



Comparison of traditional Zivzik pomegranate vinegar against commercial pomegranate vinegar: antioxidant activity and chemical composition

Abdulkерим AYBEK¹, Ebru AKKEMİK^{1,2*}

¹ Siirt University, Faculty of Engineering, Food Engineering Department, Siirt, Türkiye

² Siirt University, Science and Technology Application and Research Center, Siirt, Türkiye

Abdulkерим AYBEK ORCID No: 0000-0002-9543-8659

Ebru AKKEMİK ORCID No: 0000-0002-4177-4884

*Corresponding author: eakkemik@siirt.edu.tr

(Received: 05.05.2022, Accepted: 13.06.2022, Online Publication: 29.09.2022)

Keywords

Chlorogenic acid,
Gallic acid,
HPLC,
ICP-OES,
Potassium,
Zivzik
Pomegranate
vinegar

Abstract: The vinegar, which is well-known for its health benefits, changes its application potential and chemical qualities depending on the raw material from which it is made. In this study, physicochemical properties, total phenolic, total flavonoid, and total anthocyanin content, organic acid, sugar, and phenolic acid composition by high performance liquid chromatography (HPLC), elemental analysis by inductively coupled plasma-optical emission spectrometer (ICP-OES), and antioxidant properties of Zivzik pomegranate vinegar (ZPV) produced using traditional methods from Zivzik pomegranate varieties were compared to commercial pomegranate vinegar (CPV). Total monomeric anthocyanin, total phenolic content and total flavonoid content of ZPV was determined to be 32.39 ± 2.6425 (mg cyanidin-3-glucoside L⁻¹), 1823.529 ± 124784 (mg GAE L⁻¹) and 2.7571 ± 0.2603 (mg rutin mL⁻¹), respectively. Total monomeric anthocyanin, total phenolic content, and total flavonoid content of CPV was determined to be 1.136 ± 0.6054 (mg cyanidin-3-glucoside L⁻¹), 5764.706 ± 848.365 (mg GAE L⁻¹) and 1.3932 ± 0.0349 (mg rutin mL⁻¹), respectively. K is the most abundant element in both vinegar samples. Acetic acid is the most common organic acid found in both forms of vinegar. While chlorogenic acid was determined to be the most prevalent phenolic compound in commercial pomegranate vinegar, gallic acid was shown to be the most prevalent in Zivzik pomegranate vinegar. As a result, while it was determined that the origin and type of the raw material had a direct impact on the product quality, it was also shown that vinegar made using traditional methods was healthier than commercial vinegar.

178

Geleneksel Zivzik Nar Sirkesinin Ticari Nar Sirkesi ile Karşılaştırılması: Antioksidan Aktivite ve Kimyasal Bileşim

Anahtar

Kelimeler

Klorojenik asit,
Gallik asit,
HPLC,
ICP-OES,
Potasyum,
Zivzik Nar
sirkesi

Öz: Sağlık için oldukça faydalı olduğu bilinen sirkenin, elde edildiği hammaddeye göre kullanım potansiyeli ve kimyasal özellikleri değiştirmektedir. Bu çalışmada, nar çeşitlerinden biri olan Zivzik narından, geleneksel yöntemlerle üretilen Zivzik nar sirkesinin, fizikokimyasal özellikleri, antioksidan özellikleri, toplam fenolik, toplam flavonoid ve toplam antosiyantan içeriği, HPLC yardımıyla organik asit, şeker ve fenolik asit bileşimi ICP-OES ile element analizi yapılarak ticari nar sirkesi ile karşılaştırılmıştır. Çalışma sonucunda Zivzik nar sirkesinin ticari sirkeye göre daha fazla antioksidan olduğu bulunmuştur. Her iki sirke örneğinde de K baskın element olarak belirlenmiştir. Benzer şekilde, asetik asit, her iki sirke türünde de tespit edilen baskın organik asittir. Ticari nar sirkesinde klorojenik asit baskın fenolik bileşik iken, Zivzik nar sirkesinde gallik asidin baskın olduğu belirlendi. Sonuç olarak hammaddenin menşei ve çeşidinin elde edilen ürün kalitesine doğrudan etkisi olduğu belirlenirken, geleneksel yöntemlerle elde edilen sirkenin sağlık açısından ticari sirkeye göre daha faydalı olduğu sonucuna varıldı.

1. INTRODUCTION

With a history dating back to 3000 B.C vinegar [1] is defined as a liquid produced by ethyl alcohol and acetic acid fermentation of different raw materials [2]. As in the past, vinegar is a favorite material for health and cosmetic purposes as food stuff or disinfectant [3]. In addition, it has been known that vinegar, has antitumor [4], antibacterial [5], antidiabetic [6], antiviral properties [7] and has dermatological benefits [5].

