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Physicochemical properties of chestnut honey vinegars enriched with pollen and propolis, and their analyses of mineral, colour, and antioxidant activity

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Abstract

Honey vinegar is little known in Turkey, while pollen- or propolis-added honey vinegar is yet to be found. Therefore, the objectives of the present work were to produce chestnut honey vinegar enriched with pollen and propolis, and to analyse their physicochemical compositions, mineral contents, colours, and antioxidant activities. Eleven different honey vinegars were produced using various methods. There were significant increases in the total antioxidant activity of both pollen- and propolis-enriched vinegars, with 73 and 56% increases, respectively, compared to plain honey vinegar, and these increases were more than twice that of grape vinegar. The ferric-reducing activity and phenolic content of vinegar enriched with propolis increased by 43 and 52%, respectively, compared to plain honey vinegar, and more than threefold compared to grape vinegar. Principal component analysis revealed a strong positive correlation among all antioxidant tests and the most significant components, including antioxidant capacity, total phenolic content, and total flavonoid content. The colour difference was determined as follows: 5 < total colour difference (ΔE*ab). All vinegar samples exhibited highly variable physicochemical properties. High amounts of Na, K, Mg, and Ca were also detected. Vinegar samples containing pollen and propolis are suitable under the Turkish Food Codex legislation. The findings of the present work would encourage the production of honey vinegar enriched with pollen and propolis, as well as increase attention to honey vinegar.

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Introduction

Vinegar is a unique product manufactured biologically by fermenting agricultural liquids or sugary substances in two separate steps (alcoholic and acetic acid fermentation). During the fermentation of alcoholic beverages, yeast uses sugar to produce alcohol. Following the conversion of ethanol to acetaldehyde by alcohol dehydrogenase, acetaldehyde is converted to acetic acid by aldehyde dehydrogenase. *Acetobacter aceti*, *A. pastorianus*, and *A. hansenii* are commonly found in fruits and flowers because they can withstand high levels of sugars (sucrose, glucose, and fructose) (Plessi, 2003; Casale *et al*., 2006; Callejón *et al*., 2012).

The use of vinegar as a medicine and ingredient in human nutrition dates back to ancient times. Vinegar has several uses, including as preservative, seasoning, flavour, and healthy beverage (Ebner *et al*., 1996; Ebner and Sellmer-Wilsberg, 1999). Vinegar's medicinal properties have been attributed to the presence of essential amino acids, vitamins, minerals, organic acids, and phenolic compounds (Adams, 1998). Consuming vinegar regularly has been shown to have several benefits, including improved digestion, increased appetite, and antioxidant properties. Additionally, it can help with fatigue recovery, and maintain healthy blood pressure levels (Chou *et al*., 2015).

Vinegar also contains antioxidative compounds that positively affect human health due to its antihypertensive and antioxidant properties (Dávalos *et al*., 2005). Vinegar's antioxidant potential is due to various bioactive compounds, such as plant-derived phenolic acids and flavonoids. Phenolics' antioxidant activity involves several mechanisms, such as free radical scavenging, hydrogen donation, single electron transfer, singlet oxygen quenching,

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and metal ion chelation. In addition, they act as a substrate for radicals such as hydroxyl. Organic acids in vinegar also show bioactivity. As intermediates or products in metabolic pathways, they play an important role in biological processes. Many researchers have studied the biological activities of vinegar in this form, both *in vivo* and *in vitro*. In a significant study, the results suggested that the regeneration of liver glycogen may be facilitated by the combination of oral acetic acid and glucose (Nakao *et al*., 2001). A clinical study found that rice vinegar and acetic acid could increase blood flow (Sakakibara *et al*., 2010).

The chemical, physical, and bioactive properties of vinegar vary depending on the raw materials from which it is obtained. The vinegar's phenolic compounds and antioxidant chemicals predominantly come from the raw material. Moreover, it is known that the properties of vinegar vary with production method, region where the product is made, and climate (Raspor and Goranovic, 2008; Yetiman, 2012; Cosmulescu *et al*., 2022). The raw materials used in vinegar production are grape, wine, fruits, apple, alcohols, spices, grains, malt, beer, and honey (Yano *et al*., 1997). Honey consists of fructose and glucose (65 - 80%) as its main components, while containing less sucrose (up to 15%). Their quantities vary depending on the botanical source of nectar collected by bees. Honey is first diluted to obtain a wine containing 13 - 17% alcohol (Bahiru *et al*., 2006), and then vinegar is produced from honey wine by acetic acid bacteria. Homemade vinegar has become increasingly popular in recent years.

Pollen granules are male reproductive cells found in plant flowers (Spermatophyta). Pollens stick to bee legs and bodies during nectar collection. Bees attach pollen to pollen traps as they enter the hives, and humans collect the pollen from the traps. The material is fine and powdery in appearance. Pollen contains various phytochemicals and nutrient substances. It is also rich in carotenoids, flavonoids, and phytosterols, and is the primary food source for bees. Pollen and pollen products have been used for many beneficial applications (Stanciu *et al*., 2011).

Propolis is a brownish resinous substance collected by honeybees from various plant sources. These substances are transformed into propolis by enzymes produced by the bees. Propolis is used to seal and repair holes in the hives, and prevent the spread of microbial infections. From the past to the present, people have used propolis as a popular pharmaceutical in traditional medicine (Basim *et al*., 2006).

There are few studies on honey vinegar in the literature (Marques *et al*., 2010; Drescher *et al*., 2017). Furthermore, there has been no scientific research on chestnut honey vinegar. Therefore, the present work aimed to assess the antioxidant capabilities, bioactive compounds, and physicochemical compositions of chestnut honey vinegar enriched with pollen and propolis, which contains rich bioactive compounds.

Materials and methods

Grape vinegar from the market and plain chestnut honey vinegar were used as control samples in all analyses. Ten different vinegar samples were prepared from chestnut honey (Table 1). Before pollen and propolis were added, chestnut honey, which had 75 - 80% Brix, was diluted to a concentration of 15 - 18% Brix to ensure the proliferation of acetic acid bacteria. All samples were subjected to alcoholic fermentation until their alcohol content reached 9 - 12%. The products were fermented into vinegar. The vinegarisation process and maturation stage took approximately 120 d in sterilised glass containers under laboratory conditions. The duration required for vinegar production is contingent upon factors such as temperature, exposure to oxygen, and the specific strains of yeast and bacteria involved. The present work involved weekly measurements of acidity, and it took 120 d for the acidity to reach the desired level. Fermentation took place between 25 and 28°C in incubators. Following the maturation stage, clarification was carried out with Kieselguhr as a decantation agent for 1 - 2 d. Pollen and propolis were added to samples (Table 1), and the procedures are shown in a flow chart (Figure 1). Pollen and propolis were introduced into samples S7, S8, S9, and S10 following the formation of vinegar. Pollen and propolis were introduced at the beginning of the vinegar production process for the samples S2, S3, S5, and S6. All the samples were mixed for 24 h in a shaker, and then a filtration process was performed with 0.45 µm crossflow microfiltration. High concentrations of vinegars were diluted with distilled water at 4.0 - 4.5% acetic acid. The addition of

Table 1. Vinegar samples.

