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Bioactive components, antibacterial and antiradical properties of home-made apple and grape vinegar

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Abstract: The present study aimed to investigate major volatile compounds, organic acid, phenolic and mineral contents, and antiradical and antimicrobial properties of home-made apple/grape vinegar. Grape vinegar showed higher total acidity, organic acid content, total phenolic content (TPC), antiradical activity and lower minimum inhibitory concentration (MIC) values compared to apple vinegar. While acetic and tartaric acids were the most abundant organic acids in grape vinegar, acetic and succinic acids were the most abundant organic acids in apple vinegar. The most abundant phenolic compound was gallic acid in both grape and apple vinegar. A total of 18 and 9 volatile compounds were determined in grape vinegar samples and apple vinegar samples, respectively. The most abundant volatile compounds were acetic acid and acetoin in grape vinegar, acetic acid, ethyl acetate and 2,4,5-trimethyl-1,3-dioxolane in apple vinegar. K, Ca and Na were common minerals in both vinegar and more in the grape vinegar compared to apple vinegar. Although MIC value for grape vinegar was at 6.25% with minimum bactericidal concentration (MBC) values ranged from 6.25% to 12.50%, MIC value for apple vinegar was at 12.50% with MBC values ranged from 12.50% to 25.00% for all test bacteria. The antiradical and antibacterial activities of the vinegar samples were correlated with their TPC and organic acid contents.

Keywords: Antibacterial, antioxidant, apple, grape, vinegar.

Ev yapımı elma ve üzüm sirkelerinin kimyasal, antibakteriyel ve antiradikal özelliklerinin araştırılması

Özet: Bu çalışmada, ev yapımı elma ve üzüm sirkelerinin başlıca uçucu bileşikleri, organik asit, fenolik ve mineral içerikleri ile antiradikal ve antimikrobiyal özelliklerinin araştırılması amaçlanmıştır. Üzüm sirkesinin elma sirkesine göre daha yüksek toplam asitlik, organik asit içeriği, toplam fenolik içerik (TFİ) ve antiradikal aktiviteye sahip olduğu, aynı zamanda daha düşük minimum inhibitör konsantrasyon (MİK) değerleri gösterdiği tespit edildi. Üzüm sirkesinde, asetik ve tartarik asitler en çok bulunan organik asitti. Elma sirkesinde ise en çok bulunan organik asitler asetik ve süksinik asitlerdi. Her iki sirke çeşidinde de en fazla bulunan fenolik bileşik gallik asitti. Üzüm ve elma sirkelerinin sırasıyla 18 ve 9 adet uçucu bileşik içerdiği belirlendi. Üzüm sirkesinde asetik asit ve asetoin en çok bulunan uçucu bileşikler iken; asetik asit, etil asetat, 2,4,5-trimetil-1,3-dioksolan elma sirkesinde en fazla bulunan uçucu bileşiklerdi. K, Ca ve Na, her iki sirkede en yaygın bulunan minerallerdi. Aynı minerallerin, üzüm sirkesinde elma sirkesine kıyasla daha fazla oranda bulundukları tespit edildi. Üzüm sirkesinde test edilen bakterilere karşı MİK değerlerinin %6,25 olduğu, minimum bakterisidal konsantrasyon (MBK) değerlerinin %6,25 ile %12,50 arasında değiştiği görüldü. Elma sirkesinin test edilen bakterilere karşı MİK değerinin %12,50 olduğu, MBK değerlerinin ise tüm test bakterileri için %12,50 ile %25,00 arasında değiştiği görüldü. Yapılan çalışmada sirkelerin sahip olduğu antiradikal ve antibakteriyel aktivitelerinin, TFİ ve organik asit içerikleri ile ilişkili olduğu sonucuna varıldı.

Anahtar sözcükler: Antibakteriyel, antioksidan, elma, sirke, üzüm.

Introduction

Vinegar is a fermented product made by acetic acid bacteria that convert ethyl alcohol into acetic acid by oxidation. Vinegar can be made from fruits, cereals and vegetable and used as food supplement, tonic and nutraceutical (35, 49). Vinegar is produced from a double fermentation of any fermentable sugary substrates, and its organoleptic and chemical properties can be changed according to the type of raw materials used and fermentation methods. First step is alcoholic fermentation which is the conversion of fermentable sugars into ethanol mainly by yeast. Second step is acetic acid fermentation in which ethanol is oxidized to acetic acid aerobically by acetic acid bacteria (30, 43). In general, two different methods, traditional (slow) and submerged (quick), are used for vinegar production. Vinegar produced by slow method are high-quality in acidity and contents of aroma components but rather expensive, while vinegar produced by submerged method are quick but cheaper, which makes it the one that is most employed (14).