Different fruits, vegetables and grains are used as raw materials in vinegar making. Dimrit grape [8], black carrot [9], black mulberry [10], persimmon [11], rice [12], Trebbiano or Lambrusco grapes (balsamic vinegar) [13], and pomegranate [14] are various raw materials for vinegar making.

The quality of the vinegar depends directly on the raw material. The composition of raw materials is influenced by factors such as cultivation techniques, climate, soil, variety, etc [15]. Besides, it has been stated that vinegar could have different effects on health depending upon the raw material used. For example, it was mentioned that rice vinegar regulates blood pressure, grape vinegar facilitates and smooths digestion and grain vinegar has appetizing property [16].

There have been studies in literature dealing with the effects of factors such as fruit variety, growing conditions and harvest time on product quality. In addition to the studies investigating the effects of grape varieties on wine quality [17], there have been studies reporting that the composition of the phenolic substances in the wine produced is influenced by the variety [18]. Pomegranate has a history as old as that of vinegar [19] and has gained more recognition and importance with advances in growing techniques and food technology, especially with the advances in transportation and storage. Thus, production, consumption and trade of the pomegranate have been steadily increasing. Pomegranate is usually consumed for table, but it also has considerable consumption as sour sauce, molasses, concentrate and vinegar. Nevertheless, there have been limited number of studies on pomegranate vinegar.

The pomegranate vinegar obtained from Gapsi pomegranate, a pomegranate variety grown in Tunisia, was compared with juice, wine and commercial vinegar [14]. Another study examined phenolic substance content, antioxidant properties and color parameters of the vinegar produced from Northern Greek pomegranate genotype [20]. Antioxidant, color and phenolic substance profiles were investigated in vinegar samples obtained from Hicaz and Beynar pomegranate varieties [21]. All of these studies aimed to reveal the effect of the fruit varieties used on the product content. Raw material is the most important quality factor in vinegar production, but the cultivated variety of a certain type of plant material also has a considerable effect on the quality. Vinegar made from different varieties of the same fruit could have different quality characteristics. Even if their aromas are similar or the same, vinegars produced from different varieties may

have different compositions. As a result, the type of fruit used as raw materials would affect the final vinegar product depending upon its composition. In the present study, vinegar production was made from Zivzik pomegranate variety for the first time. Antioxidant and chemical compositions of the vinegar produced were compared to a commercial vinegar. Thus, the potential health benefits of both vinegars were compared.

2. MATERIAL AND METHOD

2.1. Material

The Zivzik Pomegranate that we used as a material in the study was purchased from pomegranate producers in Siirt in November. In addition, *Saccharomyces cerevisiae* for alcohol fermentation and vinegar mother for acid fermentation were obtained from the market. Acetonitrile, Diphenyl-2-picryl hydrazine, AlCl₃, routine, gallic acid, Folin, Ciocalteu reagent, and K-Phosphate were provided from either Sigma – Aldrich or E. Merck. All the other chemical substances used were of analytical grade and obtained from either Sigma–Aldrich or E. Merck.

2.2. Vinegar Production

Zivzik pomegranate vinegar was traditionally produced according to the Kirci (2017) method [22]. Alcoholic fermentation was carried out by adding 0.25 gL⁻¹ *Saccharomyces cerevisiae* to the fruit juice obtained by pressing the method from Zivzik pomegranate. Alcohol fermentation was terminated when the alcohol content was 7%. Later, 5% vinegar mother was added to the pomegranate solution for acid fermentation. The pomegranate solution was brought into contact with air to decrease the alcohol content and accelerate the fermentation. Fermentation was terminated when the alcohol content of the pomegranate solution was below 0.5%.

2.3. Physicochemical Analysis in Vinegar Samples

The total acidity according to the titrimetric method was made [23, 24]. Dry matter dissolved in water was read as %brix value. For this, a refractometer (HANNA-HI 96801-Refractometer) was used [24, 25]. Total dry matter analysis was done by drying in the incubator (POL-EKO-APARATURE-SLN-53-std) [8, 24]. The color analysis was measured with a colorimeter (Pencolorart USB models, 1L, Istanbul-Turkey) [24, 26].

2.4. Analysis of total monomeric anthocyanin, total phenolic substance, and total flavonoid in vinegar samples

Total monomeric anthocyanin analysis was made by modifying the pH differential method used by Fuleki & Francis (1968) [27]. Total flavonoid analysis was performed by modifying the colorimetric method of AlCl₃ in accordance with our laboratory conditions. The readings were performed at 510 nm wavelength with the Multiskan Go (Thermo 1424-7028) device. Flavonoid content was calculated as mg rutin equivalent (QE) [28,

29]. The total phenolic content of the vinegar samples was determined by used Folin and Ciocalteu reagent at 760 nm with Multiskan Go device (Thermo 1424-7028) as gallic acid equivalent [30, 31].

