Determination of the bioactive properties, mineral and phenolic composition of different solvent-based propolis extracts and their evaluation according to existing regulations

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Abstract

Introduction. The propolis is a natural remedy and a popular food supplement worldwide. Botanical origin of raw propolis, bee types, extraction methods, parameters and solvents effect the propolis extract bioactivity and attributes. In this study, 14 propolis extracts (water and ethanol based) were analyzed and compared with the results evaluated according to current regulations.

Materials and methods. The physicochemical properties (pH, titration acidity, color, °Brix), antioxidant activity (inhibition %), total phenolic content, and phenolic and mineral compositions were determined, and the results were statistically compared ($p<0.05$). Additionally, the sensory profiles of the propolis extracts were evaluated using principal component analysis.

Results and discussion. A significant ($p<0.05$) correlation was identified between the ºBrix values of the samples and their total phenolic content, suggesting that the ºBrix value could serve as an initial indicator for estimating the polyphenol content in propolis extracts. Among the phenolic compounds, chrysin, apigenin, and caffeic acid phenyl ester were found to be the most abundant. The study showed that phenolic content was generally higher in alcohol-based samples compared to water-soluble ones. None of the propolis extracts complied with the "phenolic content" requirement set out in the Turkish Food Codex (TFC) Bee Products Communiqué. Potassium and calcium were identified as the most prevalent minerals across all samples, while lead and cadmium were present within the limits specified by TFC regulations on contaminants. Although arsenic is not regulated in the national legislation, it was detected, particularly in alcohol-based samples.

Conclusions. It was recommended to establish a limit for As content in bee product regulations. Additionally, similar to the regulations in countries such as Brazil and Ukraine, it was suggested to include standards for total phenolic content or total flavonoid content as quality classification criteria in bee product regulations.

Introduction

Propolis is a natural material collected by honey bees (*Apis mellifera* L.) from various plants and is composed of resin, 50%, and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and other organic compounds such as polyphenols and terpenoids (Anjum et al., 2019; Rocha et al., 2023). Overall, more than 300 different bioactive components, including polyphenols (flavonoids, phenolic acids, and esters), phenolic aldehydes, and ketones have been identified in propolis (El-Guendouz et al., 2019). It has been proven to possess numerous beneficial pharmacological effects and has been used in complementary medicine due to its anti-inflammatory, antimicrobial, immunomodulatory, antioxidant, and antitumor activities (Mele et al., 2023). Currently, propolis is a natural remedy and a popular food supplement worldwide. It is available either in extract form or combined with other natural products, such as vitamins, minerals, or herbal syrup preparations, and as a constituent of health foods.

Raw propolis cannot be consumed directly due to its wax, resin, herbal balsam content, and bioactive compounds; therefore, it must be extracted appropriately. There are numerous studies in the literature on propolis extraction methods, parameters and solvents. The most well known form of propolis consumption is the liquid drop form. For this purpose, different solvents, such as glycol, ethyl alcohol, glycerin, essential oils and olive oil, and several extraction techniques, such as maceration, reflux, ultrasound or microwave-assisted extraction, are used. These factors, including the botanical origin of propolis, have been reported to influence the bioactivity and pharmacological properties of the final propolis extract (Özdal et al., 2023). Because propolis is a natural remedy and popular food supplement worldwide, it is possible to find many different brands of propolis from different companies in the national and international markets. These products are presented to customers by pharmacies and e-commerce platforms in a variable price range and in different forms, such as extract, tincture, capsule or powder. It is a requirement that propolis extracts, which are preferred by consumers as natural food supplements, can be offered to consumers within certain quality limits. For this purpose, several national and international regulations have been established by governmental associations and many scientific studies. In this study, the quality properties of propolis extracts consumed as food supplements were compared. They were evaluated according to the national regulations established in our country, and their suitability was discussed.

Materials and methods

Materials

The propolis extract samples were sourced from the domestic market in Turkey. Of these, seven were water-soluble (with no alcohol as the final solvent), while the remaining seven were alcohol-based propolis extracts. These samples were purchased from markets and e-commerce sites in their original commercial packaging, boxed, tightly sealed, and stored in the dark until analysis. The chemicals used in this study were obtained from Merck (Darmstadt, Germany), and the DPPH radical was sourced from Sigma (Darmstadt, Germany).

Physicochemical properties

pH meter (Ohaus) was used to measure the pH of the samples. Each sample and distilled water were homogenized (1:9, w/v) with an Ultra-Turrax homogenizer (IKA, T25, Germany). The mixture was kept for 10 min, and then titrated with 0.1 N NaOH to a pH of 8.3. The total titration acidity (TTA) was subsequently calculated. The color profile was determined by the colorimetric method (3nh Colorimeter, NR145, China) as *L*, a*,* and *b** in five replicates. The results are presented as the means and standard deviations. A refractometer (Atago, Germany) was used to determine the percentage dissolved solids of the commercial propolis samples supplied, and the measured values were given in Brix° (Keskin and Kolaylı, 2019).

Total phenolic content

The total phenolic content of the propolis extracts was determined according to the Folin–Ciocalteu assay (Shahidi and Naczk, 1995). Briefly, 0.1 mL of extract (diluted 100 times with ethanol) was transferred to a glass tube, and 0.5 mL of Folin–Ciocalteu's reagent (0.2 N) was added. After mixing the tube using a vortex, 0.4 mL of sodium carbonate solution and 4 mL of distilled water were added. After incubating the mixture in darkness for 1 h, the absorbance was recorded at 760 nm using a UV–VIS spectrophotometer (Shimadzu Corporation, Japan) against the blank prepared using ethanol instead of extracts. The total phenolic content (calculated as mg gallic acid equivalent (GAE)/100 ml) of the extract was calculated using the calibration curve [concentration = (Abs + 0.009x)/0.020] obtained using gallic acid standard solutions.

