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Research Paper / Araştırma Makalesi

Phenolic Compound Profile, Antioxidant Capacity, and Physicochemical Characteristics of Commercial Hawthorn Vinegars

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ABSTRACT

Hawthorn fruit, known for its wide range of varieties, exhibits various biological activities. Its numerous therapeutic effects are largely attributed to its bioactive compounds. This study aimed to determine the total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (via ABTS, DPPH, and FRAP assays), selected phenolic compounds, and physicochemical properties of commercial hawthorn vinegars sold in Türkiye. With the exception of one brand, all samples exhibited titratable acidity values below the acceptable limit. TPC and TFC values ranged from 86.21 to 456.42 mg GAE/L and 48.46 to 376.92 mg QE/L, respectively. Antioxidant activity values for ABTS, DPPH, and FRAP assays ranged from 111.00-722.18 mg TE/L, 36.90-381.80 mg TE/L, and 65.15-441.07 mg TE/L, respectively. Among the individual phenolic compounds identified, protocatechuic acid was the most abundant, followed by quercetin 3-β-D-glucoside, and vitexin was not detected in any vinegar sample. The study indicated that commercially available hawthorn vinegars might demonstrate significant variability in their physicochemical and bioactive properties.

Keywords: Hawthorn, Vinegar, Phenolic compound, Antioxidant activity

Ticari Alıç Sirkelerinin Fenolik Bileşik Profili, Antioksidan Kapasitesi ve Fizikokimyasal Özellikleri

ÖZ

Alıç meyvesi, geniş çeşitliliğiyle bilinir ve çeşitli biyolojik aktiviteye sahiptir. Çok sayıdaki terapötik etkisi, büyük ölçüde biyoaktif bileşiklerine atfedilir. Bu çalışma, Türkiye'de satılan ticari alıç sirkelerinin toplam fenolik madde içeriğini (TPC), toplam flavonoid madde içeriğini (TFC), antioksidan aktivitesini (ABTS, DPPH ve FRAP analizleri yoluyla), bazı fenolik bileşiklerini ve fizikokimyasal özelliklerini belirlemeyi amaçlamıştır. Bir marka hariç, tüm örnekler kabul edilebilir sınırın altında titre edilebilir asitlik değerleri göstermiştir. TPC ve TFC değerleri sırasıyla 86,21 ila 456,42 mg GAE/L ve 48,46 ila 376,92 mg QE/L arasında değişmiştir. ABTS, DPPH ve FRAP analizleri için antioksidan aktivite değerleri sırasıyla 111,00-722,18 mg TE/L, 36,90-381,80 mg TE/L ve 65,15-441,07 mg TE/L arasında belirlenmiştir. Tanımlanan bireysel fenolik bileşikler arasında, protokateşik asit en bol olanıydı, bunu kuersetin 3-β-D-glukozit takip ederken, hiçbir sirke örneğinde viteksin tespit edilememiştir. Çalışma, ticari olarak temin edilebilen alıç sirkelerinin fizikokimyasal ve biyoaktif özelliklerinde önemli değişkenlik olabileceği göstermiştir.

Anahtar Kelimeler: Alıç, Sirke, Fenolik bileşik, Antioksidan aktivite

INTRODUCTION

Some medicinal wild plants and herbs known for centuries have recently attracted increasing attention worldwide due to their potential beneficial properties on health [1]. This effect on health has been associated with the rich bioactive contents of the relevant plants [2]. One of these interesting popular medicinal plants is hawthorn (*Crataegus* spp.) which is present worldwide with about 280 species and is mostly seen in North America, Europe, and Asia in the world [3, 4]. It is known that various parts of the hawthorn plant such as leaves, flowers and fruits have been used in many traditional and modern medical practices for centuries [5]. As a result of studies conducted in the literature, it has been determined that hawthorn fruits have antidiabetic [6], antioxidant [7], cardioprotective [8], hepatoprotective [9], anticancer [10], antimicrobial [11], antihyperglycemic [12] and antidepressant [13] effects. Hawthorn fruit provides many biological effects with the bioactive substances it contains such as flavonoids, phenolic compounds, triterpenoids, proanthocyanidins [14], organic acids, sterols, vitamins, minerals and trace amounts of cardioactive amines [15]. Hawthorn fruit can be consumed fresh or dried, or it is processed into forms such as tea, jam, syrup, molasses or vinegar [14, 16].

Vinegar is a special product obtained by the conversion of fermentable sugars into ethanol by yeast, followed by the conversion of ethanol into acetic acid by acetic acid bacteria [17, 18]. Vinegar is widely used in the food industry to produce foods such as pickles, salad dressings, ketchup and mayonnaise, and to add flavor and aroma as well as extend the shelf life of these foods [19]. Historically, vinegar has been used for dealing with conditions like stomachaches, laryngitis, obesity, swelling, and fever [20]. Vinegar is a good source of many bioactive compounds including polyphenols, organic acids, melanoidins, ligustrazine, tryptophol and caffeoylsophorose which are responsible for different pharmacological and metabolic benefits [21]. The amounts and types of these significant compounds found in vinegar belong to the production raw materials, applied fermentation methods and the maturation process of the vinegar [17]. There are many types of vinegar produced from different fruits and plants on the market. One of the most attractive vinegar types in recent years is hawthorn vinegar due to its beneficial effects on health [22].