Foodborne pathogens (mainly bacteria) are the major cause of the diseases and affecting food safety and cause human illness worldwide (10). Also, some bacteria can cause food spoilage resulted in objectionable by-products in the food and altering the food's nutritional value and sensory properties such as smell, texture and appearance (20). Natural antimicrobial agents have advantages over antibiotics in terms of their antimicrobial resistance (41). The antimicrobial properties of vinegar not only make it the best alternative for antibiotics, but also make them a very useful product for cleaning fruits and vegetables, sanitizing surfaces and preventing the spoilage (35). In addition to its antimicrobial activity, several studies have indicated that vinegar has antimicrobial, antioxidant, antidiabetic, anti-inflammatory, antihypertensive, immune stimulatory and anticancer activities (13, 25, 34, 42, 49). These therapeutic activities are attributed to the presence of bioactive compounds including organic acids, phenolics, flavonoids, essential amino acids, vitamins and minerals in vinegar (8, 16). Hence, the aim of the present study was to evaluate the physicochemical parameters, major volatile compounds and mineral contents, antioxidant and antimicrobial properties of apple and grape vinegar produced with traditional spontaneous fermentation.

Materials and Methods

***Vinegar production*:** Vinegar samples produced traditionally from apples and grapes by adding Lavender honey via spontaneous fermentation were used in this study. The "Red delicious" apples, "Uluğbey Karası" grape fruits and Lavender honey were purchased from Isparta province of Turkey. The fruits were washed in clean water to remove residues, and the apples were shredded. The procedure of vinegar production is given on the Figure 1. Vinegar samples were produced using 10 L polypropylene food containers in Food Hygiene and Technology Laboratory (Burdur, Turkey). The samples were stored in 1 L polyethylene terephthalate jars and kept in the dark at 4ºC until required. The vinegar samples were filtered through a 0.45-µm membrane filter before being used in the tests. Vinegar production for each fruit was performed in triplicate.

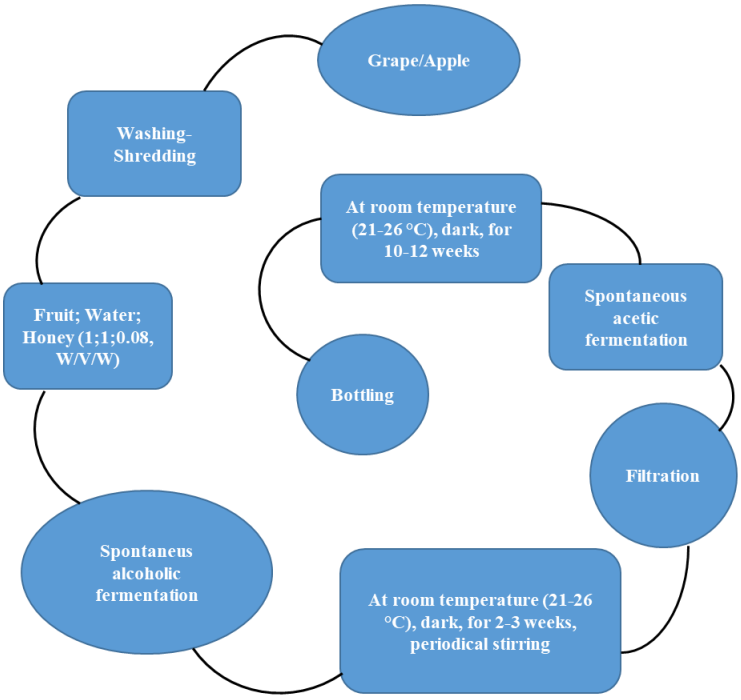

Figure 1. Vinegar production scheme.

***Physicochemical properties*:** pH values of the vinegar samples were measured with a pH meter (WTW Lab-pH Meter inoLab® pH 7110) at 25ºC ± 2ºC. Total acidity quantification of the vinegar samples was performed by titration method. Briefly, a 20 mL distilled water was mixed with 10 mL of vinegar and the diluted vinegar solution was titrated with 0.10 M NaOH. The results were expressed as acetic acid equivalent (g acetic acid/L vinegar sample) (1).

***Total phenolic content*:** Total phenolic content (TPC) of the apple and grape vinegar samples was measured by the Folin-Ciocalteu method (40). Before appropriately diluted, vinegar samples were filtered with 0.45 mm filter. The filtrate (4 mL) was mixed with Folin Ciocelteau's phenol reagent (2 mL) and Na_2CO_3 (7%, 1.6 mL). The mixture was left for 90 min at room temperature, and the absorbance was measured at 760 nm by a spectrophotometer (Thermo-MultiScan GO, ThermoScientific™ Multiskan™ GO Microplate Spectrophotometer). The standard curve was prepared using Gallic acid (methanol: water [1/99, v/v] in the range of 0.00625 - 0.1 mg/mL [$y = 6.5097x – 0.0086$] [$R^2 = 0.9956$]). Total phenolic values were expressed as mg Gallic acid equivalent (GAE)/L.