2.5. Organic Acid, Sugar, and Phenolic Acid Analyses with HPLC in Vinegar Samples

The relevant study was carried out by the Siirt University, Science and Technology Application and Research Center, by receiving service. Organic acid analysis in HPLC has been used in accordance with our laboratory conditions by modifying the Tormo and Izco (2004) method [32]. 0.5 mL of vinegar sample was diluted 1-1 ratio with 10 mM K-Phosphate pH 2.4 buffer, passed through a micropore filter (0.22 µm) and transferred to vials. HPLC-DAD (Thermo/DIONEX Ultimate 3000 series, Thermo Fisher Scientific, the US) device was used for the quantitative analysis of organic acids. Reverse phase column (C-18 Inertsil ODS-3 column, 5 µm particle, 4.6 × 250 mm ID) was used for the analysis of organic acids. In the chromatographic distinction, the mobile phase A (10 mM K-Phosphate pH 2.4) and mobile phase B (acetonitrile) were used. The column flow rate was set at 1 mL min⁻¹ and the column temperature was set to 28°C throughout the analysis. Sample injection volume was adjusted to 50 µL. Standard injection volume was adjusted to 20 µL. During the elution, changes were made to obtain a gradient of A %90, B %10 (1 min; 1mL min⁻¹), A %90, B %10 (12 min; 0.75 mL min⁻¹), and A %25, B %75 (0.75 min; 35mL min⁻¹). The chromatograms of the study were analyzed at 210 nm wavelengths. The phenolic content of the vinegar samples was made according to Akkemik et al., (2019) [33]. Vanillic acid, chlorogenic acid, gallic acid, caffeic acid, and hydroxybenzoic acid were used as standards. Organic sugar analysis of vinegar samples was determined according to TS 13359 method [34]. The study was repeated three times.

2.6. Analysis of Macro Micronutrients with ICP-OES in Vinegar Samples

The relevant study was carried out by the Siirt University, Science and Technology Application and Research Center, by receiving service. 0.5 mL of vinegar samples were taken and placed in teflon decomposition tubes. 6 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide were added on them. It was kept in the fume hood for 2 hours until the gas output was completed. Then the lids of the tubes were closed and placed in a microwave oven (Berghof Speedwave MWS-2). The microwave oven was operated in three different stage. In the first stage, it was terminated by waiting at 180°C for 10 minutes (99% power), in the second stage at 180°C for 15 minutes (99% power) and at 25°C for 5 minutes (80% power) in the last stage [35]. All samples were then placed in the ICP-OES autosampler unit (Device Perkin Elmer ICP-OES Optima 2100 DV ICP-OES; Plasma power, 1300 W; nebulizer flow, 0.80 L min⁻¹; Plasma flow, 15 L min⁻¹; auxiliary flow, 0.2 L min⁻¹; Plasma View, Axial; Sample Flow Rate, 1.50 mL min⁻¹; Delay Time, 60 sec; Source Equilibration Delay, 15 sec; Plasma Conditions,

Same for All Elements; Nebulizer Start-up, Instant). Calibration solutions were prepared at five different concentrations ranging from 25.00 to 1000.00 ppb using [36].

2.7. Antioxidant Analysis in Vinegar Samples

Free radical removal activity of DPPH (Diphenyl-2-picryl hydrazine) in vinegar samples was performed according to the Blois method in accordance with our laboratory conditions [37]. FRAP (Ferric reducing antioxidant power) analysis in vinegar samples [38, 39] was measured in Multiskango (Thermo, serial no: 1424-7028) device at 593 nm wavelength with minor modifications. Cuprac analysis based on the reduction principle of Cu²⁺ ions [40] was modified in accordance with our laboratory conditions, and the absorbance values at 450 nm wavelength were determined with the Multiskango (Thermo serial no: 1424-7028) device.

2.8. Statistical Analysis

Microsoft Excel program was used for statistical analysis of the obtained data. The mean values and standard errors of all data were calculated.

3. RESULTS AND DISCUSSION

3.1. Physicochemical Analyses in Vinegar Samples

Average pH value during acetic acid fermentation was 2.93±0.850 in vinegar samples produced from Zivzik pomegranate. In commercial pomegranate vinegar, on the other hand, the pH value was 2.83±0.10 (Table 1). pH values were reported to be between 2.61±0.04 and 3.03±0.10 for different pomegranate vinegars [14, 20, 21]. Thus, pH value of vinegar produced from Zivzik pomegranate was similar to the values reported in the literature.