The phenolic profiles and antioxidant capacities of hawthorn and hawthorn-derived products have been extensively examined in the literature, whereas comprehensive and comparative data specifically addressing commercially available hawthorn vinegars are still limited. In particular, differences in bioactive compound profiles and physicochemical characteristics among commercial products, potentially resulting from differences in raw materials, production techniques, and fermentation conditions, have not been sufficiently elucidated. Therefore, the present study aimed to comparatively evaluate the TPC, TFC, antioxidant activity (via ABTS, DPPH, and FRAP assays), selected phenolic compounds (protocatechuic acid, quercetin 3-β-D-glycoside, and vitexin), and key physicochemical properties (pH, titratable acidity, dry matter, and color values) of hawthorn vinegars from different commercial brands sold in Türkiye. The results of this study are expected to provide valuable insights into the quality variability of commercial hawthorn vinegars and to contribute to the existing literature by supporting product standardization and informed consumer choice.

MATERIALS and METHODS

Materials

In this study, hawthorn vinegars of 8 different brands offered for sale in markets or virtual shopping sites were obtained in February 2023. The production dates of all hawthorn vinegars correspond to different months within 2022. Hawthorn vinegar samples were expressed with the HV1, HV2, HV3, HV4, HV5, HV6, HV7 and HV8 codes. Vinegar samples were stored in a refrigerator at 4°C until the day of the experiment. Analyses were carried out in Tokat Gaziosmanpaşa University, Faculty of Engineering and Architecture, Department of Food Engineering Laboratories.

Chemicals and Equipment

Aluminum chloride ($AlCl_3$, 99%, kept in a tightly closed container at room temperature in a dry place), sodium hydroxide (NaOH, 99%, stored in a tightly closed container at room temperature), sodium nitrite ($NaNO_2$, 99%, stored at room temperature in a dry place), sodium carbonate (Na_2CO_3, 99%, stored in a tightly closed container at room temperature in a dry place), sodium acetate (CH_3COONa, 99%, kept in a tightly closed container at room temperature in a dry place), potassium peroxydisulfate ($K_2S_2O_8$, 99%, stored in a tightly closed container, kept locked up or in an area accessible only to qualified or authorized persons, and not near combustible materials) were supplied from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent (FC, 2N, stored at room temperature in a tightly closed container protected from light) was bought from Carlo Erba (Italy). Gallic acid (99.9%, kept in a tightly closed container at room temperature in a dry place), iron (III) chloride ($FeCl_3$, 97%, stored under inert gas, in non-metal containers, tightly closed and dry), quercetin (95%, stored in a tightly closed container at -20ºC in a dry place), ethanol (99.5%, stored in a tightly closed container in a dry and well-ventilated place, away from heat and sources of ignition) methanol (99.5%, stored in a tightly closed container in a dry and well-ventilated place, away from heat and sources of ignition), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, 98%, stored at 2-8°C in a tightly closed container), 2,2-diphenyl-1-picrylhydrazyl (DPPH, 99%, stored at 2-8°C in a tightly closed container, kept away from sources of ignition and heat) were supplied from Sigma-Aldrich (Germany). 2,4,6-Tri(2-pyridyl)-1,3,5-triazine (TPTZ, 99.81%, stored at 2-8°C in a tightly closed container, dry and protected from light) was supplied from Tokyo Chemical Industry (Tokyo, Japan). Protocatechuic acid (98%, stored at inert atmosphere and room temperature), (±)-6-hydroxy-

2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 99.70%, kept in a tightly closed container at room temperature in a dry place), quercetin 3-β-D glucoside (90%, stored in a tightly closed container at -20ºC in a dry place), vitexin (99.77%, kept in a tightly closed container at room temperature in a dry place) were bought from BLD Pharm (China).

Precise balance (Radwag, AS 220 R2, Poland), centrifuge (Hettich EBA 21, Germany), UV/VIS spectrophotometer (PG Instrument, T80+, England), Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS device, Shimadzu, LCMS-8050, Japan), pH meter (Hanna HI2211, USA), colorimeter (Minolta CR-300, Japan), vortex (Velp Scientifica, Italy), drying oven (Memmert, Germany), heated magnetic stirrer (Biosan, MSH 300, Latvia) were used at different stages during the research.

