0.09 ± 0.00	0.23 ± 0.01	0.86 ± 0.16	0.30 ± 0.02	*0.09*	*1.77*	*0.74*
Coutaric acid	0.90 ± 0.06	0.41 ± 0.03	1.85 ± 0.12	0.33 ± 0.02	0.00 ± 0.00	0.86 ± 0.04	1.27 ± 0.18	1.95 ± 0.13	*0.00*	*1.95*	*0.95*
Caffeic acid	1.16 ± 0.07	0.66 ± 0.04	1.82 ± 0.12	0.41 ± 0.03	0.11 ± 0.00	1.39 ± 0.06	1.52 ± 0.24	2.58 ± 0.17	*0.11*	*2.58*	*1.21*
Ferulic acid	0.12 ± 0.01	0.12 ± 0.01	0.21 ± 0.01	0.05 ± 0.00	0.01 ± 0.00	0.12 ± 0.01	0.17 ± 0.02	0.20 ± 0.01	*0.01*	*0.21*	*0.12*
Fertaric acid	0.47 ± 0.03	0.50 ± 0.03	0.83 ± 0.06	0.18 ± 0.01	0.03 ± 0.00	0.49 ± 0.02	0.66 ± 0.07	0.79 ± 0.05	*0.03*	*0.83*	*0.49*
Vanillic acid	1.19 ± 0.08	0.54 ± 0.04	2.44 ± 0.16	0.43 ± 0.03	0.00 ± 0.00	1.13 ± 0.05	1.68 ± 0.24	2.58 ± 0.17	*0.00*	*2.58*	*1.25*
p-Coumaric acid	0.21 ± 0.01	0.13 ± 0.01	0.43 ± 0.03	0.45 ± 0.03	0.02 ± 0.00	0.09 ± 0.00	0.26 ± 0.04	0.12 ± 0.01	*0.02*	*0.45*	*0.21*
Syringic acid	3.40 ± 0.21	1.58 ± 0.11	9.04 ± 0.61	2.65 ± 0.18	1.24 ± 0.03	1.72 ± 0.07	4.80 ± 0.17	4.16 ± 0.28	*1.24*	*9.04*	*3.57*
Total phenolic acids	*52.38 ± 3.07*	*56.27 ± 3.65*	*64.89 ± 4.37*	*40.39 ± 2.72*	*35.52 ± 0.98*	*46.47 ± 1.91*	*61.57 ± 3.41*	*70.77 ± 4.77*	*35.52*	*70.77*	*53.53*
Procyanidin B2	1.63 ± 0.12	3.11 ± 0.28	2.84 ± 0.19	1.95 ± 0.13	0.51 ± 0.01	0.09 ± 0.00	2.46 ± 0.08	1.28 ± 0.09	*0.09*	*3.11*	*1.73*
Catechin	10.19 ± 0.64	4.73 ± 0.32	27.11 ± 1.83	7.94 ± 0.53	3.73 ± 0.10	5.16 ± 0.21	14.41 ± 0.51	12.47 ± 0.84	*3.73*	*27.11*	*10.72*
Epicatechin	4.38 ± 0.28	1.50 ± 0.10	15.13 ± 1.02	4.09 ± 0.28	1.35 ± 0.04	0.57 ± 0.02	6.79 ± 0.05	3.61 ± 0.24	*0.57*	*15.13*	*4.68*
Total flavanols	*16.20 ± 1.04*	*9.34 ± 0.70*	*45.09 ± 3.04*	*13.98 ± 0.94*	*5.59 ± 0.15*	*5.82 ± 0.24*	*23.66 ± 0.38*	*17.36 ± 1.17*	*5.59*	*45.09*	*17.13*
Quercetin-3-*O*-galactoside	0.18 ± 0.01	0.04 ± 0.00	0.15 ± 0.01	0.16 ± 0.01	0.04 ± 0.00	0.39 ± 0.02	0.15 ± 0.01	0.27 ± 0.02	*0.04*	*0.39*	*0.17*
Kaempferol-3-*O*-rutinoside	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	*0.00*	*0.04*	*0.02*
Rutin	0.08 ± 0.01	0.02 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.04 ± 0.00	0.20 ± 0.01	0.05 ± 0.00	0.08 ± 0.01	*0.02*	*0.20*	*0.08*
Isorhamnetin-3-*O*-glucoside	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.06 ± 0.00	0.09 ± 0.00	0.05 ± 0.00	0.08 ± 0.00	0.08 ± 0.01	*0.05*	*0.09*	*0.07*
Quercetin	0.32 ± 0.02	0.06 ± 0.00	0.69 ± 0.05	0.06 ± 0.00	0.14 ± 0.00	0.45 ± 0.02	0.39 ± 0.05	0.53 ± 0.04	*0.06*	*0.69*	*0.33*
Total flavonols	*0.67 ± 0.04*	*0.23 ± 0.03*	*1.00 ± 0.07*	*0.36 ± 0.02*	*0.32 ± 0.01*	*1.11 ± 0.05*	*0.69 ± 0.07*	*0.99 ± 0.07*	*0.23*	*1.11*	*0.67*