Antioxidant activity

For determining the antioxidant activity (inhibition %), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used. A volume of 4900 µl of DPPH solution (0.025 g/l in ethanol) was combined with 0.3 mL of the extracts, which had been diluted 100-fold with ethanol, and the mixture was thoroughly vortexed. The mixture was then incubated in the dark for 30 minutes. Following incubation, the absorbance was measured at 517 nm using a UV–VIS spectrophotometer.The results were calculated as Trolox equivalent (µg/ml) and inhibition (%) with the following equation:

$$\text{Inhibition } \% = [1 - (\frac{Abs\ Sample}{Abs\ Control})] \cdot 100 \quad (1)$$

Phenolic composition

Phenolic content analysis was performed on an Inertsil ODS4 (3 µM, 2.1 × 50 mm) column. LC–MS/MS was carried out according to a validated method using 18 polyphenols (catechin, cinnamic acid, gallic acid, 2-5 dihyroxy benzoic acid, tannic acid, caffeic acid, trans ferulic acid, routine trihydrate, myricetin, naringenin, ellagic acid, quercetin, luteolin, chrysin, naringin, triacetin, apigenin, and caffeic acid phenyl ester). The propolis samples were diluted 100 times with ethanol and filtered. For LC–MS/MS analysis (8030 Plus, Shimadzu Corporation), a gradient phase was applied using mobile phase A (ultrapure water containing 1% formic acid) and mobile phase B (methanol containing 1‰ formic acid).The mobile flow rate was 0.4 ml/min , the injection volume was 10 µl, and the column oven

temperature was 40°C. The results are expressed as μg standard phenolic substance per ml extract sample.

Mineral composition

The mineral content of the samples was assessed using a microwave-assisted nitric acid digestion procedure (CEM MARS6, USA) followed by analysis with inductively coupled plasma–optical emission spectrometry (ICP–OES) (SpectroBlue, Germany). Approximately 1 mL of each sample was mixed with 1 ml of H_2O_2 (30% v/v) and 10 ml of HNO_3 (67% v/v) in PTFE flasks. The digestion program included heating to 200°C for 15 min, followed by a 15-min hold at 200°C. Once cooled to room temperature, the solutions were transferred to 50 ml polyethylene flasks and brought to volume with ultrapure water. The prepared samples were then filtered through microfilters and analyzed using ICP–OES (Al Khalifa and Ahmad, 2010). Calibration standards were prepared from a multi-element standard stock solution (Merck, Germany). All measurements were conducted in triplicate.

Sensory profile analysis

Propolis extracts were evaluated for turbidity (It should have a homogeneous appearance and should not contain any sediment), taste (After swallowing, a resinous and clean propolis taste should remain in the mouth), flavor (It should not contain any chemically or unusual odor notes), color/appearance (It should have a distinctive, yellowish and uniform color), and aftertaste (There should be no foul or bitter taste in the mouth after swallowing). For this purpose, the samples were coded with three-digit codes and served to panelists. 10 drops of propolis were added to plain glasses containing 100 ml of drinking water. Individuals who had previously consumed propolis, were not allergic to bee products, and did not smoke were selected as panelists. The panel group was informed about the evaluation criteria and scoring system. The samples were assessed using a hedonic scale ranging from 1 (I did not like it at all) to 5 (I liked it very much) based on sensory properties.

Statistical analysis

The data were reported as the mean±standard deviation. The statistical evaluation of the results was performed using analysis of variance (ANOVA) (IBM SPSS 1.0.0.781). After optimization, the obtained results were validated experimentally. Independent samples t-tests (SPSS 17.0.1, Chicago, IL, USA) were used for comparisons ($p<0.05$) of the two groups. Principal component analysis (PAST 4.03 Statistical Analysis App for Windows) was applied to determine product similarity and clustering tendencies according to the liking scores of the sensory evaluation results.

Results and discussion

Label Declarations and Contents of Propolis Samples

The label information and contents declared by their producers on the packages of 14 liquid propolis extracts were given in Table 1.

Table 1

Label declarations and contents of propolis samples

Sample	Volume (ml)	Form	Food supplement approval number	Final main solvent	Content	Shelf life (year)
P1	30 ml	Glass dropper bottle	+	Water	Water, propolis extract (18%)	2
P2	30 ml	Glass dropper bottle	+	Water	Pure water, raw propolis extract powder (14.4%)	3
P3	20 ml	Glass bottle	-	Water	Propolis, water	3
P4	20 ml	Glass dropper bottle	+	Water	Pure water, glycerin, propolis (20%)	3
P5	20 ml	Glass bottle	-	Water	Pure water, propolis extract (40%)	4
P6	20 ml	Glass dropper bottle	+	Water	Water, propolis extract (7.5%)	2
P7	20 ml	Glass dropper bottle	+	Water	Pure water, organic propolis	3
P8	30 ml	Glass dropper bottle	+	Ethanol	Propolis, Zn, D vitamine	3
P9	30 ml	Glass dropper bottle	+	Ethanol	Propolis (20%), ethanol	3
P10	20 ml	Glass dropper bottle	-	Ethanol	Propolis (38%), water, ethanol	2
P11	20 ml	Glass dropper bottle	-	Ethanol	Propolis (20%), water, ethanol	2
P12	20 ml	Glass dropper bottle	+	Ethanol	Propolis (30%), water, ethanol	3
P13	20 ml	Glass dropper bottle	+	Ethanol	Propolis (20%), water, ethanol	3
P14	20 ml	Glass dropper bottle	+	Ethanol	Propolis (20%), water, ethanol	3

The liquid propolis samples were classified according to the final solvent type of the end product; the samples between P1-P7 were called "water-based propolis", and the samples between P8-P14 were called "ethanol-based". Twelve of the 14 propolis samples were collected in glass bottles with droppers, and the P3 and P5 samples were collected in glass bottles without droppers. The net volumes of the samples varied between 20 and 30 ml.

According to the Turkish Food Codex (Official Gazette dated 02.05.2013 and No. 28635), "Regulation on the Import, Production, Processing and Placing on the Market of Food Supplements" (TFC, 2013), food supplements cannot be produced, processed, imported or placed on the market without approval. Despite the regulation, 10 of the 14 liquid propolis samples had a food supplement number on the label, while the approval number of 4 samples was not declared on the label.

According to the Turkish Food Codex Regulation on Labeling and Provision of Food Information to Consumers (TFC, 2017), declarations such as the main ingredient contents, net amount, origin, business registration or approval number, expiration date or shelf life must be included in the packaging. The raw propolis content (%) of extracts is important for standardization. In this study, the main components of 14 propolis samples were listed on the label, and the propolis ratio was stated on the label in 11 samples, except for the P3, P7, and P8 samples. The propolis contents of the samples varied between 7.7-40%. In the P8 example, there is mineral (Zn) and vitamin D supplementation. Shelf life was stated on the label for all samples, and this period varied between 2 and 4 years.