Physicochemical Analyses

The total dry matter (g/L), titratable acidity (g/L, expressed as acetic acid), and pH values of the hawthorn vinegar samples were determined according to the methods described by Cemeroğlu [23], following the standard procedures of Association of Official Analytical Chemists (AOAC). Specifically, total dry matter was determined by gravimetric analysis (AOAC 925.45), titratable acidity by potentiometric titration (AOAC 942.15), and pH using a digital pH meter (AOAC 981.12) [24]. The color values of the samples were measured using a colorimeter based on the International Commission on Illumination (CIE) L*, a*, b* color system. The L* (lightness), a* (redness-greenness), and b* (yellowness-blueness) values were recorded for each sample. Additionally, Chroma (C*) and Hue angle (h^o) were calculated. The color parameters were evaluated according to the standards defined by the International Commission on Illumination [25].

Total Phenolic Content

The TPC values of hawthorn vinegars were determined using FC method with slight modifications, as described by Topuz and Bayram [26]. 100 µL of hawthorn vinegar was mixed with 200 µL of 2 N FC reagent and allowed to react for 5 min at room temperature. Subsequently, 1 mL of 20% Na_2CO_3 was added to the mixture, which was stirred and incubated in the dark at room temperature for 60 min. The absorbance of the mixture was measured at 765 nm using a UV/VIS spectrophotometer. The calibration curve for TPC was constructed using gallic acid standards in the range of 0-500 mg/L (R^2=0.9912). TPC results of hawthorn vinegar samples were calculated by taking the applied dilution factors and expressed as mg gallic acid equivalent (GAE)/L.

Total Flavonoid Content

The TFC assay was performed with slight modifications to the method described by Gaafar and Salama [27]. Briefly, 500 µL of hawthorn vinegar was mixed with 2 mL of distilled water and 150 µL of 5% $NaNO_2$ and allowed to react for 5 min at room temperature. Following this, 150 µL of 10% $AlCl_3$ was added and allowed to react for 5 min at room temperature. Subsequently, 1 mL of 1 M NaOH and 1.2 mL of distilled water were added to the mixture and stirred. The absorbance was measured at 510 nm using a UV/VIS spectrophotometer. The calibration curve for TFC was constructed using quercetin standards in the range of 0-400 mg/L (R^2=0.9961). TFC results of hawthorn vinegar samples were calculated by taking the applied dilution factors and expressed as mg quercetin equivalent (QE)/L.

ABTS Cation Radical Scavenging Activity

The $ABTS^{\bullet+}$ values of hawthorn vinegars were determined method of Re et al. [28] with slight modifications. The stock solution consisted of $K_2S_2O_8$ (2.45 mM) and ABTS (7 mM). The solution was diluted with ethanol until the absorbance reached 0.700 (±0.02) at 734 nm, as measured using a spectrophotometer. 40 µL of hawthorn vinegar was mixed with 4 mL of the diluted ABTS radical solution and allowed to react in the dark for 7 min. The absorbance of the resulting mixture was measured at 734 nm using a UV/VIS spectrophotometer. The calibration curve for ABTS was constructed using Trolox standards in the range of 0-500 mg/L (R^2=0.9987). ABTS results of hawthorn vinegar samples were calculated by taking the applied dilution factors and expressed as mg Trolox equivalent (TE)/L.

DPPH Radical Scavenging Activity

The $DPPH^{\bullet}$ assay was performed according to the method described by Blasi et al. [29]. Briefly, a 0.06 mM DPPH solution was prepared in ethanol. Then, 100 µl of hawthorn vinegar was mixed with 3.9 mL of DPPH solution and allowed to react in the dark at room temperature for 30 min. The absorbance of the resulting mixture was measured at 517 nm using a UV/VIS spectrophotometer. The calibration curve for DPPH was constructed using Trolox standards in the range of 0-250 mg/L (R^2=0.9982). DPPH results of hawthorn vinegar samples were calculated by taking the applied dilution factors and expressed as mg TE/L.

Ferric Reducing Antioxidant Power

The FRAP values of hawthorn vinegars were determined previously described by Benzie and Strain [30]. Firstly, the FRAP reagent was prepared by mixing 30mM CH_3COONa buffer (pH:3.6), 10 mM TPTZ, and 20 mM $FeCl_3$ solution solution in a 10:1:1 ratio. Following this, 100 µL of hawthorn vinegar was mixed with 2.9 mL of FRAP reagent and allowed to react in the dark at room temperature for 30 min. The absorbance of the resulting mixture was measured at 593 nm using a UV/VIS spectrophotometer. The calibration curve for FRAP was constructed using Trolox standards in the range of 0-350 mg/L (R^2=0.9901). FRAP results of hawthorn vinegar samples were calculated by taking the applied dilution factors and expressed as mg TE/L.