	Vinegar type
S0	Grape vinegar
S1	Vinegar of chestnut honey
S2	Vinegar of chestnut honey + propolis
S3	Vinegar of chestnut honey + pollen
S4	Vinegar of chestnut honey + yeast
S5	Vinegar of chestnut honey + chickpea + pollen
S6	Vinegar of chestnut honey + chickpea + propolis
S7	Vinegar of chestnut honey + chickpea + addition of pollen after vinegar
S8	Vinegar of chestnut honey + chickpea + addition of propolis after vinegar
S9	Vinegar of chestnut honey + addition of pollen after vinegar
S10	Vinegar of chestnut honey + addition of propolis after vinegar

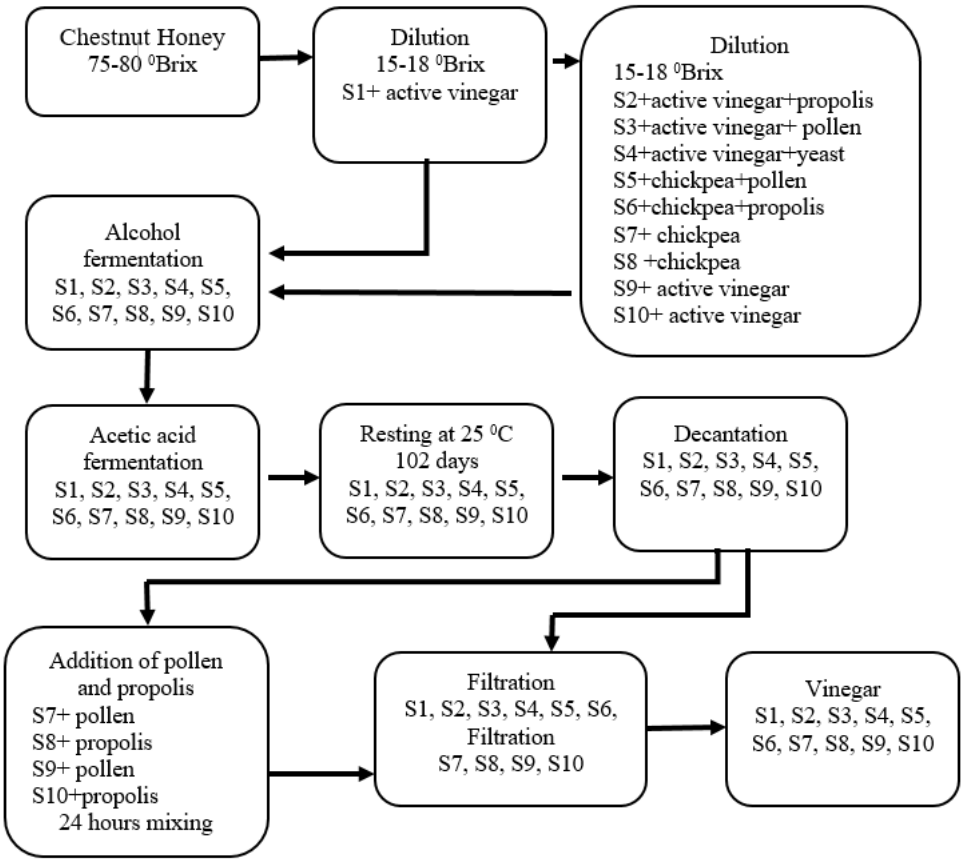

Figure 1. Production flow procedure of vinegar samples. Full name of samples are given in Table 1.

chickpeas, yeast, and active vinegar did not affect vinegar-making time. The specific strains of yeast and acetic acid bacteria used in the process may differ in their fermentation rates and efficiency. Experimenting with different species or combinations of microorganisms can lead to differences in the vinegarisation process. However, the vinegarisation process remained the same when adding chickpea, yeast, and active vinegar to the same nutrient medium, while maintaining the same temperature and all other conditions.

Chemicals and equipment

All chemicals and solvents (analytical or HPLC purity) were purchased from Merck (Darmstadt, Germany). Phenolic ingredient standards, ABTS, TPTZ, Trolox, ascorbic acid, and gallic acid standards were purchased from Sigma-Aldrich (St. Louis, Mo., USA).

Physicochemical properties

Alcohol amount

The amount of alcohol was determined following the AOAC reference table (AOAC, 2019a). The vinegar sample (100 mL) was added to a distillation flask, and a 40% (w/v) sodium hydroxide (NaOH) solution was added until it was neutralised. An estimated ¾ of the vinegar was distilled. It was then diluted to 100 mL with ultrapure water. The densities of the samples at 20°C were determined using the pyknometry method.

Oxidation number (ON) and iodine number (IN)

The vinegar sample (60 mL) was taken into a distillation flask. Following the addition of 15 mL of ultrapure water and a few pumice stones, the distillate was slowly distilled until 60 mL was collected. Next, 10 mL of sulphuric acid-water (1:3) and 10 mL of 0.1 N potassium permanganate reagent were added to 25 mL of this distillate. The mixture was left in the dark at 18°C for 30 - 35 min. Then, 5 mL of 10% potassium iodide (KI) reagent was added. The released iodine was titrated using 0.02 N sodium thiosulfate ($Na_2S_2O_3$). The indicator (a solution of starch) was introduced near the end point of the reaction. The amount of oxidation was calculated based on consumption (Ronald and Ronald, 1991).

The remaining distillate from the oxidation number analysis was used to determine the amount of iodine. First, 25 mL of increased distillate was neutralised with 10 N potassium hydroxide (KOH). Next, 10 mL of 0.1 N iodine solution was added. It was kept in the dark for 15 min, then titrated using 0.02 N $Na_2S_2O_3$ (Ronald and Ronald, 1991).

Ester analysis (EA)

The vinegar sample (100 mL) was taken into the distillation flask, a few pumice stones were added, and 30 mL of distillate was collected. Phenolphthalein was dropped into the distillate, and neutralised using 1 N KOH until a pink colour appeared. Next, 0.02 N hydrochloric acid (HCl) was added drop by drop to the sample until the pink colour disappeared. Then, 10 mL of a 0.1 KOH solution was applied to it. Afterwards, the solution was cooled in a water bath with reflux for 2 h after being soaped. Phenolphthalein was introduced in small amounts, and then titrated using 0.02 N HCl. The same experiment was repeated using pure water instead of 30 mL of distillate for the control sample (Ronald and Ronald, 1991).

Total ash

The total ash content was determined following the standard methods of AOAC (2019b). The product in the capsule was used for ash determination after the total solid content in the sample were determined. Ash determination is the continuation of total solid content determination. This sample in the porcelain capsule was taken and placed at 525 ± 10°C in the muffle furnace and burned to white or ash colour until it reached constant weight. After waiting for 20 min at the desiccator, the samples were immediately weighed.

pH

The pH values of honey vinegar samples were measured using a pH meter (Hannah, Hi, 2211-02, USA). The pH meter was first calibrated using buffer solutions of pH 4.00, 7.00, and 10.00. Vinegar samples were transferred into the 50-mL beaker, and measurements were then made (AOAC, 2019c).

Volatile acid (VA) and non-volatile acid (NVA)

Volatile acidity (VA) and non-volatile acidity (NVA) analyses were performed following the AOAC official standard methods (AOAC, 2019d; 2019e). The steam distillation apparatus was used. After the water vapour passed through the evaporation device, 50 mL of the vinegar sample was placed in the 300 mL evaporation container. In total, 200 mL of distillate was collected for 50 min. The temperature of the steam container during distillation was adjusted to maintain 25 mL of vinegar in the evaporation container. The obtained distillate was neutralised using 0.1 N NaOH and phenolphthalein. The VA and NVA were obtained using Eq. 1:

Non-volatile acid amount =
total acidity - volatile acidity (Eq. 1)

Total sugar (TS)

First, 50 mL of the sample was added to a 100 mL balloon flask. After addition 2.5 mL of each from Carrez I and Carrez II solutions, volume of the mixture was adjusted to 100 mL with ultrapure water. For hydrolysis, 25 mL of the mixture was transferred to a 100 mL balloon flask. Next, 5 mL of concentrated HCl was added and left for 30 min at 60°C. After cooling, the honey vinegar was neutralised using 5 M NaOH, and the 100 mL volumetric flask containing the solution was filled completely. After adding 15 mL of purified water to the mixture of 10 mL Fehling

(5 mL Fehling A and 5 mL Fehling B) solution, five drops of 1% methylene blue were added, and the mixture was heated on a heating magnetic stirrer (Daihan MSH-20D magnetic stirrer, Daihan Scientific, Korea). During boiling, the sample solution was poured dropwise from a burette. The titration ended when the blue colour turned tile-red at the turning point (AOAC, 2019f).

Total solid content (TSC) and total sugar-free solids (TSFS)

The TSC of honey vinegar is the weight of dried honey vinegar residue expressed as a percentage of the original honey vinegar weight (AOAC, 2019g). The TSC was determined by weighing vinegar, drying vinegar, and weighing dried vinegar residue. Honey samples were dried in the oven at 102 ± 1°C for 4 h. The TSFS was calculated by subtracting the TS from the TSC.