Table 1. Physicochemical analysis of vinegar samples

PHYSICOCHEMICAL ANALYSIS		ZIVZIK POMEGRANATE VINEGAR	COMMERCIAL POMEGRANATE VINEGAR
pH		2.95±0.1	2.83±0.1
TSS (°B)		4.80±0.100	12.17±0.058
Total dry matter (%)		2.686±0.088	7.834±0.129
g acetic acid L ⁻¹		24±0.6	41.2±0.92
Color analysis	L	8.4133±0.5992	8.1467±0.1380
	a*	1.5567±0.2318	1.6400±0.3477
	b*	-4.6300±0.5524	-4.0767±1.4008

TSS, Total soluble dry matter, L, brightness; a*, green-red color coordinate; b*, blue-yellow color coordinate

In terms of total acidity calculated based on milliequivalent weight of acetic acid, Zivzik pomegranate vinegar had an equivalent weight of 24±0.6 g L⁻¹ while commercial pomegranate vinegar had an acetic acid equivalent weight of 41.2±0.92 g L⁻¹ (Table 1). The total acidity of Zivzik pomegranate juice was reported to be 0.9753±0.0532 [41]. During the fermentation, acetic acid forms. However, if fermentation of the vinegar continues, vinegar bacteria break down the acetic acid and lead to upper oxidation. For this reason, fermentation is stopped when the acidity rate of vinegar drops to the level of 0.5%

alcohol [42, 43]. In the present study, when the alcohol content was 0.5% or below, the process was stopped. However, the expected acidity rate was not reached in vinegar sample produced from Zivzik pomegranate. The reason for this could be the break-down of the acetic acid during the experiment. In a study comparing vinegar samples produced by traditional (19 samples) and industrial methods (6 samples), it was found that all but one of the vinegars produced by traditional methods had total acidity levels of less than 40 g L⁻¹ but that the vinegars produced by industrial methods had acidity levels equal to or above 40 g L⁻¹ [24]. Similar results were also obtained in the present study.

The total dry matter and water-soluble dry matter contents of the Zivzik pomegranate vinegar appeared to be lower compared to commercial pomegranate vinegar (Table 1). The total and water-soluble dry matter contents of the vinegars produced by traditional methods were generally low in previous studies, but these contents in the vinegar produced from the Zivzik pomegranate were similar to the values reported in the literature [5, 10, 24]. It is thought that the reason for the high water-soluble dry matter and total dry matter of commercial pomegranate vinegar is due to additives used during the production of the vinegar. L, a and b values of Zivzik pomegranate vinegar and commercial vinegar were generally close to each other, and both vinegars were pale and red in color.

3.2. Total Monomeric Anthocyanin, Total Phenolic Content, and Total Flavonoid Analyses in Vinegar Samples

Table 2. Total monomeric anthocyanin, total flavonoid, and total phenolic content in vinegar samples

	TOTAL MONOMERIC ANTHOCYANIN (MG CYANIDİN-3-GLUCOSİDE L ⁻¹)	TOTAL PHENOLIC CONTENT (MG GAE L ⁻¹)	TOTAL FLAVONOID CONTENT (MG RUTİN ML ⁻¹)
Zivzik pomegranate vinegar	32.39±2.6425	1823.529±124.784	2.7571±0.2603
Commercial pomegranate vinegar	1.136±0.6054	5764.706±848.365	1.3932±0.0349

3.3. Organic Acid, Sugar, and Phenolic Acid Analyses with HPLC in Vinegar Samples

The dominant organic acid in vinegar samples was acetic acid (Table 3). However, the ratio of acetic acid in commercial pomegranate vinegar was higher. These findings confirmed the results with the titratable acetic acid. It was reported that traditional vinegar samples generally had titratable acetic acid levels of less than 40 gL⁻¹ [24]. In a study comparing the malic, citric, oxalic, and tartaric acid contents of pomegranate juice samples of different pomegranate varieties including Zivzik, it was reported that citric acid was dominant in all varieties and that the highest malic acid content was measured in Zivzik variety [41]. It was mentioned that ecological conditions and fruit maturity level were responsible for the organic acid contents in juices of different pomegranate varieties [41, 44]. Fructose, glucose and saccharose sugar contents were determined and compared in vinegar samples (Table 3). The results showed that Zivzik pomegranate vinegar had no saccharose while its fructose and glucose contents were higher than the commercial pomegranate vinegar. This once again proved that the composition of vinegar