Quantitative Analysis of Individual Phenolic Compounds

The amounts of protocatechuic acid, quercetin 3-β-D glycoside and vitexin in the hawthorn vinegars were determined by LC-MS/MS device. Hawthorn vinegars were filtered through 0.22 µm (Millex-HV) membrane filter. Filtrates were transferred to autosampler vials. Shimadzu-brand LC-MS-8050 is equipped with SIL-30AC auto-sampler, LC-30 AD pump, DGU-20A_{3R} degasser, CTO-10AS colon furnace (40°C) and LCMS-8050 mass spectrometer. The LC separation was carried out using a C18 reversed phase column (2.0 mm×150 mm, 3 µm particle size). The flow rate was set at 0.4 mL/min using (A) 1mM ammonium formate and (B) methanol as mobile phase. The following gradient program was used: t=0.00 min, 80.00% A, 20.00%B; t=3.00 min, 30.00% A, 70.00%B; t=10.30 min, 5.00% A, 95.00%B; t=10.51 min, 80.00%A, 20.00%B. The run time was 14.00 min. For MS/MS detection, the electro spray ionization (ESI) interface was used positive polarity, 250°C of desolvation line temperature, 300°C of interface temperature, 400°C block heater temperature. Nebulizer gas flow, drying gas flow and heating gas flow were 3.00, 10.00 and 15.00 L/min, respectively. All parameters of the instrument were controlled using LabSolution® software (version 4.91).

Statistical Analysis

Statistical analyses were done by using the Duncan test through the instrument of SPSS 22.0 (version 22.0, IBM, USA) statistical package program. Moreover, correlation coefficients were identified using the same program. One-way analysis of variance (ANOVA) was performed to determine the differences between groups, and Duncan's multiple range test was used to identify significant differences. The 95% confidence level ($p<0.05$) was considered significant in all analyses, and all experiments were carried out in triplicate.

RESULTS and DISCUSSION

Physicochemical Properties of Commercial Hawthorn Vinegars

Some physicochemical properties of commercial hawthorn vinegar samples are given in Table 1. It was determined that the dry matter values of hawthorn vinegar samples varied between 2.32 g/L (HV4 sample) and 12.15 g/L (HV3 sample). While there was no statistically significant difference between the HV3 and HV5 samples ($p>0.05$), there was a statistically significant difference between the other samples ($p<0.05$). The considerable variation in dry matter content among hawthorn vinegar samples may be attributed to multiple factors. Differences in raw material such as fruit maturity, sugar content, and initial composition, can significantly influence dry matter levels. Furthermore, production parameters including fermentation conditions, filtration, and storage practices are likely to contribute to the observed variability in the final vinegar products [31]. According to the Turkish Standards Institute (TS 1880 EN 13188), no limit has been established for dry matter content in vinegar [32]. In a study, the dry matter amounts of apple, white and red grape, melon, mandarin, carrot and pear vinegars were determined to be between 0.87 and 6.38 g/L [33]. In another study, Tomar et al. [15] detected dry matter value of hawthorn vinegar as 20.80 g/L.

Table 1. Physicochemical characteristics of commercial hawthorn vinegar samples

Sample	Dry matter (g/L)	pH	Titratable acidity (g/L) *
HV1	6.24±0.15^{d**}	3.35±0.01^{d}	18.29±0.21^{g}
HV2	4.27±0.01^{e}	3.75±0.01^{a}	5.70±0.14^{h}
HV3	12.15±0.15^{a}	3.52±0.01^{b}	37.05±0.07^{c}
HV4	2.32±0.09^{g}	3.02±0.01^{f}	38.09±0.55^{b}
HV5	12.01±0.05^{a}	3.31±0.01^{e}	29.14±0.06^{f}
HV6	3.59±0.09^{f}	3.02±0.01^{f}	40.14±0.31^{a}
HV7	9.47±0.30^{c}	3.36±0.00^{d}	29.79±0.01^{e}
HV8	10.65±0.26^{b}	3.43±0.00^{c}	30.62±0.11^{d}

*Acetic acid equivalent; **Superscripts in the same column show difference between vinegar samples ($p<0.05$); Results are given as mean ± standard deviation. HV: Hawthorn vinegar

The pH values of the hawthorn vinegar samples varied between 3.02 (HV4 and HV6 samples) and 3.75 (HV2 sample). While there was no statistically significant difference between the HV4 and HV6 samples ($p>0.05$), there was a statistically significant difference between the other samples ($p<0.05$). There was no limit for pH value in vinegar standards of the Turkish Standards Institute (TS 1880 EN 13188) [32]. Bıldır et al. [34] determined the pH value of hawthorn vinegar as 2.96. pH value of hawthorn vinegar was determined as 3.63 by Özdemir et al. [4]. In another study, Karadag et al. [35] determined the pH value of commercial hawthorn vinegar as 2.76.