Antioxidant activity analyses

Total flavonoid content (TFC) and total acidity (TA)

The TFC was determined following the previously used method (Kasangana *et al*., 2015). A mixture of 500 µL of the sample and 3,200 µL of methanol (HPLC purity, 30% (v/v)) was vortexed, and 150 µL of 0.5 M $NaNO_2$ solution was added. The solution was kept mixing for 5 min after adding 150 µL of 0.3 M aluminium chloride ($AlCl_3$). Then, 1 mL of 1 M NaOH solution was added and mixed for 10 min. The TFC was determined through absorbance readings using a spectrophotometer at 506 nm (UV-1800 Series Spectrophotometer, Shimadzu, Japan). Using the calibration equation obtained, the TFC results were expressed as mg quercetin equivalent (QEE)/L.

To determine the TA, 10 mL of vinegar was placed in a flask. Ultrapure water (100 mL) was then boiled and cooled beforehand, and added. A few drops of phenolphthalein indicator were then dropped. Then it was titrated using 0.5 N NaOH (AOAC, 2019d).

Ferric-reducing antioxidant power (FRAP)

A 10:1:1 mixture of 0.3 mM sodium acetate buffer solution (pH 3.6), 20 mM aqueous Ferric chloride ($FeCl_3$) solution, and 10 mM aqueous TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution were prepared. The FRAP solution (2.75 mL) was mixed with a vinegar sample (0.25 µL) and methanol (900 µL) in the spectrophotometer cuvettes, and stored at room temperature for 30 min. Then, the absorbance values were measured. The FRAP value was expressed as mg iron(II) sulphate ($FeSO_4$) equivalent/L (Benzie and Strain, 1996).

Radical cation scavenging effect (ABTS) and radical scavenging capacity (DPPH)

The determination of antioxidant capacity by ABTS assay was performed following the method described by Baltacı *et al*. (2022). The sample (150 µL) was added to the 2,850 µL ABTS study solution. The mixture was then vortexed for 1 to 6 min. The absorbance of the resulting solution was measured at 734 nm wavelength. Methanol (150 µL) was used as a blind sample. A total of 150 µL of the standards (ascorbic acid and Trolox) was used, and the same procedures were performed. Through the calibration equation obtained, ABTS outcome was expressed as mg AAE/L and % inhibition.

To determine the DPPH radical scavenging capacity, a filtered sample (0.1 mL) was added to 3 mL of DPPH solution (10 mM) in a test tube. The mixture was vigorously stirred using a vortex mixer. Then, the mixture was left to incubate in the dark for 30 min at room temperature. The absorbance of the solution was measured at 517 nm. DPPH was expressed as a percentage of inhibition capacity. Trolox and ascorbic acid were used as standards (Ahmed *et al*., 2015).

Total antioxidant activity (TAC)

The TAC (phosphomolybdate assay) was assessed following the technique described by Umamaheswari and Chatterjee (2008). First, 2,500 µL of ultrapure water was added to 500 µL of the samples. The mixture was then vortexed after the addition of 1,000 µL molybdate reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The samples were analysed in triplicates. The tubes were incubated at 95°C for 90 min with careful insulation. The same procedure was applied to the standards. The absorbance of the reaction mixture was measured at 765 nm using the spectrophotometer. Through the calibration equation obtained, the TAC results were expressed as mg AAE/L.

Total phenolic content (TPC)

The filtered sample (0.1 mL) was added to 4.5 mL of ultrapure water. To the mixture, 0.6 mL of Folin-Ciocalteu phenol reagent and 1.6 mL of sodium

carbonate (Na_2CO_3) (10%) were then added, respectively. The mixture was left to incubate in the dark for 60 min at room temperature. The absorbance of the solutions was measured at 760 nm. The results were expressed in mg gallic acid equivalent (GAE)/L (Kasangana *et al*., 2015).

Colour analysis

Colour analysis was performed using a Minolta Chromameter (CR-200) (Konica Minolta Sensing, Inc., Japan), as reported by Quek *et al*. (2007). The device was set with a white calibration plate. For the Hunter scale, L (0 - 50 indicates dark, 51 - 100 indicates light.), a (positive numbers indicate red, negative numbers indicate green), and b (positive and negative numbers indicate yellow and blue, respectively) were the colour parameters. The colours of honey vinegar were measured in special containers.

Mineral analysis

Mineral analyses were performed using an Agilent brand MP-AES according to the NMKL (1998; 2007) method. The vinegar sample (0.5 mL) and nitric acid (HNO_3) (5 mL) were added to microwave digestion vessels. At the end of the burning process, the solution was placed in a 25 mL balloon flask, and the flask was filled with ultrapure water. The calibration curves for all analytes were generated using data from five different concentrations. Tune solutions and intermediate standards were used at regular intervals to control the drift of the device after each standard. Ultrapure water analysis was performed to control cross-contamination.

Statistical analysis

All analyses were conducted at least in triplicate, and the results were presented as mean ± standard deviation. Principal component analysis (PCA) was used to evaluate the possible relationships between the studied parameters using the software package (XLSTAT Addinsoft SARL 2021).

Results and discussion

The vinegar production scheme is illustrated in Figure 1. The abbreviations of each sample are shown in Table 1. Sample S1 (plain chestnut honey vinegar) was used as a control, whereas sample S0 (grape vinegar) was used as a comparison. Table 2 lists the physicochemical features of honey vinegar samples, including alcohol content, EA, IN, ON, pH, TA, TSC, TSFS, VA, and NVA. The alcohol levels varied from 0.06 to 0.34% (v/v). EA values also contributed to the vinegar flavour, which changed from 16.60 to 21.40 mL/100 mL. The INs were determined between 1.60 and 14.93. The total ash values were between 0.51 and 0.89 g/100 mL. Pollen is rich in minerals such as calcium, potassium, magnesium, and phosphorus. When pollen was added to honey vinegar samples, it contributed to the overall ash content due to these minerals. Propolis also contains minerals, including calcium, magnesium, and zinc. When propolis was added to the vinegar samples, it increased the ash content. The high ash content of chestnut honey vinegars enriched with pollen and propolis was primarily due to the naturally occurring minerals in pollen and propolis. The ONs changed from 68.53 to 386.13. The pH levels ranged from 2.35 to 3.41. Vinegar acidity can vary naturally based on the source material and production process, potentially accounting for variations from the typical pH ranges. The acidity of honey vinegar, even after being diluted to approximately 4% acidity, is mainly caused by the existence of acetic acid and other organic acids that add to the total acidity of the solution. These acids undergo ionisation when mixed with water, resulting in the release of hydrogen ions, thus reducing the pH of the vinegar solution. The TAs were determined to be 4.09 - 4.36 g/100 mL. The ranges of VA and NVA were 7.45 - 33.28 and 8.88 - 35.03 g/L, respectively. The TSCs varied from 17.40 to 162.66 g/L. The TS values exhibited significant variation, ranging from 3.22 to 77.95 g/L, indicating a high degree of variability. The TSFS levels were between 13.12 and 151.46 g/L. Colour value analysis determined the L* values from 21.75 to 33.29, the a* values from 3.63 to 10.21, the b* values from 0.80 to 9.28, and the ΔE*ab from 14.49 to 21.26 (Table 3).

The results of antioxidant activity tests are shown in Table 4. The DPPH values were between 78.42 and 90.67% (grape vinegar comparison sample: 58.12%). DPPH activity is a parameter that varies significantly ($p < 0.05$) depending on the crude materials of the analysed samples. While TPC values were observed between 119.50 mg GAE/L (S1) and 182.86 mg GAE/L (S2 and S7), significant differences ($p < 0.05$) were observed in all vinegars. The S0 had the lowest TFC (99.81 mg GAE/L), and the highest TFC was found in S9 (142.70 mg GAE/L). The highest and lowest TACs were determined in S3

Table 2. Physicochemical properties of grape and chestnut honey vinegar samples enriched with pollen and propolis.