Zivzik pomegranate vinegar had 32.39±2.6425 mg of cyanidine 3-glucoside L-1 equivalent while commercial pomegranate had 1.136±0.6054 mg cyanidine 3-glucoside L-1 equivalent (Table 2). Although the color analyses of Zivzik pomegranate vinegar and commercial vinegar were similar, about 30 times difference was found between the two vinegars for anthocyanin rate. This finding suggests that coloring or clarification additives are used to create the red color property of the commercial vinegar sample. Rutin was used as standard to determine the flavonoid content of Zivzik pomegranate vinegar and commercial pomegranate vinegar. Zivzik pomegranate vinegar and commercial pomegranate vinegar were found to have 2.7571±0.2603 and 1.3932±0.0349 mg rutin equivalent mL⁻¹ (Table 2). Although the flavonoid content was low in the two vinegar samples examined in the present study, Zivzik pomegranate vinegar had somewhat higher flavonoid content than that of the commercial vinegar. The total phenolic matter contents of Zivzik pomegranate vinegar and commercial pomegranate vinegar were determined based on the gallic acid standard (Table 2). Furthermore, the high content of the total phenolic matter in the commercial vinegar sample compared to the Zivzik pomegranate vinegar suggested that there could be a preservative in the commercial vinegar. The total phenolic substance content was between 710.40 and 4448.0 mg gallic acid equivalent L⁻¹ in [14, 20, 21] different variety pomegranate vinegar. Literature analysis indicated that the variety is effective on phenolic matter content of pomegranate vinegar.

depends on raw materials. It is well acknowledged that various plants and fruits including pomegranate are the main sources of natural compounds behaving as exogenous antioxidants that strengthen body intrinsic antioxidant machineries for the protection of the organism against ROS-mediated damages. Gallic acid, chlorogenic acid, hydroxybenzoic acid, caffeoic acid, quercetin, chrysanthemic acid and ferulic acid (most of them were identified within the content of pomegranate in our study) are promising bioactive compounds mainly found in plants and fruits with strong antioxidant activities (45-48). Gallic acid, hydroxybenzoic acid, caffeoic acid, vanillic acid and chlorogenic acid were used as standards to determine the phenolic acid contents of Zivzik pomegranate vinegar and commercial pomegranate vinegar. The phenolic acid with the highest content in Zivzik pomegranate vinegar was gallic acid while in commercial pomegranate vinegar chlorogenic acid had the highest content (Table 3). Gallic acid was reported to be the predominant organic acid in vinegar samples produced from Beynar and Hicaz pomegranates while chlorogenic acid could not be detected in both vinegar samples [21]. These findings also pointed out the variety as an important factor in the

composition of vinegar. The findings of that study were similar to those in the present study, and very high amount

of chlorogenic acid in commercial vinegar suggested that external preservatives were added.

Table 3. Analysis of organic acid, sugar, and phenolic matter by HPLC in vinegar samples

	STANDARDS	ZIVZIK POMEGRANATE VINEGAR (PPM)	COMMERCIAL POMEGRANATE VINEGAR (PPM)
Organic acid	Tartaric acid	1444.4776±5.6134	109.4413±1.2913
	Malic acid	1306.5262±1.1746	2499.4736±4.0208
	Ascorbic acid	0.3104±0.0023	64.0754±3.7547
	Acetic acid	9553.8308±29.25	34674.5943±49.2736
	Citric acid	27.6851±0.1589	89.3851±21.825
	Total	12332.83±25.13	37436.97±71.13
Sugar	Fructose	77.0365±14.1585	14.9497±0.7483
	Glucose	87.0598±18.1468	33.3640±9.554
	Sucrose	-	7.2872
	Total	164.0962	55.6009
Phenolic acid	Gallic acid	285.4568±1.4886	131.0446±0.4778
	Chlorogenic acid	14.1489±0.1515	257.7429±1.2573
	Hydroxybenzoic acid	1.0345±0.0425	12.2285±0.0629
	Caffeic acid	1.4037±0.0005	0.6894±0.0059
	Vanillic acid	1.7866±0.0124	0.0912±0.0067
	Total	313.7477±1.8105	299.3256±7.7694

3.4. Analysis of Macro Micronutrients with ICP-OES in Vinegar Samples

In our study, the highest amount of mineral in Zivzik pomegranate vinegar was K (2382.55 ± 58.050 ppm), followed by P (94.7460 ± 4.0080 ppm), Mg (60.7500 ± 2.0160 ppm), Na (50.8960 ± 8.4870 ppm) and Ca (15.8720 ± 6.6990 ppm) (Table 4). Commercial pomegranate vinegar, on the other hand, had 2085 ± 133.652 ppm K, 211.5 ± 3.1113 ppm P, 84.323 ± 5.287 ppm Na, 62.887 ± 0.144 ppm Mg and 46.963 ± 10.892

ppm Ca (Table 4). Thus, mineral contents of the Zivzik pomegranate vinegar and commercial pomegranate vinegar were similar. Both vinegar samples had high K contents. In both vinegar samples, some metals that pose a risk factor for human health such as Ni, Pb, Fe and Cu, were found in trace amounts, while the commercial vinegar sample was also found to have Al, Cd and Cr. The presence of these heavy metals in vinegar samples emphasized the importance of soil and environmental conditions where pomegranates grow.