Physicochemical properties of propolis samples

The physical properties of the 14 propolis extract samples were given in Table 2.

The total titratable acidity values of 14 liquid propolis samples were significantly different ($p<0.05$). The difference between the total titration acidity and pH values of the water-based and alcohol-based groups was found to be significant ($p<0.05$) according to the independent variables t-test. The pH values of water-based propolis extracts varied between 3.93 and 8.26, and those of alcohol-based propolis extracts varied between 4.52 and 5.93. In general, the alcohol-based group is more acidic than the water-based group. According to the multiple variance analysis performed within the water-based and alcohol-based groups, the total titration acidity values of the propolis samples showed a significant difference within the groups ($p<0.05$).

Kara et al. (2022) found the pH of ethanolic propolis samples to be between 4.29 and 5.82 and reported that the pH may vary depending on the ethanol content in the solvent used for extraction. Additionally, propolis has an acidic pH due to the phenolic acids it contains. This suggests that the phenolic acid compositions of alcohol-based and water-based propolis extracts will differ, and alcohol-based extracts will have a higher level and greater diversity of phenolic acid profiles. The acidic pH of propolis due to phenolic acids is significant because the effects of these substances in propolis on microorganisms decrease as the pH increases (Ivančajić et al., 2010).

Table 2

Some physicochemical properties of the propolis samples

				Color			
		TTA, %	**pH**	***L****	***a****	***b****	**Brix°**
Water based	P1	0.35 ±0.07^{iiD}	8.08 ±0.09^{bA}	1.51 ±0.51cB	0.89 ±0.05^{efD}	1.68 ±0.28^{cdCD}	8.29 ±0.02^{jD}
	P2	0.00^{iE}	8.26 ±0.08^{aA}	1.73 ±0.08^{cB}	1.75 ±0.18^{defD}	2.38 ±0.20^{cdCD}	8.62 ±0.02^{iC}
	P3	2.5 ±0.14^{hC}	3.93 ±0.03^{hD}	15.70 ±0.51^{aA}	7.17 ±0.11^{aA}	20.08 ±0.37^{aA}	10.62 ±0.03^{tB}
	P4	0.00^{iE}	8.26 ±0.05^{aA}	0.42 ±0.04^{cB}	1.90 ±0.17^{cdefD}	0.56 ±0.05^{cdD}	62.69 ±0.07^{dA}
	P5	21.0 ±0.30^{aA}	4.19 ±0.03^{gC}	2.90 ±0.62^{cB}	3.62 ±0.52^{bcdeC}	4.17 ±0.92^{cC}	10.56 ±0.05^{tB}
	P6	19.3 ±0,30^{abB}	4.39 ±0.01^{fBC}	12.92 ±0.28^{aA}	5.08 ±0.20^{abcB}	14.14 ±0.17^{bB}	1.93 ±0.02^{kE}
Ethanol based	P7	17.95 ±0.20^{bB}	4.45 ±0.03^{fB}	14.97 ±4.15^{aA}	6.99 ±0.09^{aA}	20.42 ±0.58^{aA}	8.52 ±0.04^{ijC}
	P8	6.00 ±0.07^{fF}	5.93 ±0.04^{cA}	0.28 ±0.04^{cC}	0.38 ±0.21^{efB}	0.31 ±0.07^{cdC}	73.81 ±0.12^{cC}
	P9	17.10 ±0.20^{bB}	4.52 ±0.03^{fD}	2.12 ±0.61^{cC}	7.10 ±4.70^{aA}	3.49 ±1.05^{cdC}	39.64 ±0.08^{fE}
	P10	13.90 ±0.30^{dD}	4.52 ±0.01^{fD}	14.38 ±1.05^{aA}	7.51 ±0.37^{aA}	21.63 ±1.37^{aA}	86.98 ±0.03^{bB}
	P11	16.60 ±0.14^{bB}	4.79 ±0.04^{deC}	2.11 ±0.23^{cC}	7.91 ±0.62^{aA}	3.18 ±0.12^{cdC}	36.52 ±0.04^{gF}
	P12	9.7 ±0.14^{eE}	4.96 ±0.02^{dB}	6.47 ±1.20^{bB}	6.68 ±0.76^{abA}	10.21 ±1.60^{bB}	91.64 ±0.08^{aA}
	P13	15.2 ±0.30^{cC}	4.90 ±0.03^{deB}	0.15 ±0.01^{cC}	0.23 ±0.02^{fB}	0.11 ±0.1^{dC}	57.18 ±0.02^{eD}
	P14	17.7 ±0.30^{bA}	4.76 ±0.01^{eC}	1.33 ±0.13^{cC}	4.99 ±0.56^{abcdA}	2.14 ±0.23^{cdC}	35.11 ±0.02^{hG}
Between two groups	*P** value	<0.001	<0.001	0.009	0.279	0.142	0.127

* Different lowercase letters in the same column indicate that the difference between samples is statistically significant (p<0.05).

** Different capital letters in the same column indicate that the difference between groups is statistically significant (p<0.05).

Although ºBrix measurements are mostly applied to aqueous solutions, it is also used for ethanolic propolis extracts (Popova et al., 2017). In this study, it was determined that the ºBrix values of the samples were significantly different ($p<0.05$). The difference between the two water-based and alcohol-based groups was not significant ($p>0.05$) according to the independent samples t-test. In this study, the ºBrix values ranged between 1.93 and 62.69 for the water-based samples and between 35.11 and 91.64 for the alcohol-based samples. In a study, it was determined that the ºBrix values of commercial propolis samples obtained from the market varied between 0 and 100, and it was reported that this change was caused by the impurities contained in the propolis or different solvents affecting its solubility. In one study, it was reported that there was a high correlation (r^2: 0.9) between the ºBrix and total polyphenolic content of ethanolic propolis extracts, and the ºBrix value could be used to estimate the total amount of polyphenols contained in the extract (Keskin and Kolaylı, 2019).