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Phenolic acid contents in the studied samples were in accordance with some previous reported data *(21,22)* but lower than values obtained by Cerezo et al. *(4)*. As reported previously, the main phenolic acid in vinegars were gallic acid (10–64 mg/L) and tyrosol (30–109 mg/L), whereas caftaric acid (6–19 mg/L), caffeic acid (2–5 mg/L), protocatechuic acid (7–15 mg/L), ferulic acid (0.5–6 mg/L) and syringic acid (2–5 mg/L) were found in lower concentrations *(19)*. Caftaric acid *o*-quinones may be responsible for the oxidation of procyanidins with a coupled oxidation mechanism, causing the reduction of caftaric acid quinones to caftaric acid *(21)*. Natera et al. *(19)*, in a previous chemometric study of different kinds of vinegars, according to their raw materials, showed that the balsamic vinegars contain the highest amounts of gallic acid, tyrosol, catechin and caffeic acid.

Flavan-3-ols. The highest total flavanol content was detected in GV3 (45.09 mg/L) and the lowest in GV5 (5.59 mg/L). Between the flavanols, catechin had the highest concentration in all of the GV analysed. The highest level of catechin was detected in GV3 (27.11 mg/L; Table 2). Natera et al. *(19)* reported that catechin was the main flavanol in vinegars, ranged from 7 to 22 mg/L. *(22)* reported that content of catechin in industrial vinegar (27.50 mg/L) was significantly higher than that of in traditional vinegar (13.76 mg/L). A significant decrease in catechin concentration was reported during the acidification process. This could be explained by polymerization, precipitation and oxidation phenomena *(4)*. Epicatechin was the second most frequently found flavanol, varying from 0.57 mg/L in GV6 to 15.13 mg/L in GV3, followed by procyanidin B2 (0.09–3.11 mg/L).

Flavonols. Five flavonol derivatives were identified: quercetin-3-*O*-galactoside, kaempferol-3-*O*-rutinoside, rutin, isorhamnetin-3-glucoside and quercetin. Total flavonol concentration in GV ranged from 0.23 to 1.11 mg/L, with an average of 0.67 mg/L. Within the flavonols, quercetin-3-*O*-glucoside and quercetin were found to be the major compound in all vinegars analysed (Table 2). The highest levels of quercetin-3-*O*-glucoside and quercetin were detected in GV6 (0.39 mg/L) and GV3 (0.69 mg/L), respectively. Rutin was the third most dominant flavonol, varying from 0.02 mg/L in GV3 to 0.20 mg/L in GV6. Rutin is the one of the bioactive flavonoid compounds, and is present in considerable amount in plants.

Phenolic composition of apple vinegars

Flavonoids (9.48–62.37 mg/L) followed by phenolic acids (3.81–21.44 mg/L) were the most abundant compounds of the AV.