Table 4. Macro-microelement analysis in vinegar samples

Element	Zivzik pomegranate vinegar (ppm)	Commercial pomegranate vinegar (ppm)
Al (Aluminum)	-	13.503 ± 2.235
K (Potassium)	2382.55 ± 58.050	2085 ± 133.652
P (Phosphorus)	94.7460 ± 4.0080	211.5 ± 3.1113
Mg (Magnesium)	60.7500 ± 2.0160	62.887 ± 0.144
Na (Sodium)	50.8960 ± 8.4870	84.323 ± 5.287
Cd (Cadmium)	-	0.040 ± 0.0070
Cr (Chrome)	-	0.0608 ± 0.0185
Ca (Calcium)	15.8720 ± 6.6990	46.963 ± 10.892
Ni (Nickel)	5.7703 ± 0.2516	5.74567 ± 0.113
B (Boron)	1.3887 ± 0.9777	2.873 ± 0.26200
Pb (Lead)	1.1486 ± 0.0832	0.7462 ± 0.0517
Zn (Zinc)	0.7175 ± 0.2071	1.6257 ± 0.184
Fe (Iron)	0.2384 ± 0.0446	5.179 ± 2.9260
Sn (Tin)	0.1778 ± 0.0036	-
Cu (Copper)	0.1746 ± 0.0436	0.1156 ± 0.044
Bi (Bismuth)	0.0991 ± 0.1171	-
Mn (Manganese)	0.0839 ± 0.0127	0.2372 ± 0.032
Sb (Antimony)	0.0710 ± 0.0249	-
Mo (Molybdenum)	0.0656 ± 0.0082	-
Sr (Strontium)	0.0265 ± 0.0436	0.3097 ± 0.0690
Ba (Barium)	0.0172 ± 0.0128	0.0730 ± 0.0202
Li (Lithium)	0.0101 ± 0.0000	0.9247 ± 0.0209

3.5. Antioxidant analysis in vinegar samples

Antioxidant properties of vinegar samples were determined using DPPH, Cuprac and FRAP methods (Table 5). DPPH radical scavenging and copper reduction activity levels of Zivzik pomegranate vinegar were higher

than those of the commercial vinegar, while iron reduction activities of the two vinegar samples were similar. Thus, pomegranate vinegar produced by traditional methods was found to have higher antioxidant capacity than commercial vinegar.

Table 5. Antioxidant analysis of vinegar samples

	DPPH (MG TROLOX E ML ⁻¹)	CUPRAC (MG TROLOX E ML ⁻¹)	FRAP (MG TROLOX E ML ⁻¹)
Zivzik pomegranate vinegar	10.875±0.215	51.763±3.042	18.889±1.798
Commercial pomegranate vinegar	6.2297±0.405	37.225±2.044	26.683±5.256

4. CONCLUSION

There are few studies on pomegranate vinegar in the literature. The scope of this study, Zivzik pomegranate vinegar and commercial pomegranate vinegar were compared for physicochemical properties, total phenolic, total flavonoid, and total anthocyanin content, organic acid, sugar, and phenolic acid composition by HPLC, elemental analysis by ICP-OES, total monomeric anthocyanin, total phenolic content and total flavonoid content. K is the most abundant element in both vinegar samples. Acetic acid is the most common organic acid found in both forms of vinegar. While chlorogenic acid was determined to be the most prevalent phenolic compound in commercial pomegranate vinegar, gallic acid was shown to be the most prevalent in Zivzik pomegranate vinegar. As a result of the study, it was found that vinegar produced by traditional methods had a much better composition in terms of nutritional value. In addition to the importance of raw materials in vinegar production, it was revealed in the present study that the variety, ecological conditions, and fermentation process are also effective on the nutritional value of vinegars.

Acknowledgment

We thank the Science and Technology Application and Research Center at Siirt University for their contribution

Conflict of Interest

Authors declare no conflict of interest

Author(s) Contribution

All the authors contributed equally. This study is a part of the master's thesis prepared by Abdulkerim AYBEK under the supervision of Dr. Ebru AKKEMIK.