Antiradical activity: The antiradical activity of the apple and grape vinegar samples was determined as free 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (35, 39). 0.1 mL of the filtrate which was obtained by filtrating vinegar samples using 0.45 mm filter and 5 mL of 0.1 mM DPPH (Sigma-Aldrich, St. Louis, USA) solutions were mixed vigorously with vortex. The mixture incubated for 13 min at 27ºC in dark conditions. After incubation, absorbance of the final mixture was calculated at 517 nm using a spectrophotometer.

Antiradical activity (ARA, %) was calculated as below:

ARA (%) = [(ARC- ARS)/ARC] *100

ARC indicates the absorbance of control (DPPH solution) and ARS indicates the absorbance of the sample.

***Volatile compounds*:** Volatile compounds (VC) of the vinegar samples were determined using a Gas Chromatography-Mass Spectrometry GC-MS. The chromatographic analysis was performed on a Shimadzu GC-2010 Plus equipped with a mass spectrometer selective detector Shimadzu GCMS-QP2010 SE (Shimadzu, Kyoto, Japan). A three mL of vinegar sample was placed into SPME vial (Supelco 27159 15 mL clear PTFE/Siliconesepta Cap). The vial was kept for 15 minutes in a hotplate magnetic stirrer (H4000- HSE, Benchmark Scientific Inc. USA) at 45 ºC for 15 minutes, and then Fused silica SPME fiber (carboxen/ polydimethylsiloxane [CAR/PDMS]) was exposed to the headspace for 30 minutes. The desorption of absorbed volatile compounds was performed at 250 °C for 3 minutes when the fiber was inserted into the injection port and they were injected to GC-MS in the splitless mode. The GC-MS conditions and settings are shown in Table 1. The volatile compounds were compared their MS fragments with a library of mass spectra (Wiley, NIST, Tutor, FFNSC) for identification. The amount of each volatile compound was expressed as a percentage. The percentage of each compound was calculated by dividing the area of its peak to the total area under all of the peaks.

***Organic acid analysis*:** A Shimadzu HPLC system equipped with an isocratic pump (LC-10AT), a UV-VIS detector (SPD-10A set 210 nm), a column oven (CTO-10AS), a degasser (DGU-12AS), a system controller (LC-20AT) was used to determine the main organic acids in vinegar samples. Analysis was performed by the modified method described by Alhendawi et al. (2) and Krapez et al. (28). Briefly, the Supelco solid phase extraction (SPE) cartridges were conditioned with 3 mL of methanol and washed with 10 mL of distilled water. Five mL of vinegar sample were transferred to the 15-mL tubes and 2% H_3PO_4 solution was added to the tubes and the mixture was homogenized and filtered through a filter paper. One mL of filtrate was diluted with 1 mL of extraction solution (0.01 M KH_2PO_4 solution, pH adjusted to 8.00). One mL of this solution was passed through the cartridge, and the eluate was placed into a tube. The cartridge was washed with 1 mL of extraction solution. Both eluates were combined, and the aliquots of 20 µL from the eluate was injected into a HPLC system. The HPLC instrumental conditions are shown in Table 1.

Phenolic compounds: Analysis of vinegar sample was carried out by high performance liquid chromatography (HPLC) using a chromatograph equipped with an Agilent Eclipse XDB-C18 (250x4.60 mm) 5-micron column and a diode array detector (SPD-M10A, Shimadzu). The standard solutions were prepared using methanol to dissolve the chemicals to reach concentrations ranging from 0.7 to 500.0 µg/mL for gallic acid, protocatechuic acid, catechin, chlorogenic acid, caffeic acid, p-coumaric acid. The chromatographic conditions for vinegar samples were: flow rate: 0.8 mL/min; injection volume: 20 µL; column temperature: 30ºC. Methanol and acetic acid (3%) solvents were used as mobile phase (12).

***Mineral analysis*:** The concentration of mineral elements including Copper (Cu), Calcium (Ca), Chromium (Cr), Magnesium (Mg), Phosphorus (P), Potassium (K) and Sodium (Na) in vinegar samples was measured with inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin Elmer OPTIMA 5300 DV) according to EPA 6010D method (46). The ICP-OES instrumental conditions are shown in Table 1.

Table 1. GC-MS, HPLC and ICP-OES conditions and settings.