Phenolic acids. Chlorogenic acid was the major phenolic acid followed by gallic acid, caffeic acid and *p*-coumaric acid. The concentration of chlorogenic acid ranged from 3.81 to 21.44 mg/L in AV4 and AV8 respectively (Table 3). As can be seen, chlorogenic acid is the characteristic compound, comprising the largest part of the total quantified phenolic acid in all of the AV analysed. Similarly, Budak et al. *(23)* reported that gallic acid, chlorogenic acid, caffeic acid and *p*-coumaric acids were the main phenolic acids in apple cider vinegars. Nakamura et al. *(5)* investigated the changes in chlorogenic acids, caffeic acid and *p*-coumaroylquinic acid during fermentation processes of AV. They reported that the contents of chlorogenic acids, caffeic acid and *p*-coumaroylquinic acids were 101, 99 and 105% of the original in the ethanol fermentation, respectively, whereas in acetic acid fermentation, 96.2% of the original level of chlorogenic acids, 8.3% of caffeic acid and 94.7% of *p*-coumaroylquinic acids were maintained.

Flavan-3-ols. Catechins are colourless and oxidation sensitive. Normally they occur as aglycons, but sometimes they are esterified with gallic acid. Catechins, together with chlorogenic acid, are good substrates for the enzyme polyphenoloxidase. This enzyme plays a role in the browning of fruits caused by damage, such as cutting. In the presence of oxygen, these substrates form brown complexes *(24)*. The highest total flavanol content was determined in AV8 (3.65 mg/L) and the lowest in AV1 (0.73 mg/L). Among the flavanols, procyanidin B2 indicated the highest concentration in all AV analysed. The highest level of catechin was detected in AV8 (0.95 mg/L; Table 2). Reported results for catechin and epicatechin in AV were 82.6 and 17.9 mg/L by Natera et al. *(19)*. In another study on AV catechin was reported with a similar concentration (1.47 mg/L) but epicatechin and procyanidin B2 were not detected *(25)*. Owing to the very limited number of studies on bioactive constituents of apple vinegars, the results were compared with apple juice studies. Spanos et al. *(26)* found low catechin (0–4 mg/L) and 1–9 mg/L epicatechin concentrations depending upon the apple cultivar. In commercially available apple juices, none of the catechin or epicatechin compounds was detected *(27)*.

Flavonols. Total flavonol concentration in vinegars ranged from 9.48 to 62.37 mg/L, with an average of 24.60 mg/L. Within the flavonols, phloridzin and phloretin were regarded as the major compounds in apple vinegars analysed (Table 3). The highest levels of phloridzin and phloretin were detected in AV8 with concentrations of 44.35 and 7.86 mg/L, respectively. Phloridzin was detected in AV with the amount of 1.76 mg/L by Ren et al. *(25)*. AV may be distinguished from synthetic acetic acid solutions by their relatively higher content of unique phenolic compounds, such as phloridzin. Phlorizin and its derivatives have been reported as antioxidant compounds and have a range of bioactive functions, such as inhibition of lipid peroxidation, prevention of bone loss, enhancement of memory and inhibition of cancer cell growth *(28)*. In a previous study, it was reported that, after processing of apples to apple juice, generally all flavonoid concentrations decreased to low concentrations. In apples the concentration of quercetin was found as 36 mg/kg while in commercially available apple juices, only 2.5 mg/L quercetin was detected *(29)*. This loss of flavonoid content is due to degradation of these compounds during processing *(24)*.

Antioxidant and antimicrobial activity

Several studies reported that vinegars have antimicrobial and antioxidant effects with their preventive effect on hypertension and lessening of the glycemic index of carbohydrate food, etc. *(25)*. Antioxidant potential of vinegars could be related to the type of the raw materials of vinegars *(30)*. In this study, antioxidant activity of grape vinegars changed between 5.39 and 14.43 according to DPPH assay while the values ranged from 7.72 to 17.96 with respect to ABTS assay in GV5 and GV3, respectively (Table 4). The antioxidant activity of apple vinegars changed between 2.65 and 14.69 according to DPPH assay and ranged from 4.05 to 20.19 according to ABTS assay in AV4 and AV8, respectively. ANOVA analysis of antioxidant assays revealed that there were significant differences between the vinegar samples ($p < 0.05$). The antioxidant activity of vinegars was highly affected by the phenolic composition of vinegars *(31)*. Antioxidant activities of vinegars were lower than the previously