Analysis revealed that the titratable acidity values of hawthorn vinegar samples were found to range from 5.70 g/L (HV2 sample) to 40.14 g/L (HV6 sample). There was a statistically significant difference between all samples in terms of titratable acidity ($p<0.05$). Total acidity (titratable acidity) content of vinegar produced in Turkey (in water-acetic acid) should not be less 40 g/L according to TS 1880 EN 13188 [32]. According to Codex Alimentarius Commission (CAC), titratable acidity of vinegar (except wine vinegar) should not be less than 50 g/L [36]. Titratable acidity value of the only HV6 vinegar sample was in conformity with the TS 1880 EN 13188 vinegar standard. The titratable acidity value of other samples was lower than 40 g/L. The titratable acidity values of the hawthorn vinegar samples were

observed to be substantially lower than the limits recommended by Codex Alimentarius standards. The observed low titratable acidity may be attributed to raw material, fermentation parameters and post-fermentation processing [37]. In a study, titratable acidity of hawthorn vinegar was determined as 41.30 g/L by Özdemir et al. [4]. In another study, titratable acidity value as the acetic acid equivalent of hawthorn vinegar sample was determined as 25.80 g/L [34]. Titratable acidity of commercial hawthorn vinegar was determined as 22.00 g/L [38]. Karadag et al. [35] determined the titratable acidity value of commercial hawthorn vinegar as 22.90 g/L. Budak et al. [39] reported that titratable acidity value of hawthorn vinegar was 45.30 g/L.

Color Properties of Hawthorn Vinegars

Color properties of commercial hawthorn vinegar samples are provided in Table 2. L* value refers to the brightness (100=white) to darkness (0=black). While a* value indicates to the redness (+) to greenness (-), b* value indicates to the yellowness (+) to blueness (-) [17]. As with other foods, color is one of the important factors that influence consumer perception when buying vinegar [40].

The L* values of hawthorn vinegar samples ranged between 17.66 (HV8 sample) and 37.40 (HV4 sample). There was a statistically significant difference between all samples in terms of L* value ($p<0.05$). The a* values of hawthorn vinegar samples were found ranged from 0.26 (HV2 sample) to 5.73 (HV6 sample). While there was no statistically significant difference between the HV4 and HV6 samples ($p>0.05$), there was a statistically significant difference between the other samples ($p<0.05$). The b* values of hawthorn vinegar samples varied between 13.73 (HV8 sample) and 27.11 (HV7 sample). While there was no statistically significant difference between the HV1 and HV4 samples in terms of b* value ($p>0.05$), there was a statistically significant difference between the other samples ($p<0.05$).

It was determined that the C* values of the hawthorn vinegar samples ranged between 14.15 (HV8 sample) and 27.65 (HV7 sample), while the h° values varied from 69.66 (HV6 sample) to 89.18 (HV2 sample). A statistically significant difference was observed among all samples in terms of C* values ($p<0.05$), indicating variability in color saturation, whereas no significant difference was found between HV3 and HV7 in terms of hº values ($p>0.05$), with other samples differing significantly ($p<0.05$). In colorimetric analysis, C* reflects the intensity or saturation of a color, where higher values correspond to more vivid and saturated appearance, and lower values indicate duller or less intense coloration. On the other hand, h° defines the actual color tone on the CIE color circle, enabling interpretation of the dominant visual hue perceived (such as reddish, yellowish) [41]. Based on these principles, the HV7 sample exhibited the most intense and vibrant color appearance among the vinegars, whereas HV8 showed the lowest saturation, indicating a more muted visual quality. With respect to hº, lower h° values are typically associated with shifts toward red or reddish tones, and higher h° values indicate movement toward yellowish hues, which suggests that HV6 presented a more reddish color while HV2 displayed a more yellow-toned appearance. These C* and hº differences are consistent with established colorimetric interpretations linking C* to color saturation and h° to specific visual tonality in food products.

Table 2. Color values of commercial hawthorn vinegar samples

Sample Code	L*	a*	b*	C*	h°
HV1	21.15±0.03^{g**}	1.76±0.07^{f}	14.41±0.16^{f}	14.51±0.16^{g}	83.03±0.25^{b}
HV2	32.54±0.03^{c}	0.26±0.03^{g}	17.84±0.02^{d}	17.85±0.02^{d}	89.18±0.10^{a}
HV3	29.47±0.06^{d}	4.26±0.05^{d}	20.89±0.04^{c}	21.32±0.05^{c}	78.48±0.10^{d}
HV4	37.40±0.04^{a}	4.51±0.07^{c}	14.48±0.04^{f}	15.17±0.02^{f}	72.71±0.28^{f}
HV5	25.82±0.16^{f}	3.49±0.06^{e}	23.78±0.21^{b}	24.04±0.20^{b}	81.64±0.21^{c}
HV6	36.12±0.04^{b}	5.73±0.01^{a}	15.46±0.07^{e}	16.49±0.06^{e}	69.66±0.07^{g}
HV7	26.43±0.56^{e}	5.43±0.17^{b}	27.11±0.10^{a}	27.65±0.07^{a}	78.67±0.39^{d}
HV8	17.66±0.13^{h}	3.43±0.20^{e}	13.73±0.11^{g}	14.15±0.12^{h}	75.99±0.76^{e}