Sample	Alcohol (mL/100 mL)	Ester (mL/100 mL)	Iodine number (g/100 ml)	Total ash (g/100 mL)	Oxidation number	pH	Total acid (g/100 mL)	Total sugar (g/L)	Total solid content (g/L)	Total sugar free solid (g/L)	Volatile acid (g/L)	Non-volatile acid (g/L)
S0	0.30 ± 0.01^{ab}	2.68 ± 0.20^{g}	1.01 ± 0.02^{c}	0.52 ± 0.02^{b}	73.32 ± 4.93^{h}	2.70 ± 0.05^{d}	4.33 ± 0.10^{a}	3.22 ± 0.01^{f}	13.10 ± 0.05^{ı}	9.88 ± 0.05^{j}	8.30 ± 1.08^{h}	35.03 ± 0.55^{a}
S1	0.06 ± 0.02^{e}	16.60 ± 0.46^{e}	14.93 ± 0.97^{a}	0.51 ± 0.05^{d}	68.53 ± 1.00^{h}	3.41 ± 0.09^{a}	4.14 ± 0.06^{c}	4.28 ± 0.10^{f}	17.40 ± 0.50^{h}	13.12 ± 0.12^{ı}	7.45 ± 0.45^{ı}	34.41 ± 0.40^{a}
S2	0.27 ± 0.02^{b}	18.50 ± 0.31^{f}	1.87 ± 0.25^{ef}	0.78 ± 0.01^{b}	202.13 ± 2.00^{b}	2.83 ± 0.07^{cd}	4.09 ± 0.10^{c}	11.13 ± 0.10^{d}	27.10 ± 1.00^{g}	15.97 ± 0.03^{h}	16.54 ± 0.06^{f}	24.32 ± 0.10^{c}
S3	0.20 ± 0.02^{c}	21.40 ± 0.45^{a}	3.47 ± 0.47^{cd}	0.68 ± 0.01^{c}	320.50 ± 4.00^{c}	2.88 ± 0.02^{c}	4.23 ± 0.10^{abc}	9.84 ± 0.06^{e}	29.46 ± 0.54^{f}	19.63 ± 0.20^{g}	25.30 ± 0.40^{c}	17.04 ± 0.04^{e}
S4	0.20 ± 0.03^{c}	17.40 ± 0.46^{ef}	3.73 ± 0.17^{c}	0.61 ± 0.02^{c}	330.67 ± 2.00^{b}	3.38 ± 0.09^{a}	4.18 ± 0.02^{bc}	4.10 ± 0.09^{f}	30.57 ± 0.33^{ef}	26.46 ± 0.30^{e}	19.92 ± 0.07^{e}	21.90 ± 1.00^{d}
S5	0.12 ± 0.01^{d}	17.14 ± 0.81^{ef}	2.67 ± 0.23^{de}	0.68 ± 0.02^{c}	149.07 ± 8.00^{g}	2.35 ± 0.15^{e}	4.36 ± 0.15^{a}	77.95 ± 1.00^{a}	153.35 ± 1.00^{b}	75.71 ± 0.29^{b}	32.51 ± 0.40	11.41 ± 0.40^{f}
S6	0.22 ± 0.04^{c}	20.53 ± 0.38^{bc}	5.33 ± 0.47^{b}	0.88 ± 0.03^{a}	252.53 ± 2.00^{d}	2.89 ± 0.10^{c}	4.36 ± 0.10^{a}	11.91 ± 1.00^{d}	35.43 ± 1.33^{d}	23.52 ± 0.40^{f}	21.82 ± 0.08^{d}	25.79 ± 3.47^{c}
S7	0.29 ± 0.02^{b}	21.33 ± 0.41^{ab}	1.60 ± 0.70^{f}	0.62 ± 0.03^{c}	386.13 ± 2.00^{a}	2.69 ± 0.10^{d}	4.32 ± 0.06^{ab}	51.98 ± 1.01^{c}	115.57 ± 1.00^{c}	63.62 ± 0.35^{c}	25.38 ± 0.40^{c}	17.84 ± 0.06^{e}
S8	0.13 ± 0.02^{d}	20.40 ± 0.57^{c}	3.13 ± 0.68^{cd}	0.88 ± 0.04^{a}	380.27 ± 3.00^{a}	3.07 ± 0.03^{b}	4.22 ± 0.03^{abc}	11.92 ± 1.00^{d}	31.14 ± 0.76^{e}	19.22 ± 0.40^{g}	33.28 ± 0.10^{a}	8.88 ± 0.12^{g}
S9	0.34 ± 0.02^{a}	18.40 ± 0.25^{d}	2.67 ± 0.24^{de}	0.89 ± 0.09^{a}	204.80 ± 2.00^{e}	2.39 ± 0.11^{e}	4.16 ± 0.04^{c}	11.13 ± 0.10^{d}	162.66 ± 0.22^{ea}	151.46 ± 0.30^{a}	17.04 ± 0.04^{f}	24.64 ± 0.16^{c}
S10	0.27 ± 0.02^{b}	18.33 ± 0.63^{d}	3.47 ± 0.53^{cd}	0.89 ± 0.01^{a}	113.60 ± 3.00^{g}	2.35 ± 0.15^{e}	4.34 ± 0.06^{ab}	63.52 ± 1.00^{b}	115.57 ± 1.00^{c}	52.02 ± 0.55^{d}	14.45 ± 0.05^{g}	28.83 ± 0.07^{b}

Values are mean ± standard deviation. Means followed by different lowercase superscripts within similar columns are significantly ($p < 0.05$) different. Full name of samples are given in Table 1. S1 served as control. Sample S0 served as comparison.

Table 3. Colour properties of grape and chestnut honey vinegar samples enriched with pollen and propolis.

Sample	L*	a*	b*	ΔE^*_{ab}
S0	16.58 ± 0.15^{f}	7.10 ± 0.05^{b}	14.11 ± 0.09^{a}	14.36 ± 0.13^{d}
S1	21.75 ± 0.10^{e}	10.21 ± 0.20^{a}	0.96 ± 0.30^{e}	14.49 ± 0.25^{d}
S2	33.29 ± 0.83^{a}	5.91 ± 0.40^{c}	3.80 ± 0.19^{c}	19.70 ± 0.80^{d}
S3	30.41 ± 0.40^{bc}	4.03 ± 0.59^{e}	8.83 ± 0.00^{b}	15.14 ± 0.48^{d}
S4	26.79 ± 0.20^{d}	3.88 ± 0.10^{e}	3.21 ± 0.20^{c}	15.29 ± 0.03^{d}
S5	31.19 ± 0.40^{b}	4.40 ± 0.20^{de}	1.48 ± 0.45^{e}	19.52 ± 0.32^{b}
S6	30.35 ± 0.30^{bc}	3.76 ± 0.20^{e}	9.28 ± 0.20^{b}	14.99 ± 0.30^{d}
S7	29.55 ± 0.50^{c}	4.05 ± 0.10^{e}	2.10 ± 0.20^{d}	17.95 ± 0.50^{c}
S8	30.75 ± 0.20^{bc}	3.63 ± 0.30^{e}	9.15 ± 0.10^{b}	15.43 ± 0.26^{d}
S9	29.52 ± 0.45^{c}	4.08 ± 0.15^{e}	2.11 ± 0.10^{d}	17.92 ± 0.34^{c}
S10	32.98 ± 1.00^{a}	4.89 ± 0.10^{d}	0.80 ± 0.05^{f}	21.26 ± 0.73^{a}

Values are mean ± standard deviation. Means followed by different lowercase superscripts within similar columns are significantly ($p < 0.05$) different. Full name of samples are given in Table 1. S1 served as control. Sample S0 served as comparison.

Table 4. Antioxidant properties of grape vinegar and chestnut honey vinegar samples enriched with pollen and propolis.