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Table 3. Phenolic compounds of apple vinegars (mg/L ± standard deviation)

	AV1	AV2	AV3	AV4	AV5	AV6	AV7	AV8	*Minimum*	*Maximum*	*Average*
Gallic acid	0.96 ± 0.01	1.52 ± 0.02	0.47 ± 0.01	0.48 ± 0.01	0.64 ± 0.01	0.86 ± 0.01	0.89 ± 0.01	2.57 ± 0.04	*0.47*	*2.57*	*1.05*
Protocatechuic acid	1.52 ± 0.02	4.82 ± 0.07	1.51 ± 0.02	1.15 ± 0.01	1.26 ± 0.02	2.12 ± 0.02	2.21 ± 0.01	6.35 ± 0.05	*1.15*	*6.35*	*2.62*
Chlorogenic acid	3.90 ± 0.05	12.36 ± 0.17	3.86 ± 0.05	2.96 ± 0.04	3.24 ± 0.05	5.43 ± 0.04	5.67 ± 0.06	16.29 ± 0.13	*2.96*	*16.29*	*6.71*
Caffeic acid	0.39 ± 0.01	1.36 ± 0.02	0.42 ± 0.01	0.19 ± 0.00	0.33 ± 0.00	0.59 ± 0.01	0.62 ± 0.01	1.77 ± 0.02	*0.19*	*1.77*	*0.71*
Vanillic acid	1.28 ± 0.02	2.02 ± 0.03	0.63 ± 0.01	0.64 ± 0.01	0.85 ± 0.01	1.14 ± 0.02	1.18 ± 0.02	3.42 ± 0.05	*0.63*	*3.42*	*1.40*
p-Coumaric acid	0.35 ± 0.00	0.42 ± 0.01	0.13 ± 0.00	0.18 ± 0.00	0.22 ± 0.00	0.27 ± 0.00	0.29 ± 0.02	0.81 ± 0.01	*0.13*	*0.81*	*0.33*
Total phenolic acids	*8.39 ± 0.12*	*22.50 ± 0.32*	*7.03 ± 0.10*	*5.60 ± 0.08*	*6.54 ± 0.09*	*10.41 ± 0.02*	*10.86 ± 0.03*	*31.22 ± 0.06*	*3.81*	*21.44*	*8.80*
Procyanidin B2	0.54 ± 0.01	0.41 ± 0.02	0.12 ± 0.00	0.79 ± 0.01	0.48 ± 0.01	0.45 ± 0.01	0.40 ± 0.01	1.35 ± 0.04	*0.12*	*1.35*	*0.57*
Catechin	0.14 ± 0.01	0.27 ± 0.00	0.19 ± 0.01	0.64 ± 0.01	0.32 ± 0.00	0.32 ± 0.02	0.24 ± 0.00	0.95 ± 0.06	*0.14*	*0.95*	*0.38*
Epicatechin	0.04 ± 0.00	0.23 ± 0.02	0.48 ± 0.01	1.06 ± 0.01	0.53 ± 0.01	0.45 ± 0.01	0.34 ± 0.00	1.36 ± 0.03	*0.04*	*1.36*	*0.56*
Total flavanols	*0.73 ± 0.03*	*0.91 ± 0.05*	*0.79 ± 0.02*	*2.48 ± 0.03*	*1.33 ± 0.02*	*1.22 ± 0.02*	*0.99 ± 0.01*	*3.65 ± 0.05*	*0.73*	*3.65*	*1.51*