	Conditions	Setting
GC/MS	GC	Shimadzu GC- 2010 Plus
	MS	Shimadzu GCMS-QP2010 SE
	Detector and injector port temperature	250°C
	Column flow	1.61 min/mL
	Ionization method	Electron ionization (EI), 70 eV
	Carrier gas	Helium
	Column	Restek Rxi-5Sil MS (30 m * 0.25 mm, 0.25 um (Restek, 13623)
	Oven temperature	Held at 40°C for 2 mins, raised by 4°C/min to 250°C and held for 5 min at 250°C
	Used libraries	Wiley, NIST, Tutor, FFNSC
	SPME conditions	Sample was kept in the SPME vial for 15 min at 45ºC and then the fiber (Fused silica SPME fiber (CAR/PDMS) (Supelco, 57318) exposed to the headspace for 30 min. Following, the desorption was performed in 250ºC.
	SPME vial	Supelco 27159 15 mL clear PTFE /Siliconesepta Cap
HPLC	Instrument	Shimadzu HPLC system
	Detector	SPD-10Avp UV-VIS detector (210 nm)
	System controller	LC- 20AT prominence
	Auto sampler	SIL–20AC prominence
	Pump	LC- 20AT prominence
	Degasser	DGU- 20A5 prominence
	Column oven	Shimadzu CTO-10ASvp
	Column	Teknokroma Tracer Extrasil ODS-2, 250 mm × 4.6 mm id, 5 μm (TR-016059)
	Column temperature	30°C
	Mobil phase	H3PO4/H2O (pH: 2.2)
	Injection volume	20 μL
	Flow rate	0.8 mL/min
ICP-OES	Plasma gas flow	15 L/min
	Auxiliary gas flow	0.2 L/min
	Nebulizer gas flow	0.6 L/min
	Power	1450 watt
	Torch cassette position	-3
	Pump speed	1.5 mL/min
	Purge	Normal
	Resolution	Normal
	Integration time	10 seconds minimum/20 seconds maximum
	Read delay	60 sec

***Minimum inhibitory concentration (MIC)*:** The MIC and MBC of vinegar samples against 11 microorganisms including *Staphylococcus aureus* (ATCC 25923), Methicillin-Resistant *S. aureus* (ATCC 43300) (MRSA), *Bacillus cereus* (ATCC 33019), *Listeria monocytogenes* (Refik Saydam Laboratory Culture Collection, Turkey, RSKK 472), *L. monocytogenes* (RSKK 02028), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas fluorescens* (ATCC 13525), *Escherichia coli* O157:H7 (ATCC 35150), *Salmonella* Enteritidis *(*ATCC 13076), *Salmonella* Enteritidis Phage Type 4 (NCTC 13349) and *S.* Typhimurium (ATCC 14088) were determined. The MIC values of the vinegar samples was determined by using microdilution method in 96-well microplates according to the CLSI guidelines (17). The bacterial strains were cultivated in Mueller-Hinton agar (MERCK 105437) and incubated for 18-24 h at 37°C for *S. aureus*, *B. cereus*, *E. coli* O157:H7 and *Salmonella* strains, or at 30°C for *P. fluorescens* for growth. Each bacterial cell was transferred into 0.9% sterile saline buffer and adjusted to 0.5 McFarland scale which represents a concentration of approximately 1.5 x 10^8 CFU/mL. The two-fold serial dilutions of vinegar samples (50%, 25%, 12.5%, 6.75%, 3.12%, 1.56% and 0.78%) were prepared in Mueller-Hinton broth (MH, MERCK 110293) and dispensed into wells of the microplate. 20 μL of the bacterial culture was inoculated into each well. Three control tubes were maintained for each test batch (media control, organism control and extract control). After incubating the plates at 30°C for *P. fluorescens* and at 37°C for the other bacterial strains for 24 h, microbial growth (turbidity) was determined at 600 nm using a

microplate reader (Epoch, BioTek, USA). The lowest concentration of the vinegar where no visible microbial growth was selected as the MIC value.

***Minimum bactericidal concentration (MBC)*:** The MBC was determined by subculturing the suspension (10 µL) from each well in the plate on Mueller-Hinton agar. The plates were incubated at 37°C for *S. aureus*, *B. cereus*, *E. coli* O157:H7 and *Salmonella* strains, or at 30°C for *P. fluorescens* strains for 24 h. MCB values were identified by determining the lowest concentration of vinegar samples that completely killed the growth of culture (3).

Results

The pH values of apple vinegar and grape vinegar were 3.03±0.01 and 2.94±0.01, respectively. The total acidity for grape vinegar (2.43 g/100 mL) was higher than apple vinegar (0.99 g/100 mL). The pH values of the vinegar samples were in good correlation with the total acidity (Table 2).