**Superscripts in the same column show difference between vinegar samples ($p<0.05$); Results are given as mean ± standard deviation. HV: Hawthorn vinegar

The L*, a* and b* value of yellow hawthorn fruit (*Crataegus tanacetifolia*) vinegar was determined as 27.80, 1.33 and 0.30, respectively [15]. In a study, the color values of home-made hawthorn vinegar were determined as L* (18.10), a* (1.96) b* (10.67) [42]. In another study, the L*, a* and b* value of hawthorn vinegar samples was determined as 31.4, 20.48 and 40.08 [43]. Karadag et al. [35] determined the L*, a* and b* values of commercial hawthorn vinegar as 61.30, 4.47 and 33.10, respectively.

Total Phenolic and Flavonoid Contents of Hawthorn Vinegars

The bioactive properties of vinegars may vary depending on the type of raw material used vinegar production [35]. In addition, geographical regions, soil structure, climatic conditions and vinegar production method also affect bioactive properties of vinegars [44]. TPC values of the commercial hawthorn vinegar samples ranged between 86.21 mg GAE/L (HV4 samples) and 456.42 mg GAE/L (HV2 sample) (Table 3). There was a statistically significant difference between all samples in terms of TPC values ($p<0.05$). This case indicates a substantial variation in phenolic

concentration among products, reflecting differences in raw material quality and processing conditions. Higher TPC values suggest a greater presence of phenolic compounds, which are associated with antioxidant capacity and potential health-promoting properties [45]. This range is comparable to those reported for other fruit vinegars, confirming hawthorn vinegar as a potential source of dietary phenolics [46]. It was determined that the TFC values of hawthorn vinegar samples varied between 48.46 mg QE/L (HV4 sample) and 376.92 mg QE/L (HV8 sample). While there was no statistically significant difference between the HV5-HV7, HV1-HV3 samples in terms of TFC values ($p>0.05$), there was a statistically significant difference between the other samples ($p<0.05$). The coefficient of Pearson's correlation between TPC and TFC of hawthorn vinegar samples was determined as 0.92.

Table 3. TPC and TFC values of commercial hawthorn vinegar samples

Sample Code*	TPC (mg GAE/L)	TFC (mg QE/L)
HV1	263.29±9.13^{f*}	208.85±1.09^{d}
HV2	456.42±4.12^{a}	363.46±6.53^{b}
HV3	394.75±7.66^{c}	196.92±1.63^{d}
HV4	86.21±0.29^{h}	48.46±1.63^{f}
HV5	286.21±5.60^{e}	226.54±9.79^{c}
HV6	106.83±6.48^{g}	89.23±2.72^{e}
HV7	307.04±12.08^{d}	231.54±4.90^{c}
HV8	421.83±2.95^{b}	376.92±7.07^{a}

*HV: Hawthorn vinegar, TPC: Total phenolic compound, TFC: Total flavonoid content; **Superscripts in the same column show difference between vinegar samples ($p<0.05$); Results are given as mean ± standard deviation.

TPC value of hawthorn vinegar was determined as 2420.73 mg GAE/L by Özdemir et al. [4]. In a study, Kadas et al. [43] determined TPC content of hawthorn vinegar as 502 mg GAE/L. TPC value of yellow hawtorn fruit (*Crataegus tanacetifolia*) vinegar was determined as 751.11 mg GAE/L by Özdemir et al. [4]. Akgün et al. [47] determined the TPC values of commercial hawthorn vinegars (*C. tanacetifolia*) from two different companies as 328.46 and 467.59 mg GAE/mL. TPC value of commercial hawthorn vinegar was determined as 280 mg GAE/L [38]. Karadag et al. [35] determined the TPC value of commercial hawthorn vinegar as 647.00 mg GAE/L. Budak et al. [39] reported that TPC value of hawthorn vinegar was 2420. 73 mg GAE/L. Güzel [46] determined the TFC values of yellow hawthorn vinegar and red hawthorn vinegar as 61.06 and 119.04 mg QE/L, respectively. In another study, TFC value of hawthorn vinegar sample was determined as 290.44 mg QE/L [34].

It is seen that the TPC and TFC values of the hawthorn vinegars we obtain from the market and analyzed in the literature are different from each other. This situation may be related to the geographical region, soil structure, climatic conditions, as well as the type of hawthorn fruit and the vinegar production method [34, 44].