Luteolin-*O*-glucoside	0.59 ± 0.02	0.53 ± 0.01	1.16 ± 0.02	0.30 ± 0.00	0.69 ± 0.01	0.66 ± 0.03	0.73 ± 0.02	1.98 ± 0.08	*0.30*	*1.98*	*0.83*
Isorhamnetin-3-*O*-rutinoside	0.20 ± 0.01	0.40 ± 0.02	0.13 ± 0.01	0.10 ± 0.00	0.14 ± 0.00	0.21 ± 0.01	0.22 ± 0.02	0.63 ± 0.04	*0.10*	*0.63*	*0.25*
Isorhamnetin-3-*O*-glucoside	0.17 ± 0.01	0.26 ± 0.00	0.08 ± 0.01	0.09 ± 0.00	0.11 ± 0.00	0.16 ± 0.02	0.15 ± 0.00	0.48 ± 0.05	*0.08*	*0.48*	*0.19*
Kaempferol-3-*O*-rutinoside	0.06 ± 0.00	0.11 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.07 ± 0.02	0.06 ± 0.01	0.20 ± 0.05	*0.03*	*0.20*	*0.08*
Quercetin-3-*O*-rhamnoside	0.40 ± 0.01	2.53 ± 0.04	0.79 ± 0.01	0.20 ± 0.00	0.46 ± 0.01	1.14 ± 0.23	0.56 ± 0.65	3.41 ± 0.70	*0.20*	*3.41*	*1.19*
Quercetin	0.41 ± 0.01	0.96 ± 0.01	0.29 ± 0.01	0.20 ± 0.00	0.30 ± 0.00	0.47 ± 0.01	0.49 ± 0.01	1.41 ± 0.02	*0.20*	*1.41*	*0.57*
Rutin	0.09 ± 0.01	0.17 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.06 ± 0.00	0.10 ± 0.02	0.10 ± 0.02	0.29 ± 0.05	*0.04*	*0.29*	*0.11*
Luteolin	0.52 ± 0.02	0.42 ± 0.01	0.91 ± 0.01	0.27 ± 0.00	0.57 ± 0.01	0.54 ± 0.02	0.60 ± 0.02	1.63 ± 0.07	*0.27*	*1.63*	*0.68*
Apigenin	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.02 ± 0.00	0.04 ± 0.00	0.04 ± 0.01	0.05 ± 0.00	0.13 ± 0.02	*0.02*	*0.13*	*0.05*
Phloretin	1.19 ± 0.02	6.63 ± 0.09	2.09 ± 0.05	0.59 ± 0.01	1.28 ± 0.02	2.62 ± 0.04	2.76 ± 0.04	7.86 ± 0.11	*0.59*	*7.86*	*3.13*
Phloridzin	15.27 ± 0.21	11.32 ± 0.16	14.44 ± 0.14	7.64 ± 0.11	15.94 ± 0.22	14.78 ± 0.21	16.44 ± 0.23	44.35 ± 0.62	*7.64*	*44.35*	*17.52*
Total flavonols	*18.93 ± 0.22*	*23.36 ± 0.36*	*20.03 ± 0.23*	*9.48 ± 0.13*	*19.64 ± 0.28*	*20.79 ± 0.61*	*22.16 ± 0.30*	*62.37 ± 1.82*	*9.48*	*62.37*	*24.60*