The concentrations of phenolic compounds present in grape vinegar and apple vinegar were 26.7 µg/mL and 6.83 µg/mL, respectively. Gallic acid was found to be the major phenolic acid in the vinegar. Although the vinegar had low p-coumaric acid concentrations, chlorogenic acid was not detected in apple vinegar. The TPC values of grape vinegar and apple vinegar were 498.36 mg GAE/L and 209.10 mg GAE/L respectively. TPC values belonging to different dilutions (1/1; 1/2; 1/4; 1/8) of grape and apple vinegar were given in Table 2. A proportional decrease depending on the dilution was observed. Antiradical activity of grape vinegar and apple vinegar measured 30.14% and 16.44% and were indicated as DPPH free radical scavenging abilities (Table 2).

The results of major organic acids, phenolic acids and mineral compounds of the vinegar samples are shown in Table 3. The main organic acid in grape and apple vinegar was acetic acid, accounting for 79.9% and 84.2% of the organic acid present in grape vinegar and apple vinegar, respectively. The level of organic acids (22283.8 µg/g) in grape vinegar was higher than apple vinegar (9003.8 µg/g). While tartaric acid (3769.4 µg/g) was the second most abundant organic acid, lactic acid was not detected in grape vinegar. Whereas citric acid content in grape vinegar was low, being 0.3%, malic acid content in apple vinegar was low, being 1.1%.

Table 2. Physicochemical properties, total phenolic contents and antiradical activity of grape and apple vinegars.

Parameters	Dilutions	Apple vinegar	Grape vinegar
pH	-	3.03±0.23	2.94±0.11
Total Acidity (g/100 mL)	-	0.99±0.05	2.43±0.14
TPC (mg GAE/L)	100%	209.10±5.97	498.36±7.55
	50%	117.11±3.07	322.15±5.07
	25%	79.49±2.29	153.60±2.25
	12.5%	34.42±1.02	81.58±1.05
DPPH (%)	100%	16.44±0.12	30.14±0.75
	10%	10.40±0.66	9.87±0.10

Values are expressed as mean±SD. TPC: Total phenolic content, DPPH: 2.2-diphenyl-1-picrylhydrazyl.

Table 3. Major organic acids, phenolic acids and mineral compounds of the vinegars.

Groups	Compounds	Grape vinegar	Apple vinegar
Organic acids (µg/g)	Tartaric	3769.4 (16.9%)	110.8 (1.2%)
	Malic	323.8 (1.4%)	101.8 (1.1%)
	Lactic	ND*	467.9 (5.2%)
	Acetic	17815.8 (79.9%)	7584.4 (84.2%)
	Citric	67 (0.3%)	167.1 (1.8%)
	Succinic	307.7 (1.3%)	571.8 (6.3)
Phenolic compounds (µg/mL)	Gallic acid	10.8	4.5
	Protocatechuic acid	2.4	0.7
	Catechin	4.4	1.3
	Chlorogenic acid	3.1	*
	Caffeic Acid	5.2	0.3
	P-coumaric acid	0.8	0.03
Mineral content (mg/g)	Cu	<0.006 µg/g	<0.006 µg/g
	Mg	5.696±0.4846	5.389±0.2275
	Cr	<0.005 µg/g	<0.005 µg/g
	Ca	30.04±0.522	6.715±0.2967
	K	153.8±5.22	131.2±4.29
	Na	21.85±1.502	15.37±0.734
	P	10.90±0.087	6.901±0.1194

*ND: Non-Detected.

K, Ca, Na, P and Mg were the most abundant minerals found in the vinegar (Table 3). Grape vinegar was richer than apple vinegar in terms of the amounts of these minerals. The concentrations of Cu and Cr in the vinegar were <0.006 and <0.005 µg/g, respectively.

Volatile compounds of the vinegars were presented in Table 4. A total of 9 and 18 volatile compounds were found in apple and grape vinegar, respectively. While acetic acid and acetoin were the most abundant in apple vinegar, acetic acid, ethyl acetate and 2,4,5-trimethyl-1,3-dioxolane were the most abundant in grape vinegar. Acetic acid constituted 67.50% and 62.37% of apple and grape vinegar, respectively.

Table 5 and Figure 2 show the antibacterial activity of the vinegar against test bacteria. The MIC value of the vinegar was determined against eleven microorganisms (six Gr (+) and five Gr (-) bacteria). Although MIC value for grape vinegar was at 6.25% with MBC values ranged from 6.25% to 12.5%, MIC value for apple vinegar was at 12.5% with MBC values ranged from 12.5% to 25% for all test bacteria. Both grape (6.25%) and apple vinegar (12.5%) had similar MIC and MBC values on *B. cereus*, *S.* Enteritidis, *S.* Enteritidis PT4 and *S.* Typhimurium. The MBC values of grape vinegar and apple vinegar for *L. monocytogenes*, *E. faecalis*, MRSA, *S. aureus*, *P. fluorescens* and *E. coli* O157:H7 were twice the MIC values.