Sample	TAC	TPC	TFC	FRAP	ABTS	DPPH
S0	133.01 ± 0.11^{f}	110.17 ± 1.09^{e}	99.81 ± 0.20^{e}	55.11 ± 0.91^{h}	46.38 ± 1.67^{e}	58.12 ± 2.97^{f}
S1	177.25 ± 0.91^{f}	119.50 ± 2.52^{de}	100.15 ± 1.56^{e}	168.56 ± 0.60^{g}	50.36 ± 3.95^{e}	78.42 ± 2.09^{e}
S2	277.13 ± 2.29^{c}	182.86 ± 1.87^{a}	117.96 ± 1.74^{d}	242.38 ± 3.82^{a}	72.73 ± 2.70^{d}	90.67 ± 4.62^{b}
S3	306.31 ± 2.52^{a}	121.52 ± 2.65^{cd}	103.51 ± 3.79^{e}	198.45 ± 0.90^{de}	75.34 ± 3.50^{d}	84.15 ± 0.00^{cd}
S4	301.99 ± 0.99^{a}	182.23 ± 3.47^{a}	120.97 ± 1.97^{cd}	215.79 ± 3.61^{c}	93.16 ± 1.86^{b}	83.25 ± 3.86^{cd}
S5	228.33 ± 5.39^{d}	128.13 ± 7.77^{cd}	127.68 ± 2.08^{bc}	182.86 ± 5.29^{f}	90.59 ± 2.36^{bc}	86.41 ± 1.54^{cde}
S6	220.52 ± 3.61^{d}	130.11 ± 0.34^{c}	104.40 ± 3.79^{e}	217.41 ± 2.65^{bc}	92.59 ± 3.34^{b}	86.12 ± 2.67^{cde}
S7	287.21 ± 5.43^{b}	182.86 ± 2.80^{a}	135.77 ± 4.58^{ab}	225.53 ± 4.00^{b}	85.49 ± 3.20^{d}	88.32 ± 1.43^{bc}
S8	278.26 ± 0.87^{bc}	174.59 ± 3.61^{ab}	132.82 ± 5.49^{b}	202.33 ± 1.54^{d}	85.94 ± 2.65^{d}	89.43 ± 1.18^{bc}
S9	305.47 ± 3.57^{a}	121.50 ± 1.32^{cd}	142.70 ± 2.65^{a}	198.57 ± 1.03^{de}	88.69 ± 1.76^{bc}	87.96 ± 1.35^{bc}
S10	301.71 ± 3.21^{a}	171.51 ± 3.61^{b}	129.38 ± 4.58^{bc}	191.75 ± 6.05^{ef}	90.39 ± 2.72^{bc}	89.66 ± 2.07^{bc}
AA	-	-	-	-	99.38 ± 0.54^{a}	98.67 ± 0.56^{ah}
Trolox	-	-	-	-	98.75 ± 1.48^{a}	98.16 ± 1.15^{a}

Values are mean ± standard deviation. Means followed by different lowercase superscripts within similar columns are significantly ($p < 0.05$) different. Full name of samples are given in Table 1. TAC (total antioxidant capacity) and FRAP (ferric-reducing antioxidant power assay) were expressed as mg AAE/L. TPC (total phenolic content) and TFC (total flavonoid content) were expressed as mg GAE/L. DPPH (radical scavenging capacity) and ABTS (radical-scavenging activity) were expressed as inhibition %. S1 served as control. Sample S0 served as comparison.

(306.31 mg AAE/L) and S1 (177.25 AAE/L), whereas the comparison sample was measured at 133.01 mg AAE/L. ABTS activity ranged from 50.36% (S1) to 93.16% (S4). The FRAP activities of the vinegars were determined to be between 168.56 and 225.53 mg AAE/L.

Table 5 shows the mineral analysis results (expressed as mg/L) for chestnut honey vinegar samples enriched with pollen and propolis. The mineral analyses of the samples are summarised as follows = (S1 to S10): Fe (iron) ranged from 23.50 mg/L in S1 to 155.43 mg/L in S10; Mn (manganese)

Table 5. Mineral contents of chestnut honey vinegar samples enriched with pollen and propolis.

Sample	Fe	Mn	Cu	Al	Zn	Na	K	Ca	Mg	Total mineral
S1	23.50 ± 2.00^{g}	11.00 ± 3.61^{b}	1.50 ± 0.36^{c}	122.33 ± 6.11^{e}	6.73 ± 0.86^{h}	223.00 ± 17.58^{h}	3933.33 ± 49.33^{g}	492.97 ± 6.03^{h}	101.00 ± 6.46^{h}	4884.67 ± 334.63^{f}
S2	73.50 ± 5.55^{d}	15.13 ± 3.21^{ab}	3.97 ± 0.70^{a}	122.00 ± 6.24^{e}	25.43 ± 4.35^{e}	1135.00 ± 26.46^{a}	4830.00 ± 87.18^{d}	1473.00 ± 20.00^{c}	180.00 ± 18.03^{f}	4920.40 ± 108.91^{f}
S3	24.10 ± 3.61^{g}	13.53 ± 4.91^{ab}	3.83 ± 0.61^{a}	107.00 ± 9.17^{f}	38.93 ± 0.95^{c}	518.00 ± 8.00^{f}	5236.67 ± 50.33^{c}	575.00 ± 18.03^{g}	232.00 ± 6.24^{e}	7863.03 ± 127.44^{c}
S4	97.77 ± 2.10^{c}	11.27 ± 2.08^{b}	3.47 ± 0.38^{ab}	73.33 ± 4.93^{g}	30.40 ± 0.87^{d}	575.00 ± 18.03^{e}	4446.67 ± 40.41^{ef}	546.67 ± 37.86^{g}	248.13 ± 1.70^{de}	6754.07 ± 63.10^{d}
S5	55.20 ± 4.00^{e}	15.07 ± 4.19^{ab}	2.63 ± 0.38^{b}	62.67 ± 5.51^{d}	10.80 ± 0.87^{g}	689.67 ± 8.52^{c}	4486.67 ± 167.73^{e}	858.33 ± 8.02^{d}	307.00 ± 8.89^{b}	6037.70 ± 101.31^{e}
S6	117.07 ± 2.47^{b}	17.37 ± 2.37^{a}	3.90 ± 0.20^{a}	137.97 ± 1.75^{d}	15.13 ± 0.67^{f}	928.33 ± 8.02^{b}	6600.00 ± 264.58^{a}	790.00 ± 18.03^{e}	277.00 ± 6.24^{c}	6493.03 ± 188.35^{d}
S7	41.87 ± 0.90^{f}	16.60 ± 2.65^{ab}	3.47 ± 0.38^{ab}	172.67 ± 6.51^{b}	23.60 ± 0.87^{e}	415.00 ± 8.72^{g}	4233.33 ± 28.87^{f}	777.97 ± 1.75^{e}	336.00 ± 2.65^{e}	8891.77 ± 282.17^{a}
S8	50.87 ± 4.04^{e}	13.67 ± 1.21^{ab}	4.00 ± 0.78^{a}	196.00 ± 2.65^{a}	7.47 ± 0.45^{h}	662.63 ± 6.37^{d}	6146.67 ± 117.19^{b}	1627.63 ± 2.03^{b}	145.97 ± 3.55^{g}	6025.50 ± 46.26^{e}
S9	39.10 ± 7.81^{f}	14.27 ± 1.10^{ab}	3.40 ± 0.44^{ab}	157.80 ± 1.84^{c}	67.80 ± 1.85^{a}	573.00 ± 8.89^{e}	4766.67 ± 125.83^{d}	652.00 ± 6.24^{f}	230.00 ± 9.54^{e}	8859.90 ± 111.91^{a}
S10	155.43 ± 4.00^{a}	16.23 ± 0.59^{ab}	3.47 ± 0.38^{ab}	127.00 ± 1.80^{e}	56.07 ± 0.74^{b}	648.33 ± 20.21^{d}	5283.33 ± 189.30^{c}	1968.33 ± 23.63^{a}	256.97 ± 2.59^{d}	6509.03 ± 126.92^{d}

Values are mean ± standard deviation. Means followed by different lowercase superscripts within similar columns are significantly ($p < 0.05$) different. Full name of samples are given in Table 1. Results of minerals were expressed as mg/L %. LOQ = 0.01 mg/L.