While the differences between the samples were found to be significant in terms of the L*, a* and b* values, the difference between the two groups based on water and alcohol was significant for the L* value ($p>0.05$) but was not found to be significant in terms of a* and b* values ($p>0.05$). In addition to the effect of solvent and process parameters on the final product color of liquid propolis extracts, the botanical origin and region of the raw propolis used as a raw material are also important. In fact, color can be used as a distinguishing feature in identifying origin. Contieri et al. (2022) examined the color qualities of propolis in their study. They reported that although commercial propolis extracts should have amber tones, reddish or greenish according to Brazilian legislation, all samples exhibited a light tone, and samples were detected with "reddish pink and gray tones" that did not match the characteristic color.

Antioxidant activity and total phenolic content

The total phenolic content and antioxidant activity of the 14 propolis extract samples were given in Table 3.

The difference between the total phenolic content and antioxidant activity of all the samples was determined as statistically significant ($p<0.05$). Moreover, they varied within a wide range of data. The lowest Trolox Equivalent Antioxidant Capacity (TEAC) was 88.9 µg/ml (P5), the highest was 24205.6 µg/ml (P10), the lowest total phenolic content was 372.22 µg GAE/ml (P5), and the highest was 30333.33 µg GAE/ml (P12). The differences between the two sample groups, water-based and alcohol-based, were found to be significant ($p<0.05$) according to the independent variables t-test. The antioxidant activity and total phenolic content of alcohol-based propolis were greater than those of water-based propolis. Keskin (2018) determined the total phenolic content to be 16.13-178.38 mg GAE/g in the raw propolis samples collected from Turkey when ethanol was used as a solvent, similar to the findings of this research. On the other hand, they reported that the total phenolic content was much lower (0.07-0.22 mg GAE/g propolis) when water was used as a solvent. Kubiliene et al. (2015) reported that the total phenolic content of propolis obtained from Lithuania was 12.7 mg GAE/ml because of alcohol extraction, 1.6 mg GAE/ ml because of water extraction, and 0.5 mg GAE/ml because of olive oil extraction.

The flora plays a crucial role in the antioxidant activity of propolis. Shehata et al. (2018) observed significant variations in the chemical compositions of brown and green propolis collected from different regions, such as Egypt, China, Bulgaria, and Brazil. Studies indicate that the variety, geographical location, climate, and vegetation of the region where propolis is collected are essential in determining its antioxidant capacity.

Table 3

Antioxidant activity and total phenolic content values

	Samples	Antioxidant activity		Total phenolic content
		Inhibition (%)	TEAC*** (μg/ml)	GAE (μg/ml)
Water based	P1	26.15±0.31^{fB}	6155.6±88.89^{fB}	3250.00±50.00^{gB}
	P2	43.15±0.86^{eB}	11011.1±244.44^{eB}	3427.78±16.67^{gB}
	P3	8.74±0.21^{hB}	1183.3±61.11^{hB}	916.67±5.56^{ijB}
	P4	49.38±0.27^{dB}	12788.9±77.78 ^{dB}	7511.11±133.33^{eB}
	P5	4.90±0.31^{iB}	88.9±88.89^{iB}	372.22±5.56^{jB}
	P6	6.05±0.25^{iB}	416.7±72.22^{iB}	1066.67±66.67^{iB}
	P7	22.18±0.39^{gB}	5022.2±111.11^{gB}	2094.44±127.78^{hB}
Ethanol based	P8	78.05±0.74^{cA}	20977.8±211.11^{cA}	10138.89±72.22^{dA}
	P9	84.71±0.51^{bA}	22877.8±144.44^{bA}	18472.22±150.00^{bA}
	P10	89.36±0.10^{aA}	24205.6±27.78^{aA}	29961.11±116.67^{aA}
	P11	87.24±0.43^{aA}	23600.0±122.22^{aA}	13444.44±33.33^{cA}
	P12	83.74±0.47^{bA}	22600.0±133.33^{bA}	30333.33±222.22^{aA}
	P13	82.57±0.04^{bA}	22266.7±11.11^{bA}	5983.33±127.78^{fA}
	P14	89.28±0.02^{aA}	24183.3±5.56^{aA}	13372.22±227.78^{cA}

* Different lowercase letters in the same column indicate that the difference between samples is statistically significant (p<0.05).

** Different capital letters in the same column indicate that the difference between groups is statistically significant (p<0.05).

TEAC - Trolox Equivalent Antioxidant Capacity; * GAE - gallic acid equivalent.

The extraction method and conditions, such as solvent type and extraction time, also affect the extraction efficiency and final product properties. In a study conducted by Mokhtar (2019), the use of ethanol as a solvent in maceration extraction resulted in higher phenolic and flavonoid contents than did the use of water. It has also been reported that the highest bioactivity was achieved with a solid/liquid ratio of 1/5. The chemical composition and bioactivity of propolis vary according to seasonality, the flora of the region where hives are located, dominant botanical origin, bee species, and extraction type and parameters (Calegari et al., 2017).

In this study, the botanical origin or geographical location of the commercially available propolis extracts was unknown. However, according to the results, it is possible that the substance used as the extraction solvent and the extraction process used accordingly have an effect on the antioxidant capacity of the final product. On the other hand, the ratio of crude propolis in the propolis extract may also have an effect on antioxidant activity. However,

although the P5 sample was the product with the highest propolis content (40%) according to the label information, it was found to have the lowest antioxidant activity. The antioxidant activities of propolis extracts, which are water-based and commercially available for consumption, show a very heterogeneous distribution. Although many different solvents and processes have been tested for the extraction of bioactive substances from propolis, studies have shown that alcohol extraction is the best method and that alternative solvents and methods used in the production of nonalcoholic extracts are insufficient.

It was reported that there is a positive correlation between the total phenolic content and the antioxidant activity of propolis (Aliyazıcioglu et al., 2013). Many studies have shown that, in addition to antioxidant capacity, all other biological activities, such as antimicrobial, antitumoral, anti-inflammatory, antiviral and antifungal activities, increase in proportion to the amount of phenolic substances (Kolaylı et al., 2010; Tezcan et al., 2011). In this study, a positive correlation was detected between the antioxidant activity (inhibition %) and total phenolic contents (μg/ml) of the examined samples according to the Pearson correlation test and was found to be statistically significant ($p<0.05$). It is known that the positions of the hydroxyl groups of flavonoids, which are phenolic components, change their antioxidant capacity (Cai et al., 2006). As a result, the presence of a specific flavonoid can increase the total amount of phenolic substances, which can increase the total antioxidant capacity.