Antioxidant Activity of Commercial Hawthorn Vinegars

Phenolic compounds found in vinegar have an important effect in reducing oxidative stress in the body, regulating lipid metabolism, preventing cardiovascular diseases and delaying aging, thanks to their high antioxidant activity [48]. Additionally, the carotenoids, phytosterols, vitamins C and vitamin E found in vinegar also contribute to vinegar's antioxidant activity [49]. It was determined that ABTS values of the commercial hawthorn vinegar samples ranged between 111.00 mg TE/L (HV4 samples) and 722.18 mg TE/L (HV2 sample) (Table 4). There was a statistically significant difference between all samples in terms of ABTS values ($p<0.05$). DPPH values of the commercial hawthorn vinegar samples varied between 36.90 mg TE/L (HV4 samples) and 381.80 mg TE/L (HV2 sample). While there was no statistically significant difference between the HV3-HV8, HV5-HV6 samples in terms of DPPH values ($p>0.05$), there was a statistically significant difference between the other samples ($p<0.05$). FRAP values of the commercial hawthorn vinegar samples ranged between 65.15 mg TE/L (HV4 samples) and 441.07 mg TE/L (HV2 sample). The coefficients of Pearson's correlation between TPC and ABTS, DPPH, FRAP were 0.83, 0.70, 0.92, respectively. For ABTS, DPPH and FRAP methods, the highest antioxidant activity was detected in the HV4 sample, and the lowest antioxidant activity was detected in the HV2 sample. The reason why samples have different values according to the antioxidant activity analysis method is due to the use of different radicals in the analysis and the tendency of these radicals to react with different possible antioxidant groups in the sample [4].

The antioxidant activity of hawthorn vinegar was determined as 13.01 mmol TE/L according to the ABTS method by Özdemir et al. [4]. The antioxidant activity of yellow hawthorn fruit (*C. tanacetifolia*) vinegar was determined as 86.23 mg TE/L by Tomar et al. [15]. The antioxidant activities of commercial hawthorn vinegar by ABTS, DPPH and FRAP methods were determined as 380, 410 and 450 mg TE/L [38]. Güzel [46] determined the ABTS, DPPH and FRAP values of yellow hawthorn vinegar and red hawthorn vinegar as (4.40 and 8.45 mmol TE/L), (1.32 and 4.96 mmol TE/L) and (3.59 and 28.50 mmol TE/L), respectively. Karadag et al. [35] determined the FRAP value of commercial hawthorn vinegar as 544.00 mg/L. Budak et al. [39] reported that ABTS value of hawthorn vinegar was 23.01 mmol TE/L.

Table 4. Antioxidant activity of commercial hawthorn vinegar samples

Sample Code*	ABTS (mg TE/L)	DPPH (mg TE/L)	FRAP (mg TE/L)
HV1	326.00±9.00^{d**}	114.65±1.77^{c}	271.00±6.21^{c}
HV2	722.18±25.71^{a}	381.80±12.73^{a}	441.07±5.52^{a}
HV3	376.45±0.64^{c}	130.15±1.06^{b}	275.02±3.28^{c}
HV4	111.00±1.93^{h}	36.90±0.71^{f}	65.15±2.07^{f}
HV5	189.64±2.57^{f}	50.90±0.71^{e}	179.90±9.49^{e}
HV6	158.27±0.64^{g}	52.40±1.41^{e}	71.00±3.10^{f}
HV7	249.18±12.21^{e}	67.65±1.06^{d}	196.00±8.11^{d}
HV8	404.18±3.86^{b}	136.15±1.06^{b}	309.05±1.38^{b}

**HV: Hawthorn vinegar, ABTS: Cation radical scavenging activity, DPPH: Free radical scavenging activity, FRAP: Ferric reducing antioxidant power; **Superscripts in the same column show difference between vinegar samples ($p<0.05$); Results are given as mean ± standard deviation.

Individual Phenolic Compounds in Commercial Hawthorn Vinegars

The amount of individual phenolic compounds (protocatechuic acid, quercetin 3-β-D glucoside and vitexin) obtained from commercial brands were determined with LC-MS/MS. The selection of protocatechuic acid, quercetin 3-β-D-glucoside, and vitexin in this study was based on their reported abundance and biological relevance in hawthorn and hawthorn-derived products [50]. Protocatechuic acid is a common phenolic acid widely detected in many fruits and is known to contribute significantly to antioxidant activity [51]. Quercetin 3-β-D-glucoside, a major flavonol glycoside, has been frequently reported as one of the predominant flavonoids in hawthorn leaf, playing an important role in its functional and health-promoting properties [52]. Vitexin is an apigenin flavone glucoside naturally present in several plants, including hawthorn and it has been widely reported to exhibit multiple bioactive properties [53]. Therefore, the determination of these three phenolic compounds provides valuable information regarding the phenolic profile, nutritional quality, and authenticity of commercial hawthorn vinegar samples.