ranged from 11.00 mg/L in S1 to 17.37 mg/L in S6; Cu (copper) ranged from 1.50 mg/L in S1 to 4.00 mg/L in S8; Al (aluminium) ranged from 62.67 mg/L in S5 to 196.00 mg/L in S8; Zn (zinc) ranged from 6.73 mg/L in S1 to 67.80 mg/L in S9; Na (sodium) ranged from 223.00 mg/L in S1 to 1135.00 mg/L in S2; K (potassium) ranged from 3933.33 mg/L in S1 to 6600.00 mg/L in S6; Ca (calcium) ranged from 492.97 mg/L in S1 to 1968.33 mg/L in S10; and Mg (magnesium) ranged from 101.00 mg/L in S1 to 336.00 mg/L in S7. Other elements like Co (cobalt), Ni (nickel), Cr (chromium), Cd (cadmium), and Pb (lead) were reported as "< LOQ" (below the limit of quantification, which was 0.01 mg/L), indicating they were not detected at measurable levels in these samples. The mineral contents varied significantly across samples, likely due to differences in the composition of pollen, propolis, and possibly other factors related to production and processing. Samples enriched with pollen and propolis generally showed higher levels of minerals compared to the chestnut honey vinegar (S1), indicating that these additives contributed to the overall mineral contents, particularly in samples like S6, S8, S9, and S10.

PCA analysis, one of the analytical approaches, was used to define the multivariate analysis data. Honey vinegar samples were characterised by significant correlations between the 13 variables (Table 6). The physicochemical properties of honey vinegar (Table 2) were evaluated with PCA to analyse the similarities between the samples and variables (Figure 2). First and second principal component (PC1 and PC2) together explained 59.74% of the total variance clustered in four coloured circles (red, blue, green, and black). There was a wide variation in physicochemical parameters owing to differences in features related to raw materials and honey vinegar. Some foods contain weak acids that do not significantly affect pH, even if their acidity increases. Without generating a substantial pH alteration, these mild acids can contribute to the overall acidity. The composition of foods such as vinegar and honey are complex due to the presence of various organic acids, sugars, and other compounds. The interactions between these components can result in unexpected relationships between pH and total acidity, as they can affect pH and acidity in non-linear ways.

Gerbi *et al*. (1998) and Sahin *et al*. (1977) reported that the amount of alcohol in vinegar ranged from 0.20 to 0.70 mL/100 mL, which was in conformity with Turkish regulation (TSE, 2016). During alcoholic fermentation in the present work, the produced alcohol was entirely converted into acetic acid by aerobic fermentation, which was below Turkish regulation. There were statistical differences ($p < 0.05$) compared to the control samples in alcohol amount measurements; however, these were in negligible ranges.

The EAs of vinegar enriched with pollen and propolis were slightly higher than the control samples ($p < 0.05$), whereas the results were compatible with previous studies (Oguntoyinbo *et al*., 2011; Ullah *et al*., 2014). The EA correlated highly positively with ON and VA, as shown in Table 6. In PCA, EA was high in propolis-enriched vinegar.

In earlier studies, the INs of vinegar were investigated (Oguntoyinbo *et al*., 2011; Ullah *et al*., 2014), and the results were higher than the values of the present work. In general, the INs of vinegar containing pollen were lower than those of vinegar containing propolis. This may be related to the presence of more than 300 compounds in the propolis structure (Kuropatnicki *et al*., 2013).

Multivariate analysis of PC1's mineral capacity of vinegar explained 38.01% *versus* PC2, which explained 18.22% (Figure 3). Three groupings were obtained from cluster analysis- Group 1: S1; Group 2: S3, S4, S5, S7, and S9; and Group 3: S2, S6, S8, and S10. Figure 3 shows that the mineral parameters clustered our vinegar samples into pollen, propolis, and control samples. The total ash and total mineral contents showed a positive correlation (Table 6). Honey vinegar was found to be very rich in Mg, Na, K, and Ca minerals. Ash and mineral contents were higher in pollen- and propolis-enriched vinegars than in control samples. Quantities of Cd and Pb in vinegar samples could not be determined (< LOQ). These results were within the Turkish Food Codex (2011) maximum limit. Gerbi *et al*. (1998) reported that the total ash values of wine vinegar changed from 0.20 - 0.26 g/100 mL. Ash and mineral contents were found to be high in vinegar-containing pollen owing to the high mineral content (Stanciu *et al*., 2011).

The ON values were determined with the titrimetric method, and found to be 424 in previous research (Ullah *et al*., 2014). It has been reported that oxidation occurs because of the triggering of polyphenolic compounds by oxygen (Cline, 2003). Compared to control samples, ON results were higher in vinegar containing pollen and propolis. This can be explained by the rich content of polyphenolic compounds in pollen and propolis.

Table 6. Correlations (r_s values) between physicochemical analyses of vinegar.

Variables	Alcohol	Ester amount	Iodine number	Total ash	Oxidation number	pH	Total acid	Total sugar	Total solid content	Total sugar free solid	Volatile acid	Non-volatile acid	Total mineral
Alcohol	1	-0.243	-0.593	-0.121	0.049	-0.506	0.129	-0.022	0.287	0.404	-0.252	0.259	0.277
Ester Amount		1	-0.041	0.514	0.614	0.109	-0.071	0.219	0.260	0.214	0.560	-0.549	0.564
Iodine Number			1	0.146	-0.470	0.602	-0.172	-0.327	-0.389	-0.321	-0.517	0.521	-0.514
Total Ash				1	-0.159	-0.339	0.182	0.567	0.657	0.534	0.127	-0.059	0.310
Oxidation Number					1	0.203	-0.035	-0.069	-0.027	0.007	0.706	-0.714	0.529
pH						1	-0.280	-0.642	-0.764	-0.632	-0.154	0.128	-0.285
Total Acid							1	0.478	0.235	0.017	0.223	-0.065	0.246
Total Sugar								1	0.727	0.355	0.401	-0.357	0.362
Total Solid Content									1	0.900	0.294	-0.288	0.211
Total Sugar Free Solid										1	0.146	-0.166	0.056
Volatile Acid											1	-0.971	0.458
Non-Volatile Acid												1	-0.386
Total Mineral													1

Values in bold are different from 0 with a significance level alpha = 0.05. Significant correlations are displayed in bold. Correlation coefficients vary between -1 and 1. The closer it is to 1 or -1, the stronger the link is between two variables. Negative values indicate negative correlation, and positive values indicate positive correlation. Values close to zero indicate the absence of correlation.

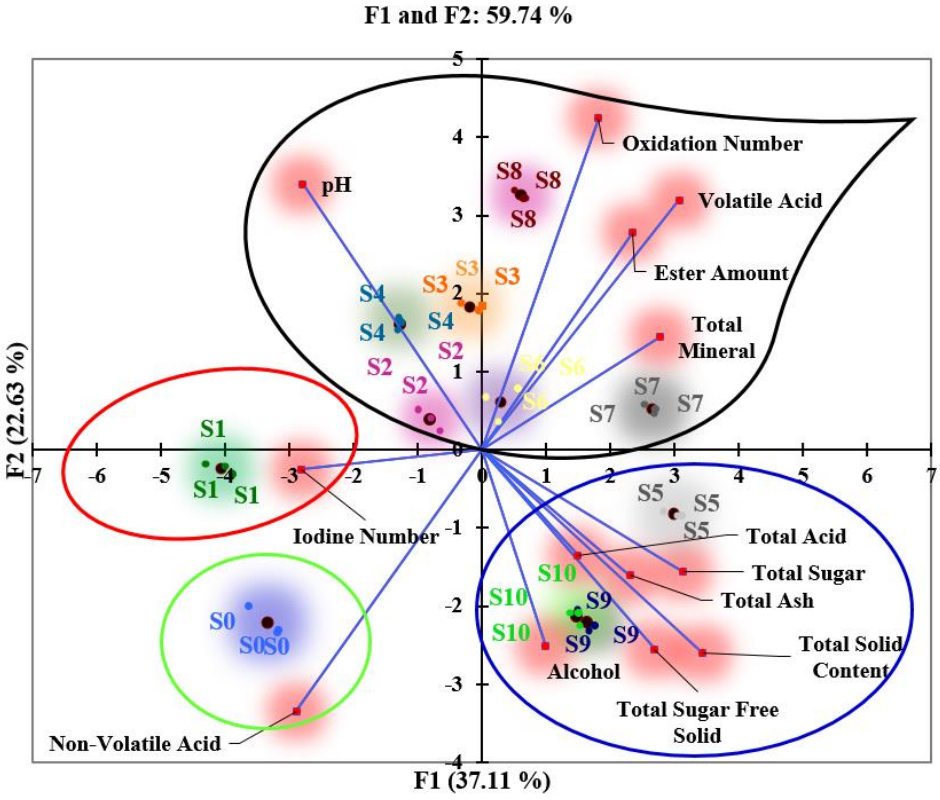

Figure 2. Multivariate analysis of physicochemical properties of vinegar samples. Full name of samples are given in Table 1.