Identification of phenolic compounds

In this study, the phenolic compounds in propolis extracts were characterized via LC–MS/MS. The results were given in Table 4a and b. Phenolic compounds, which are secondary metabolites of plant origin and are found in many natural products, are agents responsible for antioxidant activity (do Nascimento et al., 2016; Zhang et al., 2018). According to previous studies, at least 300 different propolis compounds have been identified; their biological activities are attributed mainly to phenolic components, such as flavonoids (flavonols, flavones, flavonones, dihydroflavonols, and chalcones); aromatic aldehydes; terpenes; alcohols; and beta-steroids. Phenolic compounds have biological activities, such as antioxidant potential, because they have hydroxyl groups and aromatic compounds in their chemical structures (Milene Angelo and Jorge, 2007; Gülcin, 2012). According to different studies, the main phenolic compounds identified in propolis are hydroxycinnamic and hydroxybenzoic acids (Calegari et al., 2017; Xavier et al., 2017), chrysin, galangin, pinocembrin, apigenin, quercetin, luteolin, ferulic acid, benzoic acid, cinnamic acid, flavones, flavonols, and flavanones (Kar et al., 2019; Peter et al., 2017; Woźniak et al., 2020). In this study, catechin, myricetin, naringenin, quercetin, rutin trihydrate, chrysin, apigenin and CAPE were detected in all the samples.

Luteolin was not detected in water-based samples (except P7) but was detected in all alcohol-based samples. Triacetin was detected in all the samples except P8. In general, the total flavonoid content of alcohol-based propolis extracts was greater than that of the water-based samples in this study. Several studies have shown that the ethanol ratio in the solvent affects the phenolic compound composition of propolis extracts. While the phenolic acid content is high in solvents with high water content, no significant change is observed in solvents containing 70% or more ethyl alcohol (Kara et al., 2022).

In terms of phenolic contents of the chestnut propolis extracts, CAPE, apigenin and chrysin were determined to be the most abundant compounds, followed by quercetin and naringenin. The beneficial biological activities of propolis, such as antimicrobial, anti-inflammatory, antiulcer, and anticancer properties, are closely related to its bioactive compounds. Flavonoids are effective against various bacteria and protect against ulcers

(Ruiz-Hurtado et al., 2021). While chrysin is known to have anti-inflammatory and antineoplastic effects and functions as an important antioxidant and hepatoprotective agent (liver protector) (Shahbaz et al., 2023), another major component of propolis that causes its anticancer effect is caffeic acid phenyl ester (CAPE) (Keskin, 2018). The sample with the highest CAPE, chrysin, quercetin and naringenin contents among the alcohol-based samples was P10, which had the highest raw propolis content (38%). A study comparing the phenolic compositions and antioxidant activities of propolis samples extracted with different ethanol concentrations and extraction conditions revealed caffeic acid to be the main component in extracts prepared with solvents containing 0-40% ethanol and revealed chrysin and pinocembrin to be the main components in extracts containing 60% or more ethanol (Kara et al., 2022). Therefore, the percentage of raw propolis and alcohol used in the preparation of the extract has an effect on the phenolic composition of the final product.

Table 4b shows the phenolic acid contents of the liquid propolis samples. Although tannic acid could not be detected in any water-based chestnut propolis extracts, it was detected only in P9 and P13 in the alcohol-based samples. The major phenolic acids in the water-based samples were trans ferulic, caffeic, cinnamic and gallic acids, while in the alcohol-based samples, they were trans ferulic, caffeic, cinnamic and ellagic acids. P4 from water-based samples and P10 from alcohol-based samples were the propolis extracts with the highest phenolic acid content. In this study, the P4 and P10 samples, which used chestnut propolis on their labels, had the highest CAPE, ferulic acid, and ellagic acid contents and the highest antioxidant activity (inhibition %). In another study, propolis samples containing caffeic acid phenyl ester (CAPE) presented stronger radical scavenging activity (Sulaiman et al., 2014). This indicates that the botanical origin of propolis used as a raw material has an impact on the bioactivity of the propolis extract.

According to the Turkish Food Codex Bee Products Communiqué, liquid propolis consumed as a food supplement (coumaric acid (total of o, m and p-), pinobanksin, galangin, cinnamic acid and 3-4 dimetoksi cinnamic acid (total), chrysin, pinosembrin, caffeic acid, CAPE, caempferol, ferulic acid (total of izo and trans), quercetin, naringenin, rutin, apigenin, protocatechuic acid and artepilin C) must contain at least 5 phenolic substances at a minimum level of 500 mg/l. In Tables 5a and 4b, these phenolic compounds foreseen in the Communiqué were marked (*). As seen from the tables, none of the propolis extracts obtained from the national market meet the specified phenolic substance limits in the codex. Moreover, it is considered that the phenolic compound composition alone is insufficient in the bee products regulation. In addition, similar to the regulations in various countries concerning propolis, it is necessary to establish the total phenolic content or total flavonoid content as a quality criterion.

Mineral composition and heavy metal contents

Propolis is an important food supplement for human nutrition because of its antibacterial, antifungal, antiviral, and antioxidant activities. Therefore, it is crucial to determine propolis's content in terms of essential minerals and heavy metals. In this study, the concentrations of several elements (Ca, Mg, K, Mn, Fe and Zn) and heavy metals (Hg, As, Cd and Pb) in propolis extracts were determined using ICP–OES (Table 5). Heavy metals and other elements demonstrated a heterogeneous distribution in propolis extracts. Therefore, the difference between the means of the two alcohol and water-based groups was not found to be statistically significant ($p<0.05$) in terms of Ca, Mg, K or Mn. Fe and Zn were found to be significant ($p<0.05$) because these two elements were below the detection limits in most of the propolis samples. The amount of Zn in the P8 sample was found to be very high

because it was already declared on the label as Zn supplemented. While the K content was found to be highest for all the samples, Fe and Zn were rarely found. The mineral contents of the two groups, regarding the solvent type, could not be compared clearly because the data were quite heterogeneous. Although P7 is water-based and P13 is alcohol-based, they have similar contents. Soós et al. (2019) determined that the elemental content of propolis extracts is significantly affected by the composition of the extraction solvent, and in most cases, the extraction time has a large impact. It has been reported that most of the analyzed elements dissolve better in aqueous solutions than in solvents containing ethanol; however, with a few exceptions, elements essential to the human body are also well extracted in solvents containing 50% or 80% (v/v) ethanol. Görkem (2022) reported that in commercial propolis drop samples, in addition to Fe and Na, other elements (Ca, Mg, K, Al, B, Cu, Mn, P and Zn) were found to be more abundant in a sample extracted with water than in samples extracted with alcohol and water + glycerol. Ristivojević et al. (2023) determined that microelements were variable, macroelements (Na, Ca, Mg, K and P) were more common, and the Ca content was the highest in twentytwo propolis samples that differed in terms of geographical and botanical origins in Serbia. Mineral diversity is passed on to the composition of propolis by transferring the mineral composition of the soil to the plants from which the propolis is obtained. Therefore, plant sources strongly influence the elemental composition of propolis [38]. As a result, it is thought that the botanical origin, parameters such as time, temperature, and several applications in propolis extraction impact the elemental distribution and concentrations.