Table 4. The volatile compounds of traditional apple and grape vinegars.

Compounds	Apple vinegar (%)	Grape vinegar (%)
2,3-Butanedione	1.88	ND*
2-Methylbutanal	5.97	ND*
Acetic acid	67.50	62.37
Acetoin	22.72	0.84
3-Methyl-1-butanol	0.41	0.65
2-Methyl-1-butanol	0.36	ND*
6-Methyl-5-hepten-2-one	0.19	ND*
n-Hexanoic acid	0.27	ND*
Phenylethyl Alcohol	0.71	ND*
Acetaldehyde	ND*	0.66
Ethyl alcohol	ND*	0.40
Butan-3-Enoic Acid Methyl Ester	ND*	0.35
Methyl acetate	ND*	1.02
Ethyl Acetate	ND*	27.72
3-Methylbutanal	ND*	0.10
2,4,5-trimethyl-1,3-dioxolane	ND*	20.06
1-Butanol, 2-methyl-, (.+/-.)	ND*	0.83
Isobutyrate <ethyl->	ND*	0.14
Isobutyl acetate	ND*	1.08
Furfural	ND*	0.23
Isovalerate <ethyl->	ND*	0.30
iso-Valeric Acid	ND*	0.38
Isoamyl acetate	ND*	1.46
11-Butanol, 2-methyl-, acetate	ND*	1.39

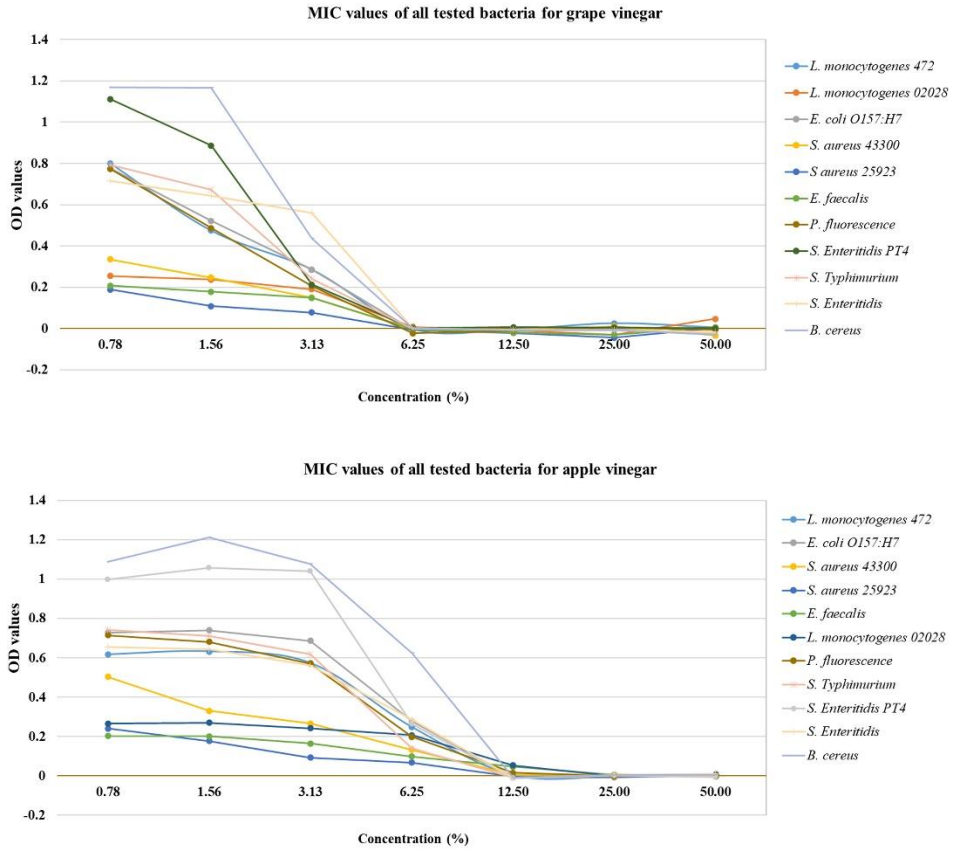

Figure 2. MIC values of grape and apple vinegars for food pathogens.

Table 5. Antimicrobial activity of home-made apple and grape vinegar against some pathogens.