Main validation data for phenolic compounds determined by LC-MS/MS method are given Table 5. The R^2 value and retention time of protocatechuic acid were determined as 0.9993 and 5.601 min, respectively, while the limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.4802 µg/kg and 4.9342 µg/kg. The precursor m/z of protocatechuic acid was identified as 153.00, and the product m/z value was determined to be 109.10. The R^2 value and retention time of quercetin 3-β-D-glucoside were determined as 0.9985 and 6.664 min, respectively, while the LOD and LOQ were found to be 2.3221 µg/kg and 7.7405 µg/kg. The precursor m/z of quercetin 3-β-D-glucoside was identified as 462.80, and the product m/z value was determined to be 301.10. The R^2 value and retention time of vitexin were determined as 0.9994 and 6.364 min, respectively, while the LOD and LOQ were found to be 2.0397 µg/kg and 6.7991 µg/kg. The precursor m/z of vitexin was identified as 430.80, and the product m/z value was determined to be 311.20.

Table 5. Main validation data for phenolic compounds determined by LC-MS/MS method

Data	Protocatechuic acid	Quercetin-3-β-D glucoside	Vitexin
R^2	0.999343	0.998587	0.99944
LOD	1.480289	2.322153	2.039739
LOQ	4.934297	7.74051	6.799129
Precursor m/z	153.00	462.80	430.80
Product m/z	109.10	301.10	311.20
Retention time (min)	5.601	6.664	6.364
Equation	y=(508.539)x+(554.499)	y=(667.514)x+(5756.29)	y=(2263.22)x+(336.927)

The amount of phenolic compounds of commercial hawthorn vinegars were given Table 6. Protocatechuic acid values of the commercial hawthorn vinegar samples varied between 6.35 mg/L (HV4 samples) and 29.48 mg/L (HV1 sample). Protocatechuic acid could not be detected for HV2 and HV8 samples. Quercetin-3-β-D glucoside was determined as 0.36, 1.15 and 0.95 mg/L for HV1, HV5 and HV7 samples, respectively. Quercetin-3-β-D glucoside was not detected in other samples. Moreover, vitexin compound was not detected in any sample.

Bakir et al. [38] determined the amount of protocatechuic acid as 5.4 mg/L for commercial hawthorn vinegar. Karadag et al. [35] determined the protocatechuic acid content of commercial hawthorn vinegar as 6.25 mg/L. Öğüt et al. [54] determined the quercetin amounts for control hawthorn vinegar and ultrasound-treated hawthorn vinegar as 2.75 and 0.19 mg/L, respectively. Quercetin could not be detected in heat-treated hawthorn vinegar. Vitexin compound is found in hawthorn, passion flowers, mung bean, beetroot, bamboo, otter flowers, and gaillardia [53]. However, vitexin was not detected in any hawthorn vinegar bought on the market.

Table 6. Individual phenolic compounds of commercial hawthorn vinegar samples

Sample Code*	Protocatechuic acid (mg/L)	Quercetin-3-β-D glucoside (mg/L)	Vitexin (mg/L)
HV1	29.48±0.00[a**]	0.36±0.00[c]	ND
HV2	ND	ND	ND
HV3	14.96±0.00[b]	ND	ND
HV4	6.35±0.00[f]	ND	ND
HV5	9.30±0.00[d]	1.15±0.00[a]	ND
HV6	14.04±0.00[c]	ND	ND
HV7	7.41±0.00[e]	0.95±0.00[b]	ND
HV8	ND	ND	ND

*HV: Hawthorn vinegar, ND: Not detected; **Superscripts in the same column show difference between vinegar samples ($p<0.05$); Results are given as mean ± standard deviation.

CONCLUSION

In this present study, hawthorn vinegars from 8 different brands sold in the market were analyzed. Titratable acidity value of the hawthorn vinegar samples (except HV6 sample) were not in conformity with the TS 1880 EN 13188 vinegar standard. Hawthorn vinegar samples were quite different from each other in terms of pH value, dry matter and color values. In particular, the titratable acidity value of the HV2 vinegar sample was determined to be quite low. It was determined that the HV2 sample, which has a very low titratable acidity value, had the highest total phenolic compound and antioxidant activity values. Protocatechuic acid was detected mostly in vinegars, followed by quercetin 3-β-D glucoside. Vitexin was not detected in any hawthorn vinegar sample. As a result, it has been determined that hawthorn vinegars of different brands sold in the market are quite different from each other in terms of both physicochemical and bioactive properties. In addition to the hawthorn fruit, other parameters such as hawthorn variety, hawthorn growing conditions, vinegar processing method should be taken into consideration as important factors in the physicochemical and bioactive properties of hawthorn vinegar production. Based on the results of this study, future research is recommended to investigate in more detail the effects of hawthorn vinegar's fruit variety, cultivation conditions, production methods, and storage conditions on its biological and physicochemical properties. Furthermore, *in vitro* and *in vivo* studies evaluating the health effects of different phenolic compounds would be valuable in elucidating the potential of hawthorn vinegar as a functional food.

AUTHOR CONTRIBUTIONS

Semra Topuz Türker: Methodology, Formal analysis, Software, Writing-original draft; Mustafa Bayram: Supervision, Conceptualization, Writing-review & editing

CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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