The heavy metal contents of the samples were given in Table 5b. The heavy metal contamination limits for food supplements in the Turkish Food Codex Regulation of Contaminants (TFC, 2023) are 3 mg/kg for Pb, 1.0 mg/kg for Cd, and 0.1 mg/kg for Hg. There is no limit value for arsenic (As). Although arsenic was not detected in water-based propolis extracts, it was detected in all alcohol-based propolis extracts except for the P8 and P13 samples. The lowest amount of arsenic was 1031.25 µg/kg (P10), and the highest was 2445.80 µg/kg (P11).

Since the Hg contents of all propolis samples in this study were below the detection limit (0.4 mg/kg), they could not be evaluated statistically, and comparisons could not be made according to the TFC Regulation of Contaminants. The Cd values of the samples were determined to be the lowest at 185.6 µg/kg (P8) and the highest at 232.40 µg/kg (P12), and since all samples were below the limit value (<1 mg/kg), they were appropriate according to the contaminant regulations. The differences between the samples were not significant in the water-based group ($p>0.05$) but were significant in the alcohol-based group ($p<0.05$). The Pb values of the samples were determined to be the lowest at 502.7 µg/kg (P1) and the highest at 855.2 µg/kg (P8), and since all samples were below the limit value (<3 mg/kg), they were appropriate according to the contaminant regulations. The Pb content of the water-based group was lower than that of the alcohol-based group ($p<0.05$).

Because plant sources and soil strongly influence the elemental composition of propolis, the basic propolis content can be used to develop reliable traceability methods and distinctive features of the geographical areas where it is produced to indicate environmental pollution (Golubkina et al., 2016). However, the detection of As, especially in alcohol-based samples, in this study suggested that extract production factors such as solvent qualities, in addition to plant and soil qualities, are also effective for detecting heavy metal contamination.

Table 4

Flavonoid (a) and phenolic acid (b) contents of liquid propolis samples

	(ppb)	Catechin	Mirisetin	Naringenin*	Quercetin*	Rutin*	Luteolin	Chrysin *	Apigenin*	CAPE*	Triacetin	Total flavonoid (ppm)
Water based	P1	1698.28	1109.37	241.54	723.62	480.69	-----	163171.53	52191.19	1582.39	1938.53	223.137
	P2	425.66	1216.36	822.65	820.53	495.27	-----	83540.17	61255.76	2475.28	3338.48	154.390
	P3	2278.98	1231.35	29137.31	12119.98	1211.38	-----	42383.26	15946.98	30759.63	2662.11	137.731
	P4	1594.73	1223.18	50326.08	1226.59	1302.52	-----	183255.37	62563.74	302348.06	2677.54	606.518
	P5	668.87	108.82	1275.95	163.28	50.16	-----	12132.26	3441.37	65.90	5351.08	232.576
	P6	171.21	1109.21	15384.22	1505.74	503.31	-----	56712.49	11724.32	44490.57	2773.36	134.374
	P7	1554.47	2538.24	31700.26	28273.76	8653.00	1409.32	31654.46	38448.91	62663.48	2259.10	209.155
Ethanol based	P8	501.66	1894.13	41203.99	43532.22	30997.50	5330.91	88643.11	122669.46	136380.31	-----	471.153
	P9	1517.46	7192.16	92555.68	110944.03	25826.11	11508.03	83669.99	103957.15	431734.70	1733.27	870.639
	P10	1006.02	27877.75	187138.01	335933.89	4582.85	68736.66	173957.97	97849.53	804898.84	3481.76	1705.463
	P11	1030.00	1516.66	91773.18	154905.19	6606.80	40295.97	125398.19	152157.10	476212.41	2430.03	1052.326
	P12	1464.30	8707.65	146142.00	122668.44	2088.98	112053.92	172442.71	313710.50	697909.15	11001.99	1588.190
	P13	823.28	2051.87	67871.83	42315.71	12538.39	11459.01	84767.58	73791.34	222212.11	3534.82	521.366
	P14	1195.56	2283.71	63196.52	62623.03	3083.76	856.75	107240.42	43526.17	474079.80	9511.22	767.597

a

	(ppb)	Cinnamik acid*	Gallic acid	Tannic acid	Cafeic acid*	2-5, Dihydroxy benzoic acid	Trans ferulic acid*	Ellagic acid	Total phenolic acid (ppm)
Water based	P1	58450.54	13753.26	-----	4782.78	6.79	4473.17	2347.21	83.814
	P2	53905.98	18036.81	-----	8577.11	205.52	5541.38	9614.61	95.881
	P3	17967.96	2426.63	-----	120729.46	-----	9274.57	10387.19	160.786
	P4	81810.10	4044.50	-----	114837.86	50.59	728639.38	2074.71	931.457
	P5	584.38	4898.46	-----	4198.82	28.49	1557.38	223.80	11.491
	P6	8631.01	6729.90	-----	147326.29	-----	75817.91	1848.80	240.354
	P7	10538.64	4279.65	-----	352788.11	208.05	257101.52	24222.41	649.138
Ethanol based	P8	99732.59	20968.94	-----	25871.21	763.58	57368.14	46999.98	251.705
	P9	213153.99	9250.30	176506.07	407085.56	91.28	71362.26	96421.70	973.871
	P10	96462.05	5914.59	-----	515156.69	308.98	1348264.31	251768.28	2217.875
	P11	250661.62	10022.72	-----	317015.62	39.28	140639.74	142636.50	861.016
	P12	141871.22	4392.37	-----	356322.53	-----	260053.17	120861.73	883.501
	P13	87747.12	11753.58	53549.92	524699.46	267.67	141010.21	33164.36	852.192
	P14	184581.14	24595.99	-----	451022.94	146.21	874694.26	56777.39	1591.818