	Strains	Grape vinegar		Apple vinegar	
		MIC (%)	MBC (%)	MIC (%)	MBC (%)
Gr(+)	*B. cereus* ATCC 33019	6.25	6.25	12.5	12.5
	L. monocytogenes RSK 472	6.25	12.5	12.5	25
	L. monocytogenes RSK 02028	6.25	12.5	12.5	25
	E. faecalis ATCC 29212	6.25	12.5	12.5	25
	S. aureus ATCC 43300	6.25	12.5	12.5	25
	MRSA ATCC 25923	6.25	12.5	12.5	25
Gr(-)	*S.* Enteritidis ATCC 13076	6.25	6.25	12.5	12.5
	S. Enteritidis PT4 NCTC 13349	6.25	6.25	12.5	12.5
	S. Typhimurium ATCC 14088	6.25	6.25	12.5	12.5
	P. fluorescens ATCC 13525	6.25	12.5	12.5	25
	E. coli O157:H7 ATCC 35150	6.25	12.5	12.5	25

MRSA: Methicillin-resistant *Staphylococcus aureus*.

Discussion and Conclusion

The search for potential alternatives to antibiotic has become more important due to the increasing occurrence of antimicrobial resistance among bacteria (41). Plants or plant extracts may contain active ingredients having antibacterial properties (27, 45). Vinegar is fermented traditional plant-based products and have several functional therapeutic properties such as antimicrobial, antioxidant due to the presence of active substances from plants. A wide variety of different vinegars are produced from raw materials of different agricultural origin containing starch and sugars around the world (35, 49).

Depending on its origin and production methods, pH values of vinegar may differ. In this study, pH values in grape and apple vinegar varied from 2.36 to 3.27 which were in line with previous studies (1, 18). pH value was lower in grape vinegar than in apple vinegar. Total acidity is an important indicator for assessing the quality of vinegar. Although commercial vinegar should comply with the national standard, it is not always possible to obtain standard acidity values for home-made vinegar. In the current study, the vinegar samples did not comply with regulatory limits for total acidity (total acidity ≥40g/L) (44).

In general, our findings showed that K, Ca and Na were the most abundant minerals present in both vinegar, and grape vinegar was rich in the minerals ranked as K, Ca, Na, Mg and P in a descending order, respectively, compared to apple vinegar. The results were consistent with previous study (1,35). Cu level of <0.006 µg/g in the vinegar samples was in conformity with the maximum limit (for Cu+Zn), which was 10 mg/L, approved by Turkish Food Codex (4).

Phenolic compounds in vinegar mainly derived from raw material used in the preparation of the vinegar and are the major ingredients for the antioxidant activities of the vinegar. The phenolic compounds contain one or more hydroxyl groups attached directly to the aromatic ring and are acknowledged as strong antioxidants playing an important role in pharmacological properties such as antibacterial (29, 50). Many studies demonstrated that presence of phenolic compounds in vinegar promoted their antibacterial activity (7, 37). Production methods and raw material used in the preparation of vinegar may result in differences in the phenolic composition of the vinegar (8, 12). In addition, it has been reported that vinegar made from Uluğbey Karası grapes exhibited high antioxidant activity due to its phenolic substances and red delicious apple had high phenolic compounds compared to other kinds of apples (12, 15).

The findings of this study showed that the TPC value for the grape vinegar were near 2.5 times higher than the apple vinegar. Similarly, the concentrations of all phenolic compounds were higher in the grape vinegar compared to the apple vinegar. Ozturk et al. (35) measured TPC and antioxidant activity of traditional home-made vinegar collected from different regions of Turkey. They obtain variable result in terms of TPC and antiradical activity levels. Although the TPC and antiradical activities measured in grape and apple vinegar samples were in a wide range, similar to our study, in general they found higher TPC and antiradical activity in grape vinegar than apple vinegar. These differences may be result of different type and composition of the raw material of vinegar.

Kelebek et al. (26) analyzed three batches of eight apple vinegar samples and eight grape vinegar samples of different brands, produced with biological fermentation. The grape vinegar was produced from grapes obtained from the Aegean region, while the apple vinegar was produced from apples obtained from Central Anatolia Region. The concentration of gallic acid ranged in grape and apple vinegar from 7.45-21.84 and 0.47-2.57 mg/L,

respectively. We measured gallic acid values 10.8 mg/L in grape vinegar and 4.5 mg/L in apple vinegar. The gallic acid content in grape vinegar depends on the origin of the wine and on the enological techniques to which it has been subjected. Their result also showed that antioxidant activity of grape vinegar changed between 5.39 and 14.43 and the antioxidant activity of apple vinegar changed between 2.65 and 14.69 (mMTrolox/L). Contrary to their findings, we measured higher antiradical activity in grape vinegar than apple vinegar. Liu et al. (31) determined the antioxidant activities, TPC of 23 commonly-consumed fruit vinegar. The TPC values of fruit vinegar ranged from 29.64 to 3216.60 mg GAE/L. The fruit vinegar with the highest TPC values were balsamic vinegar (3216.60 mg GAE/L). Although TPC values were higher than our results, similarly, they also report that red wine vinegar had higher (ranged between 993.51- 654.95 mg GAE/L) TPC values than apple vinegar (495.52 mg GAE/L).