REFERENCES

- Yetiman AE. Identification of acetic acid bacteria in vinegar microflora by molecular techniques. Graduate School of Natural and Applied Sciences, Kayseri: University of Erciyes; 2012.
- Turkey National Standard-TSE, Vinegar-product made from liquids of agricultural origin - definitions, requirements, marking (Vol. TS 1880 EN 13188/D1:2016), Ankara. 2016.
- Muller MF. Gençlik ve Sağlık İksiri Sirke. Dharma Yayıncılıarı, İstanbul, 2009.
- Abe K, Kushibiki T, Matsue H, Furukawa K.I, Motomura S. Generation of Antitumor Active Neutral Medium-Sized α -Glycan in Apple Vinegar Fermentation. Bioscience, Biotechnology and Biochemistry. 2007;71(9):2124-2129.
- Kadaş Z. Determination of bioactive properties and metabolic effectsof hawthorn vinegar. Graduate School of Natural and Applied Sciences, Bolu: University of Abant İzzet Baysal; 2011.
- Johnston CS, Quagliano S, White S. Vinegar ingestion at mealtime reduced fasting blood glucose concentrations in healthy adults at risk for type 2 diabetes. Journal of Functional Foods, 2013;5(4):2007-2011.
- Samanidou VF, Antoniou CV, Papadoyannis IN. Gradient Rf-Hplc Determination of Free Phenolic Acids in Wines and Wine Vinegar Samples After Spe, With Photodiode Array Identification. Journal of Liquid Chromatography & Related Technologies, 2001;24 (14):2161-2176.
- Unal E. A study on vinegar production from Dimrit grape by different methods. Graduate School of Natural and Applied Sciences, Adana: Çukurova University; 2007.
- Öztürk S. A research on the production of vinegar from black carrot. Graduate School of Natural and Applied Sciences, Ankara: Ankara University; 2015.
- Marangoz FI. Effect on bioactive compounds and antioxidant properties of mulberry fruit of vinegar product processing. Graduate School of Natural and Applied Sciences, Çanakkale: Çanakkale Onsekiz Mart University; 2016.
- Sakanaka S, Ishihara Y. Comparison of antioxidant properties of persimmon vinegar and some other commercial vinegars in radical scavenging assays and on lipid oxidation in tuna homogenates. Food Chemistry, 2008;107 (2):739-744.
- Nishidai S, Nakamura Y, Torikai K, Yamamoto M, Ishihara N. et al., Kurosu, a traditional vinegar produced from unpolished rice. Suppresses lipid peroxidation in vitro and in Mouse ski. Bioscience Biotechnology and Biochemistry, 2000;64 (9):1909-1914.
- Anonymous, <https://s3.amazonaws.com/gourmet-living/Balsamic+Vinegar+FAQ.pdf> Last Accessed: 30 June 2019.
- Kharchoufi S, Gomez J, Lasanta C, Castro R, Sainz F. et al., Benchmarking laboratory-scale pomegranate vinegar against commercial wine vinegars: antioxidant activity and chemical composition. Journal of the Science of Food and Agriculture, 2018;98(12):4749-4758.
- Morales ML, Tesyafe W, Garcia-Parrilla MC, Casas JA, Troncoso AM. Sherry wine vinegar: Physicochemical changes during the acetification process. Journal of the Science in Food and Agriculture, 2001;81:611-619.
- Budak N, Güzel-Seydim ZB. Sirke üretimi ve bazı fonksiyonel özellikleri. Gıda Teknolojisi, 2010;14(11):85-88.