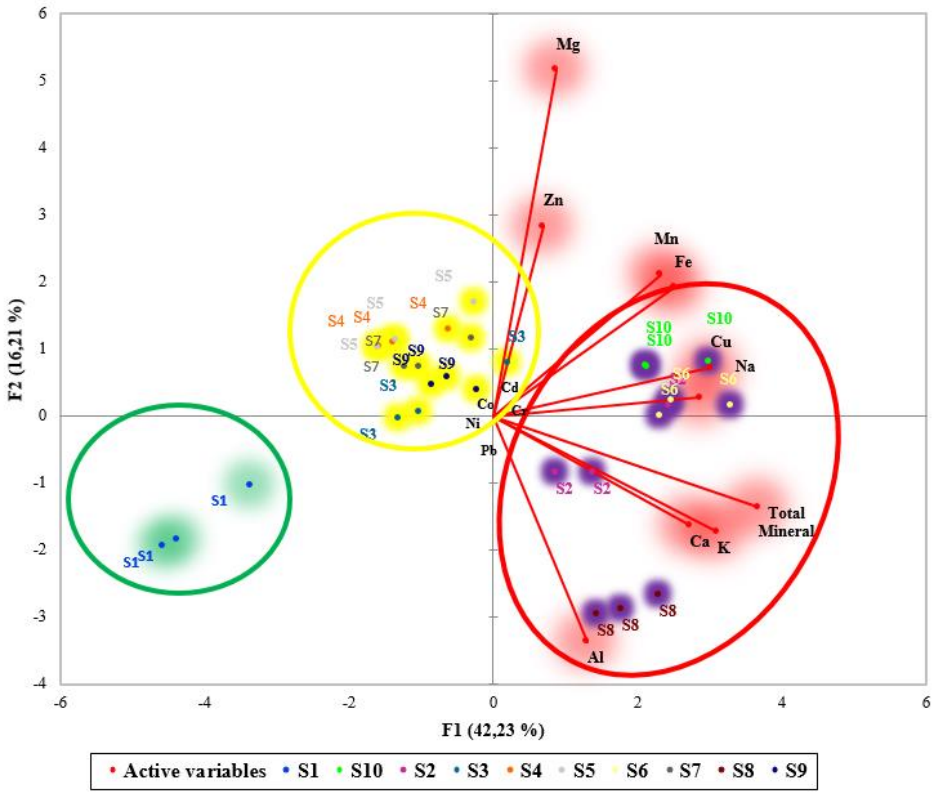

Figure 3. Multivariate analysis of mineral capacity of vinegar samples. Full name of samples are given in Table 1.

The determined pH values and TA amounts of the honey vinegar samples were consistent with other studies. The TAs of vinegars in these earlier studies were 2.0 - 6.0 g/100 mL (Chen *et al*., 2012), 0.32 - 9.63 g/100 mL (Cosmulescu *et al*., 2022), and 5.40 - 6.60 g/100 mL (Gerbi *et al*., 1998). TA and pH values showed a negative correlation (Table 6). All the vinegar samples were compatible with the Turkish Food Codex (2011) regarding TA, which specifies the minimum acidity value as 40 g/L for vinegar (TSE, 2016). The acidity of vinegar is caused by the presence of acetic acid. Short-chain volatile organic acids affect vinegar's aroma and quality. These volatile acids (mainly acetic, propionic, and butyric acids) come from raw materials, or are generated by fermentation (Yang and Choong, 2001). VA levels showed a weak positive correlation with TA. On the other hand, there was a weak negative correlation between pH and NVA. Higher levels of VA were detected in vinegar enriched with pollen and propolis. Non-volatile acids typically include organic acids such as citric, malic, and tartaric acids. These acids contribute to the TA of the solution, and can affect its pH. As the concentration of NVA increased, the pH decreased, resulting in a positive correlation between pH and NVA.

The TSC values of earlier studies for vinegar were 13.7 - 102.6 g/L (Budak, 2010), 21.4 - 44.6 g/L (Aykin, 2013), and 163.8 g/L (Gerbi *et al*., 1998), which were closer to the values detected among vinegars in the present work. The differences in TSCs of vinegar samples in both past and present work were due to the solid content of vinegar enriched with pollen and propolis. The Turkish standards for vinegars prescribe no TSC limit (TSE, 2016). The quantity of TSC in honey vinegar is closely related to microorganism fermentative activity, and the sugar is unfermented in high-sugar vinegar. The TSFS is related to soluble solids (without sugar), including salt, free amino acids, protein, and other components. The TSC parameters had a strong positive correlation between the TS and TSFS (R = 0.727, 0.900; Table 6), as seen in PCA analysis (Figure 2). The levels of TSC, TS, and TSFS were higher in vinegar enriched with pollen and propolis than in control samples.

Figure 4 shows the colour separation of vinegar samples based on pollen or propolis enrichment. In PCA, four ellipses represent the differences in vinegar colour, and strong negative and positive correlations exist between them. Based on six different traditional apple vinegar samples carried out in the study of Ozturk *et al*. (2015), the mean L* value

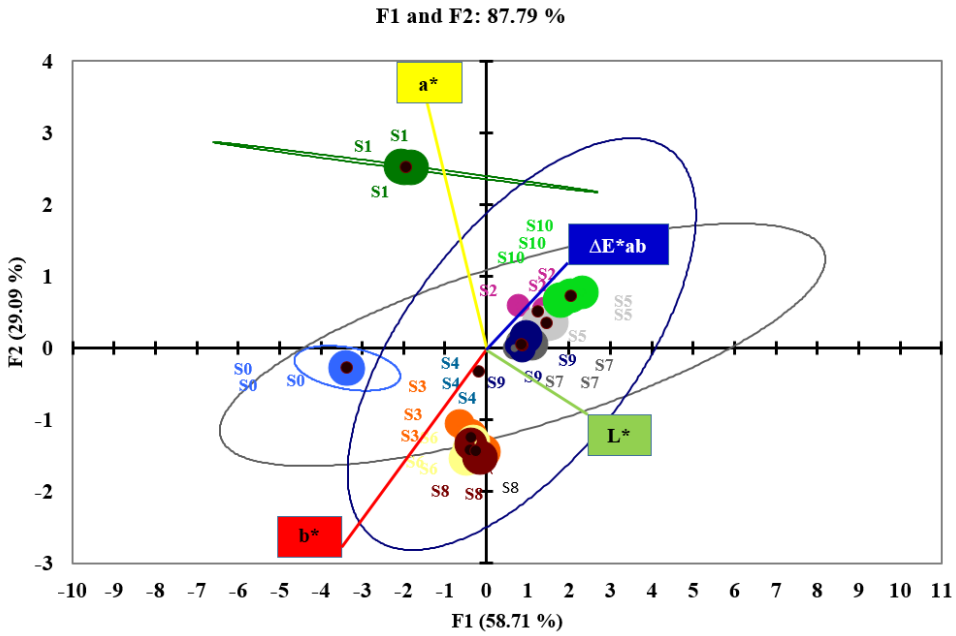

Figure 4. Multivariate analysis of colour of vinegar samples. Full name of samples are given in Table 1.

was between 4.58 and 20.15, and the a* value was between 0.09 and 6.66, respectively. In contrast, the b* value was between 3.71 and 11.98. Other than the L* values, which were slightly lower than the present work's results, the a* and b* values were in the similar range with the present work. The ΔE*ab (colour difference) was more distinguishable in vinegar enriched with pollen and propolis than in control vinegar. When the ΔE*ab values of vinegar from different raw materials were evaluated, it was determined that 5 < ΔE*ab (Mokrzycki and Tatol, 2011). Therefore, colours can be observed in all vinegar samples. The differences between the studies can be attributed to the natural colour of the raw materials. When buying vinegar, colour is critical to consumer perception (Bakir *et al*., 2017).