The results of this study indicated that grape vinegar displayed significantly higher TPC values and antiradical activity compared to apple vinegar. In addition, TPC values and antiradical activity were found to be higher in 1/1 and 1/2 diluted grape vinegar than in 1/1 diluted apple vinegar, probably due to the phenolic contents, as we measured gallic acid, protocatechuic acid, catechin, chlorogenic acid, caffeic acid, p-coumaric acid values much higher in grape vinegar. In this study, the effects of dilution of vinegar on antiradical activity and TPC were also examined. TPC values decreased in both grape and apple vinegar in proportion to dilution (Table 2). Antiradical activity also decreased depending on the dilution (Table 2). Interestingly, antiradical activity did not reduce at the same rate of the dilution. While there was a 36% reduction of antiradical activity of apple vinegar, it was measured as approximately 67.2% in the grape vinegar in 1/10 dilution. The changes in antiradical activity depending on dilution of samples were similar to the results of the study conducted by Aydin and Gokisik (5). Many studies indicated that the antioxidant capacity of vinegar was highly correlated with their phenolic contents being affected by the raw materials and manufacturing processes (6, 47). Similarly, a correlation was found between phenolic content and antiradical activity in this study.

Organic acids, volatile compounds and other fermentation products in vinegar play a role on its organoleptic and antimicrobial properties (13, 43). Organic acids are used in food industry applications to control pathogenic bacteria (36). All test microorganisms were found sensitive to both vinegar samples and grape vinegar has exhibited higher antibacterial activity than apple vinegar. Amongst the tested bacteria, While *Salmonella* spp. and *B. cereus* were more sensitive to both grape vinegar and apple vinegar, *S. aureus*, MRSA, *L. monocytogenes*, *E. faecalis*, *P. fluorescens* and *E. coli* O157:H7 were less sensitive to both grape vinegar and apple vinegar. A large number of studies indicated that home-made vinegar had antibacterial activity on a wide range of food pathogens (19, 23, 24, 35, 38). Vinegar is rich in polyphenols such as gallic, protocatechuic, chlorogenic, caffeic acids and organic acids such as citric, malic, tartaric, lactic, acetic and succinic acids, which are responsible for antimicrobial activity (31, 48). Organic acids act on bacteria via destruction of the outer membrane of bacteria, inhibition of macromolecular synthesis and increase in intracellular osmotic pressure (16). Polyphenols show antibacterial activity by alteration in the permeability of the bacterial cell wall, in various intracellular functions and the cell wall rigidity (11). In this study, the high antimicrobial activity of grape vinegar may be related to having high level of organic acid and phenolic compounds in grape vinegar compared to apple vinegar. Antibacterial potential of the vinegar depends on the amount of the organic acid and phenolic compounds and the findings of this study are consistent with previous studies (23, 24, 35, 38). Bioactive properties of the vinegar can change according to the type and composition of the raw material. The differences in the antibacterial activity between apple and grape vinegar were attributed to their different organic acids and other compositions present in the vinegar.

In this study, the honey, which is a monofloral honey produced in the lavender fields in Isparta province, was included before the initial fermentation step (Figure 1). The honey is rich in antioxidants including phenolic acids, organic acids and flavonoids that exhibit antioxidant activity (9). Honey also exhibits antibacterial activity against a large diversity of bacteria due to its high sugar content and low pH level (3, 32). In the current study, the honey added in the vinegar production process may have contributed to the antiradical and antibacterial activity of the vinegar due to its components such as sugars, flavonoid, phenolic acid and organic acid.

The antibacterial activity of the vinegar varies for each bacterial species, and the activity depends on the raw material used for vinegar production. In this study, home-made grape vinegar contained higher amounts of organic acids and phenolic compounds than apple vinegar, therefore it had a stronger antibacterial effect on food pathogens. The antibacterial activity was in a positive correlation with the concentration of phenolic compounds and organic acids. The vinegar samples had high antiradical activity; grape vinegar showed stronger activity than apple vinegar. Antiradical capacity of the vinegar was related with their phenolic, volatile and organic acid contents. Our findings confirmed that home-made vinegar had significant bacteriostatic and bactericidal activities on several pathogenic bacteria.

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Ethical Statement

This study does not present any ethical concerns.

Conflicts of Interest

The authors declare no conflict of interest.

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