* Phenolic compounds that must be present in a propolis extract according to the Turkish Food Codex Bee Products Communiqué

b

Table 5

Mineral (a) and heavy metal (b) contents of the liquid propolis samples

		Ca (mg/kg)	Mg (μg/kg)	K (mg/kg)	Mn (μg/kg)	Fe (μg/kg)	Zn (μg/kg)
Water based	P1	59.40 ±1.00^{deB}	4628.35 ±167.40^{aD}	179.45 ±10.0^{ghC}	< 0.076	884.90 ±43.40^{bA}	< 0.554
	P2	27.20 ±0.30^{deB}	1615.2 ±9.75^{aEF}	151.00 ±3.20^{hD}	< 0.076	118.05 ±1.50^{dC}	< 0.554
	P3	24.30 ±0.15^{deB}	1225.45 ±5.75^{aF}	99.80 ±0.95^{iE}	< 0.076	< 1.243	< 0.554
	P4	28.00 ±0.30^{deB}	2350.45 ±18.70^{aE}	160.4 ±3.^{20hCD}	36.45 ±0.080^{efC}	< 1.243	< 0.554
	P5	57.55 ±0.20^{deB}	11003.6 ±15.10^{aB}	58.05 ±1.10^{iF}	< 0.076	< 1.243	< 0.554
	P6	212.15 ±3.50^{abA}	8038.75 ±63.45^{aC}	235.00 ±3.65^{fB}	231.05 ±4.6^{cB}	263.45 ±2.95^{cB}	127.6 ±7.50
	P7	257.75 ±30^{aA}	35350.3 ±406.12^{aA}	1097.65 ±6.35^{bA}	449.5 ±29.64^{aA}	< -5.616	89.15 ±34.84
Ethanol based	P8	111.6 ±1.55^{cC}	12863.75 ±178.35^{aA}	642.7 ±4.20^{dC}	132.35 ±2.40^{dD}	5472.4 ±38.25^{a}	15582.8 ±91.00
	P9	30.3 ±0.05^{deE}	2596.5 ±32.50^{aA}	153.6 ±1.70^{hF}	< 0.076	< 1.243	< 0.554
	P10	155.4 ±0.85^{cB}	15635.75 ±166.8^{aA}	720.2 ±2.30^{cB}	363.75 ±2.80^{fF}	< 1.243	< 0.554
	P11	16.30 ±0.15^{eF}	2373.00 ±21.30^{aA}	198.70 ±2.05^{gE}	< 0.076	< 1.243	< 0.554
	P12	63.05 ±0.40^{dD}	18029.00 ±2.42^{aA}	571.8 ±4.95^{eD}	61.55 ±0.03^{eE}	< 1.243	< 0.554
	P13	209.30 ±2.75^{bA}	51504.50 ±133.50^{aA}	1831.00 ±6.65^{aA}	403.40 ±2.95^{bA}	< 1.243	142.3 ±8.45
	P14	57.5 ±0.55^{deD}	5767.10 ±46.05^{aA}	194.4 ±0.35^{gE}	225.15 ±2.50^{cC}	< 1.243	< 0.554
	*p**	0.140	0.817	0.238	0.940	0.007	0.001

a

		Hg (mg/kg)	As (µg/kg)	Cd (µg/kg)	Pb (µg/kg)
Water based	P1	<0.4	< 3.034	199.80 ±4.10^{aA}	502.7 ±51.60^{eB}
	P2	<0.4	< 3.034	211.40 ±1.75^{aA}	662.35 ±17.35^{deAB}
	P3	<0.4	< 3.034	214.15 ±1.05^{aA}	684.00 ±13.05^{cdAB}
	P4	<0.4	< 3.034	197.85 ±0.05^{aA}	688.30 ±21.70^{bcdAB}
	P5	<0.4	< 3.034	227.90 ±1.65^{aA}	832.1 ±41.00^{abcA}
	P6	<0.4	< 3.034	223.30 ±1.80^{aA}	812.60 ±0.10^{abcdA}
	P7	<0.4	< 3.034	227.40 ±47.84^{aA}	824.45 ±70.20^{abcdA}
Ethanol based	P8	<0.4	< 3.034	185.60 ±0.10^{aE}	855.15 ±22.75^{aA}
	P9	<0.4	1898.35 ±182.80^{bAB}	211.00 ±2.80^{aD}	761.9 ±19.75^{abcdB}
	P10	<0.4	1031.25 ±56.3^{cC}	227.25 ±1.90^{aAB}	806.3 ±14.7^{abcdAB}
	P11	<0.4	2445.80 ±76.95^{aA}	209.40 ±1.10^{aD}	764.20 ±17.10^{abcdB}
	P12	<0.4	1129.25 ±3.51^{cC}	232.40 ±0.08^{aA}	805.40 ±0.20^{abcdAB}
	P13	<0.4	< 3.034	216.45 ±2.15^{aCD}	849.45 ±9.40^{abA}
	P14	<0.4	1336.75 ±182.1^{cBC}	221.15 ±1.45^{aBC}	781.15 ±7.60^{abcdAB}
	*P**	-	<0.001	0.416	0.006

b

* Different lowercase letters in the same column indicate that the difference between samples is statistically significant (p<0.05).

** Different capital letters in the same column indicate that the difference between groups is statistically significant (p<0.05).