In PCA analyses of the TAC, TPC, TFC, FRAP, DPPH, and ABTS, PC1 explained 70.19% of the variance, while PC2 accounted for 10.36% of the variance. In other words, PC1 and PC2 explained 80.55% of the initial variability in total (Figure 5). There were profile differences in the results of TAC, TPC, TFC, FRAP, ABTS, and DPPH analyses among vinegar samples. Three different clusters (red, green, and blue-coloured circles), separate from each other, occurred (Figure 5). There was a strong positive correlation between the antioxidant analysis results based on Spearman's rank correlation coefficients such as TAC-TFC: 0.618; TAC-FRAP: 0.767; TAC-FRAP: 0.703; TAC-DPPH: 0.774; TPC-FRAP: 0.612; TPC-DPPH: 0.545; TFC-ABTS: 0.615; TFC-DPPH: 0.605; FRAP-ABTS: 0.707; FRAP-DPPH: 0.914; and ABTS-DPPH: 0.755.

The bioactivity properties of the honey vinegar samples showed a wide range of values (Table 4), and a strong positive correlation was detected between them (Figure 5). The highest TAC, TPC, FRAP, TFC, ABTS, and DPPH levels were obtained in honey vinegar enriched with pollen and propolis. However, grape vinegar (S0) and plain honey vinegar (S1) exhibited lower activity.

DPPH activities varied significantly ($p < 0.05$) depending on the source materials of the analysed vinegar samples. The highest DPPH activities were determined in vinegar enriched with pollen and propolis (Table 4). When comparing the DPPH activities of the vinegar samples with those reported by Ozturk *et al*. (2015) and Kahraman *et al*. (2022), the vinegars enriched with pollen and propolis exhibited greater DPPH values. DPPH results were relatively high for propolis, or pollen-added vinegar, compared to Trolox and AA standards.

Significant differences ($p < 0.05$) were found in TPC values in the present work. Ozturk *et al*. (2015), Bakir *et al*. (2017), and Kahraman *et al*. (2022) reported higher TPC values in their studies: 42.0 – 2,228.8, 240.0 – 2,550.0, and 34.4 - 498.3 mg GAE/L, respectively. The functional features of honey vinegar are related to the number of natural antioxidants derived from pollen and propolis. The antioxidant impacts of vinegar were connected to the presence of phenolic acids, flavonoids, and Maillard

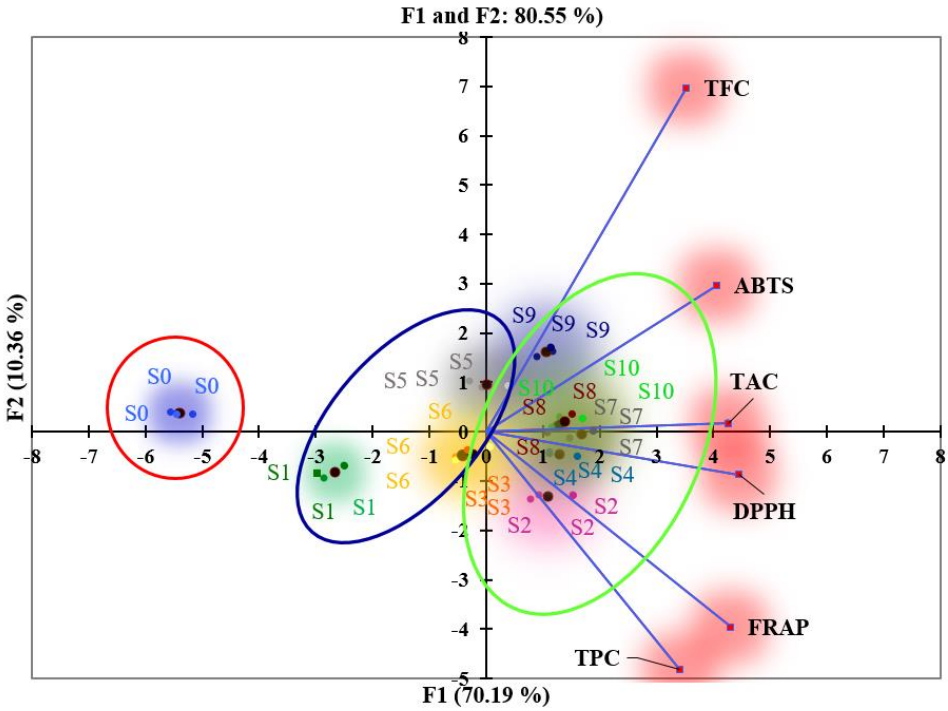

Figure 5. Multivariate analysis of antioxidant capacity of vinegar samples. Full name of samples are given in Table 1.

reaction products in vinegar composition (Aykin, 2013).

Honey vinegar flavonoids may come from honey, pollen, or propolis. Flavonoids have a low molecular weight, and add antioxidant properties to honey vinegar. Bakir *et al*. (2017) pointed out that the TFC of vinegar samples fluctuated between 2.4 and 96.0 mg catechin equivalent (CE)/100 mL, whereas the TFC range in the present work was between 99.8 and 142.7 mg GAE/L. The TFC of honey vinegar was significantly influenced by pollen and propolis ($p < 0.05$).

The TAC changed significantly depending on the enrichment substances ($p < 0.05$). The TAC values detected in honey vinegar samples showed significant differences based on applied enrichment processes (Table 1). Aydin and Gokisik (2019) measured the TAC values in Isabella grape (*Vitis labrusca* L.) vinegar as 113.12 ± 0.011 mg AAE/L.

Enriched chestnut honey vinegar exhibited intense ABTS radical scavenging activity. The ABTS activity of the vinegar is summarised in Figure 5. The ABTS-S0 correlation matrix was 0.648, while the ABTS-S4 matrix was 0.274. A strong positive correlation matrix was identified for all samples, except S1. Aydin and Gokisik (2019) determined the ABTS values of the grape vinegar (% inhibition) sample as 63.71, which was consistent with the results of the present work. AA and Trolox standards exhibited high ABTS radical scavenging activity (99.38% and 98.75%).

The vinegar showed higher FRAP activity than other antioxidant activities except TAC. Similar results for the FRAP content of different vinegar samples in previous study have been reported to be between 24.0 and 210 mg Trolox/100 mL (Bakir *et al*., 2017).

Conclusion

The present work demonstrated the production of honey vinegars using chestnut honey, and their incorporation with pollen and propolis. The incorporation of pollen and propolis had an impact on the composition, antioxidant activity, and physicochemical qualities of chestnut honey vinegar. Chestnut honey vinegar samples incorporated with pollen and propolis had higher TACs. In addition, the activities of FRAP, TPC, and TFC increased in the chestnut honey vinegar samples incorporated with pollen and propolis. Therefore, it can be inferred that chestnut honey vinegar incorporated with pollen and propolis has the potential to serve as a functional beverage or a daily flavouring enhancer. The ash and mineral levels were higher in chestnut honey vinegar samples incorporated with pollen and propolis

compared to the control samples. Multi-component analysis was conducted successfully on all chestnut honey vinegar samples incorporated with pollen and propolis. The correlations of the physicochemical parameters varied significantly due to changes in the characteristics of the raw materials and honey vinegar. Results from the PCA demonstrated that the majority of chestnut honey vinegar samples incorporated with pollen and propolis displayed a strong positive correlation with their bioactivities, whereas the control and comparison samples exhibited a lower level of bioactivity.

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