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Table 4. Antimicrobial and antioxidant activity of vinegar samples

	Antimicrobial activity (zone of inhibition, mm)			Antioxidant activity (mMTrolox/L)	
Sample codes	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeroginosa*	DPPH	ABTS
GV1	10.50 ± 0.55^{BC}	10.33 ± 0.82^{ef}	11.83 ± 0.98^{f}	11.59 ± 0.78^{b}	15.83 ± 0.62^{ab}
GV2	10.00 ± 1.90^{bcd}	9.33 ± 1.03^{g}	13.00 ± 1.10^{de}	8.56 ± 0.57^{c}	11.87 ± 0.62^{c}
GV3	11.00 ± 0.00^{b}	9.83 ± 0.98^{fg}	13.50 ± 1.64^{cd}	14.43 ± 0.97^{a}	17.96 ± 1.34^{a}
GV4	9.67 ± 1.21^{cd}	11.17 ± 0.75^{de}	13.50 ± 1.05^{cd}	7.11 ± 0.48^{d}	10.03 ± 0.64^{cd}
GV5	13.00 ± 0.89^{a}	12.33 ± 1.37^{c}	13.33 ± 0.52^{cde}	5.39 ± 0.15^{e}	7.72 ± 0.20^{e}
GV6	12.50 ± 1.87^{a}	14.67 ± 0.52^{a}	15.33 ± 0.52^{ab}	6.94 ± 0.29^{d}	9.44 ± 0.12^{de}
GV7	13.33 ± 0.82^{a}	13.83 ± 0.75^{ab}	15.50 ± 0.55^{a}	11.17 ± 0.50^{b}	14.93 ± 1.44^{b}
GV8	11.00 ± 0.63^{b}	13.50 ± 0.55^{b}	13.83 ± 0.98^{cd}	9.00 ± 0.54^{c}	10.93 ± 1.14^{cd}
AV1	13.33 ± 0.52^{a}	14.00 ± 0.00^{ab}	13.83 ± 0.75^{cd}	4.24 ± 0.05^{d}	5.93 ± 0.28^{d}
AV2	10.50 ± 0.55^{BC}	12.00 ± 0.63^{cd}	12.33 ± 0.82^{ef}	6.71 ± 0.11^{b}	9.49 ± 0.14^{b}
AV3	12.50 ± 0.55^{a}	14.00 ± 0.89^{ab}	14.33 ± 0.52^{BC}	4.32 ± 0.05^{d}	6.29 ± 0.07^{d}
AV4	10.67 ± 0.52^{BC}	11.83 ± 0.98^{cd}	13.50 ± 0.55^{cd}	2.65 ± 0.04^{e}	4.05 ± 0.05^{e}
AV5	10.67 ± 0.52^{BC}	12.17 ± 1.17^{c}	13.83 ± 1.47^{cd}	4.27 ± 0.06^{d}	6.22 ± 0.08^{d}
AV6	9.83 ± 0.75^{cd}	12.17 ± 0.41^{c}	13.33 ± 0.52^{cde}	4.90 ± 0.37^{c}	7.06 ± 0.49^{c}
AV7	9.00 ± 0.00^{d}	10.17 ± 0.75^{fg}	12.33 ± 1.21^{ef}	5.14 ± 0.15^{c}	7.39 ± 0.20^{c}
AV8	No zone	No zone	No zone	14.69 ± 0.30^{a}	20.19 ± 0.41^{a}

All values are means ± standard deviations.
Values with different letters (a–g) within a row differ significantly ($p < 0.05$).

reported antioxidant activities of vinegars produced with different fruits. Matsuura et al. *(32)* reported that the antioxidant activity of plum vinegar extract of cherry blossom had a greater superoxide anion scavenging activity with a value of 39.65 mmol Trolox equivalent/L compared with red wine, which is a well-known strong antioxidant. LC–MS analysis showed that cyanidin-3-glucoside, cyanidin-3-rutinoside and caffeic acid were the major components in the phenolic extract prepared from plum vinegar extract of cherry blossom, and they possessed superoxide anion scavenging activity. Caffeic acid was found to be mainly responsible for the scavenging activity of phenolic extract with a minor contribution from cyanidin-3-glucoside and cyanidin-3-rutinoside. In the study reported previously *(33)*, total phenolics, total anthocyanins, antioxidant activities and antiradical activity of blueberry liquid products such as blueberry vinegars were investigated. According to the study, antiradical activity (DPPH assay) results ranged from 52.0 to 65.6%. Antiradical and antioxidant activities (beta-carotene bleaching assay and ferric thiocyanate assay) of vinegars were highly correlated with their phenolic content.