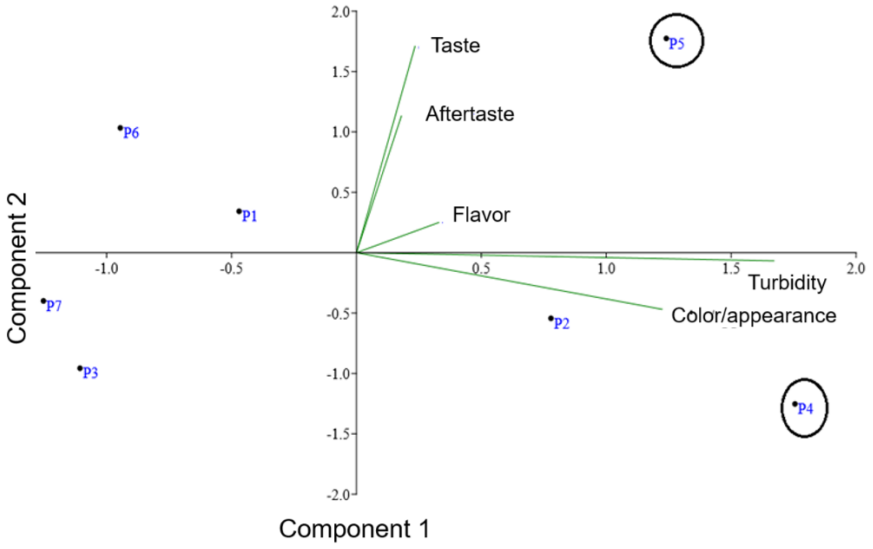
It is crucial that propolis extracts offered for consumption as food supplements adhere to common criteria and are standardized. For the standardization of these products, not only the phenolic substance composition but also the characteristics of the raw propolis ratio, total polyphenol content, phenolic composition, contaminant limits (such as heavy metals and pesticides), solvent types, packaging, and labeling must be determined. This study found that alcohol- and water-based commercial propolis offered by different companies varied significantly in terms of label declarations, physical and bioactive properties, mineral and heavy metal contents, and sensory properties. While alcohol-based extracts generally show higher values, especially in terms of bioactive properties, intragroup variability exists in both alcohol- and water-based groups. Standardization of the content of these products will only be achievable by complying with legislation, ensuring consistency in consumption recommendations, pricing, and labeling. Given the variability in propolis extracts offered for commercial consumption, the publication of common legislation containing quality limits has become important and a priority. This study recommends that unusual situations, such as the detection of arsenic in alcohol-based extracts, be taken into consideration, relevant regulations be revised, and the results of this and similar scientific studies be used to establish quality limits in legislation. Similar to regulations in various countries, such as Brazil, it is recommended to establish a limit for arsenic (As) content in our national legislation for propolis.

Sensory Properties

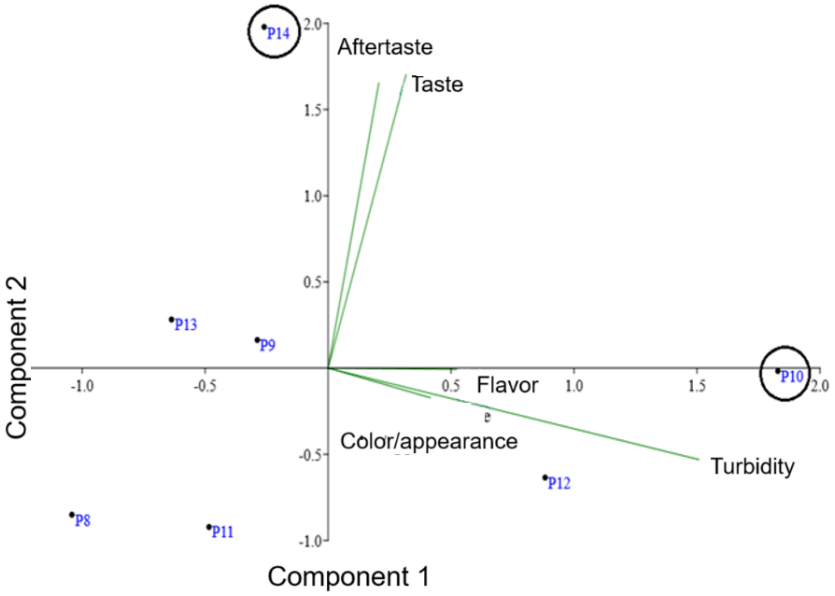
Water and alcohol-based propolis extracts were prepared in separate groups at a concentration of 10 drops/100 ml of water and were evaluated for sensory activity by panelists at different times. The sensory evaluation was based on five basic criteria: taste, flavor, turbidity, color/appearance, and aftertaste. The panelists rated each criterion on a scale from 1 (I did not like it at all) to 5 (I liked it very much). The average scores for each criterion were calculated, and principal component analysis (PAST 4.03 Statistical Analysis App for Windows) was applied to determine product similarity and clustering tendencies according to the sensory evaluation results.

To determine the clustering tendency and similarities of the ethanol-based and water-based propolis samples according to their sensory analysis scores, the resulting water-based products (Figure 1a) and alcohol-based products (Figure 1b) were evaluated by principal component analysis. For water-based products, the most popular product was P4 in terms of color/appearance and turbidity qualities and P5 in terms of taste, flavor, and aftertaste properties. Although these samples did not show a clustering tendency with the others, the P1 and P6 samples and the P3 and P7 samples were found to be similar and showed clustering.

When the ethanol-based products were evaluated, P10 was the most liked product in terms of color/appearance, turbidity, and flavor properties, while P14 was the most liked product in terms of taste, aroma, and aftertaste properties. Although these samples did not show a clustering tendency with the others, the P9 and P13 samples and the P8 and P11 samples were found to be similar and showed clustering among themselves.



a



b

Figure 2. Principal component analysis graphs of water-based (a) and alcohol-based (b) propolis samples

Conclusions

It is crucial that propolis extracts offered as food supplements meet common criteria and are standardized. For effective standardization, it is necessary to determine key characteristics such as the raw propolis content, total polyphenol content, phenolic composition, contaminant limits (e.g., heavy metals and pesticides), solvent types, packaging, and labeling requirements. Although the Bee Products Communiqué has only recently been published, it is considered necessary to review the characteristics and limit values of raw propolis and propolis extracts. This study found that commercial alcohol- and water-based propolis extracts offered by various companies vary significantly in terms of label declarations, physical and bioactive properties, mineral and heavy metal contents, and sensory characteristics. While alcohol-based extracts generally exhibited higher values, particularly in bioactive properties, variability was observed within both alcohol- and water-based groups. To achieve standardization in consumption recommendations, pricing, and labeling, compliance with finalized legislation is essential. Given the variability in commercially available propolis extracts, the publication of legislation with quality limits has become a priority. This study highlights the need for unusual findings, such as the detection of arsenic in alcohol-based extracts, to be addressed in the relevant regulations. Revising these regulations based on the results of this and similar scientific studies will be critical for establishing quality standards in future legislation.

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