- [17] Bekar T. The Effects of Grape Growing on Quality Wine, Turkish Journal of Agricultural and Natural Sciences, 2016;3(4):255–264.
- [18] Kelebek H. Researches on the phenolic compounds? profile of öküzgözü, bogazkere and kalecik karası cultivars grown in different regions and their wines. Graduate School of Natural and Applied Sciences, Adana: Çukurova University; 2009.
- [19] Godara NR, Godara RK. Assesment of New Germplast of Pomegranate at Hisar. Haryana Journal of Horticultural Sciences. 1991; 20 (3-4):197-202.
- [20] Ordoudi SA, Mantzouridou F, Daftsiou E, Malo C, Hatzidimitriou E. et. al., Pomegranate juice functional constituents after alcoholic and acetic acid fermentation. Journal of Functional Foods, 2014;(8): 161-168.
- [21] Budak HN. Antioxidant activity and phenolic contents Pomegranate vinegar, Agro FOOD Industry Hi Tech. 2015;26(5):68-72.
- [22] Şengül H. Functional vinegar production from güvem (*Prunus spinosa*) fruit. Graduate School of Natural and Applied Sciences, Tekirdağ: Namık Kemal University; 2017.
- [23] Cemeroğlu B. Gıda Analizleri, Genişletilmiş 2. Baskı. Gıda Teknolojisi Derneği Yayınları, No:34 Bizim Grup Basımevi. Ankara, 2010.
- [24] Ibrahim MZ. Physicochemical and microbiological properties of industrial and traditional homemade vinegar. Graduate School of Natural and Applied Sciences, Kahramanmaraş: Kahramanmaraş Sutcu İmam University; 2019.
- [25] Akbaş M, Cabaroğlu T. A Research on The Determination of Compositions of Grape Vinegars Produced in Turkey and Their Conformity to Food Legislation. Gida. 2009;35(3):183-188.
- [26] Şengün İY, Kılıç G. Microflora, Bioactive Components and Health Effects of Various Kinds of Vinegars. Akademik Gıda 2019;17(1):89-101.
- [27] Fuleki T, Francis F. Quantitative methods for anthocyanins. Journal of Food Science, 1968;33:72-83.
- [28] Park YS, Jung ST, Kang SG, Heo BK, Arancibia-Avila P. et. al., Antioxidants and proteins in ethylene-treated kiwifruits. Food Chemistry, 2008;107(2):193-206.
- [29] Dörtkardeş M. Determination of antioxidant capacities of *Salvia Hasankeyfensis* Dirmenci, Celep & O.Güner, *Stachys Mardinensis* (Post) R.R. Mill, *Ferulago Bernardii* L.Tomkovich & M.Pimenov ve *Hymenocrater Bituminosus* Fisch. & C.A.Mey. Graduate School of Natural and Applied Sciences, Siirt: Siirt University; 2019.
- [30] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. American Journal of Enology and Viticulture, 1965;16:144-158.
- [31] Slinkard K, Singleton VL. Total Phenol Analyses: Automationand Comparison with Manual Methods, American Journal of Enologyand Viticulture, 1977;28:49-55.
- [32] Tormo M, Izco JM. Alternative reversed-phase high-performance liquid chromatography method to analyse organic acids in dairy products, Journal of Chromatography A, 2004;1033:305–310.
- [33] Akkemik E, Aybek A, Felek I. Effects of Cefan Melon (Cucumis Melo L.) Seed Extracts on Human Erythrocyte Carbonic Anhydrase I-II Enzymes, Applied Ecology and Environmental Research, 2019;17(6):14699-14713.
- [34] Turkey National Standard-TSE, TS 13359, Honey-Fructose, glucose, sucrose, turanose and maltose content determination - High performance liquid chromatography (hplc) method, Ankara, 2008.
- [35] Anonymous http://www.onlinecas.com/Berghof/mWS3/Microonde%20MWS2/applications%20MWS2/AR_MW_S-2_Food-Pharma-Cosmetics_140405.pdf 14.07.20-15:30
- [36] Anonymous https://www.perkinelmer.com/PDFs/downloads/AT_L_BarnesFoodAtomicSpec.pdf 14.07.20-15:32
- [37] Blois MS. Antioxidant determinations by the use of a stable free radical. Nature, 1958;29:1199-1200.
- [38] Benzie IFF, Strain JJ. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Analytical Biochemistry, 1996;239:70-76.
- [39] Benzie IFF, Szeto YT. Total antioxidant capacity of teas by the ferric reducing/antioxidant power (FRAP) assay. Journal of Agricultural and Food Chemistry, 1999;47:633–636.
- [40] Apak R, Güçlü K, Demirata B, Özyürek M, Çelik S.E. et al., Comparative evaluation of varius total antioxidant capacity assays applied to phenolic compounds with the cuprac assay. Molecules, 2007;12:1496-1547.
- [41] Vardin H, Karaaslan M, Yılmaz F, İzol G, Cesur Ö. et al., Zivzık ve Görümlü Narlarının Özelliklerinin ve Katma Değerli Ürünlerle İşlenebilirliğinin Belirlenmesi Projesi. Şanlıurfa. 2012.
- [42] Turker I. Sirke Teknolojisi ve Teknikte Laktik Asit Fermantasyonları. Ankara Üniversitesi Basımevi, Ankara. 1963.
- [43] Özkaya H, Şahin E, Türker İ, Gıda Bilimi ve Teknolojisi, Ankara Üniversitesi Ziraat Fakültesi Yayınları, Ders Kitabı, Ankara, 1991.
- [44] Saxena AK, Manan J.K, Berry SK. Pomegranates; postharvest Technology, Chemistry and Processing. Indian Food Packer. 1987;41(4):43-60.
- [45] Kucukler, S., Darendelioglu, E., Caglayan, C., Ayna, A., Yıldırım, S., & Kandemir, F. M. (2020). Zingerone attenuates vancomycin-induced hepatotoxicity in rats through regulation of oxidative stress, inflammation and apoptosis. Life Sciences, 259, 118382.
- [46] Aykutoglu, G., Tartik, M., Darendelioglu, E., Ayna, A., & Baydas, G. (2020). Melatonin and vitamin E alleviate homocysteine-induced oxidative injury and apoptosis in endothelial cells. Molecular Biology Reports, 47(7), 5285-5293.
- [47] Ayna, A., Özbolat, S. N., & Darendelioglu, E. (2020). Quercetin, chrysins, caffeic acid and ferulic acid ameliorate cyclophosphamide-induced toxicities in SH-SY5Y cells. Molecular Biology Reports, 47(11), 8535-8543.

- [48] Caglayan, C., Kandemir, F. M., Darendelioğlu, E., Küçükler, S., & Ayna, A. (2021). Hesperidin protects liver and kidney against sodium fluoride-induced toxicity through anti-apoptotic and anti-autophagic mechanisms. *Life Sciences*, 281, 119730.