PCA was performed in order to check the validity of phenolic compounds, phenol content and antioxidant capacity results to discriminate vinegar samples according to the type of the raw material. The PCA model was constructed with four PCs accounting for 93.7% of total variability. PC1 and PC2 explain 59.8 and 25.4% of total variance, respectively. A PCA biplot including score and loading plots with two principal components is illustrated in Fig. 2. GV are on the right side of the graph while AV are scattered on the left side of the graph. AV are characterized by luteolin, rutin, luteolin-*O*-glucoside, phloridzin, phloretin, apigenin, quercetin-3-*O*-rhamnoside, isorhamnetin-3-*O*-rutinoside, *p*-coumaric acid, chlorogenic acid, caffeic acid and TPC. GV are characterized by vanillic acid, gallic acid, tyrosol, protocatechuic acid, caftaric acid, coutaric acid, ferulic acid, fertaric acid, syringic acid, procyanidin

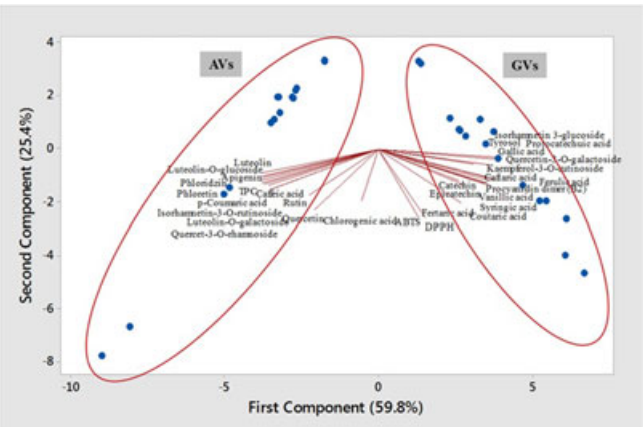

Figure 2. Principal components analysis biplot for the first two principal components of vinegar phenolic compounds. [Colour figure can be viewed at wileyonlinelibrary.com]

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B2, catechin, epicatechin, quercetin-3-*O*-galactoside, kaempferol-3-*O*-rutinoside, isorhamnetin 3-glucoside and quercetin. In the study reported by Yu et al. *(34)*, PCA was applied for discrimination and characterization of Zhenjiang aromatic vinegars according to their volatile compounds analysed using headspace solid-phase microextraction/gas chromatography–mass spectrometry. Nine alcohols, 13 acids, 16 esters, five aldehydes, four ketones and eight heterocycle compounds were identified in the samples.

The antimicrobial activities of different vinegars were tested against *S. aureus*, *E. coli* and *P. aeruginosa* strains. Zones of inhibition are given in Table 4. Statistical analyses revealed that there were significant differences between the vinegars according to their antimicrobial activities. The results indicated that AV1 and GV7 exhibited the highest antimicrobial activity against *S. aureus*, GV6 showed the highest inhibitory effect on *E. coli* and GV7 had the highest antimicrobial effect on *P. aeruginosa*. AV8 did not show any inhibitory effect on bacterial strains. Antimicrobial activities of sweet lime peel, sweet lime fruit–peel combined and sweet lime fruit vinegars were tested on *E. coli*, *S. typhi* and *Klebsiella* species. Sweet lime fruit–peel combined vinegar exhibited the highest inhibitory effect on *E. coli* and *S. typhi*, while sweet lime peel vinegar showed the highest inhibition of *Klebsiella* species *(11)*. Ozturk et al. *(30)* compared home-made vinegars with industrial vinegars with respect to their antimicrobial properties and demonstrated that Turkish traditional vinegars showed lower antimicrobial activity compared with industrial vinegars.

Conclusion

In conclusion, gallic acid, tyrosol, protocatechuic acid, caftaric acid, catechin, epicatechin and syringic acid constituted the highest proportion of the total phenolic contents in GV. Chlorogenic acid, phloridzin and phloretin were found to be the major phenolic compounds in AV. With respect to antimicrobial and antioxidant activity results, GV showed higher antimicrobial and antioxidant activity than AV. With regard to phenolic composition of vinegars with their antioxidant capacities, two separate groups were obtained and characterized the vinegars with PCA based on the type of raw material. The results we obtained in this study extend our knowledge about the composition of vinegars obtained from different raw materials consumed in Turkey and allow the consumer to compare vinegar brands with the highest contents of beneficial compounds.

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