

Determination of Antioxidant Capacities with the Phenolic and Flavonoid Contents of Royal Jelly Mixtures

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Abstract

Royal jelly, which is used in functional foods due to its antioxidant capacity, has started to be widely used. In this study, the antioxidant capacities and phenolic contents of royal jelly mixtures were determined. Total phenolic substance contents (TPC) of royal jelly mixtures were specified using the Folin-Ciocalteu procedure, total flavonoid substance contents (TFC) by aluminium chloride colourimetric method, phenolic compound contents with LC-MS/MS and antioxidant activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. TPC ranged from 0.6 to 38.2 mg GAE/100 g, and the mean value was determined as 11.08 mg GAE/100 g. TFC ranged from 0.1 to 30.06 mg QE/100 g and a mean value of 9.2 mg QE/100 g, and DPPH ranged from 4.4 to 93.0 mg TE/mL and a mean value of 29.11 mg TE/mL. TPC, TFC and DPPH activities were highest in S6 and S7 samples, and lowest in S1 and S2 samples. Caffeic acid phenethyl ester was the highest yielding phenolic compound in all samples, and Luteolin was the lowest yielding compound. The highest phenolic compound contents were determined in the S6 and S7 samples. As a result, it has been observed that food products containing bee products are rich in phenolic contents and have high antioxidant properties. TPC, TFC, DPPH activity and phenolic contents were found to change as the type and ratio of bee products in the food changed.

Introduction

The oxidation reaction, which occurs as a result of the interaction between the oxygen in the air and the food components, can often cause undesirable problems such as colour, taste and odour changes and a decrease in nutritional value. The substances that can be found naturally in the food that prevent these undesirable problems or that can be added externally are defined as antioxidant substances (Köksel, 2007). For example; Royal jelly is food with natural antioxidant properties. The amount of antioxidant substances in the content of bee products varies depending on the plant source from which the nectar is produced, seasonal and environmental factors (Kılıç Altun & Aydemir, 2021; Spilioti et al., 2014). In addition to these, in addition to enzymes such as catalase, glucose oxidase, and peroxidase, the antioxidant feature of bee products is

obtained from phenolic acids (benzoic, ferulic, coumaric and caffeic acid), flavonoids, tocopherols, carotenoids and vitamins such as thiamine, ascorbic acid and riboflavin originates (Khalil et al., 2012). It has been stated that the amount of phenolic substances contained in bee products and their antioxidant properties are related, and the antioxidant properties increase due to the increase in the total amount of phenolic substances (Alzahrani et al., 2012).

Natural diets rich in flavonoids and phenolic content with antioxidant effects have increased the interest in nutrition and food science in recent years. Natural phenolic and flavonoid compounds are secondary metabolites holding an aromatic ring with at least one hydroxyl group (Köksel, 2007). Phenolic compounds because their hydroxyl groups can directly contribute to the antioxidant effect, and some even stimulate the synthesis of endogenous antioxidant

molecules in the cell (Gomes et al., 2010; Kahraman et al., 2010). Research reports show that phenolic compounds prevent metal inactivation, free radical inhibition in biological systems, peroxide decomposition and oxidative disease burden (Özmen & Akin, 2006).

Beekeeping, which has been carried out without the need for much capital since ancient times, is one of the most important agricultural activities worldwide, which provides a return in a short time with honey and other bee products (Bölüktepe & Yılmaz, 2008). As a result of beekeeping, which is one of the oldest agricultural activities in the world in the historical process, various bee products such as honey, beeswax, pollen, royal jelly, bee venom and propolis are obtained. These products, which are used as foodstuffs, are also widely used in the treatment of many diseases.

Bee products have been used as a natural medicine, immune booster and antioxidant in order not to be exposed to the chemical effects of drugs for the protection of health and the treatment of diseases, and have been preferred by people since ancient times. Today, various bee products such as honey, pollen, royal jelly, propolis and foods containing bee products appear in markets, advertisements and breakfast rooms as functional foods. In this case, the nutritional values, nutritional contents, and product characteristics of bee products come to the fore. High total phenolic and flavonoid contents in bee products are associated with high nutritional and nutritive values and antioxidant capacity. Royal jelly is the special food of the queen honey bee larva and is a special bee product secreted from the hypopharyngeal and mandibular glands of worker honey bees between the sixth and twelfth days of their lives (Haydak, 1970; Pavel et al., 2011). Today, royal jelly is included in many functional foods due to its many benefits. This study aimed The aim of this study was to determine the antioxidant capacities and phenolic compositions of mixtures containing royal jelly.

Materials and Methods

Collection of Royal Jelly Mixtures

In this research, 7 samples of commercial royal jelly mixes were selected from products offered in pharmacies and marketplaces in Türkiye (Erzurum) in 2022. While the samples were being collected, care was taken that they were not from the same brand or the same content. The contents of the product are based on the information written on the packaging of the products. The purchased royal jelly mixtures were brought to the laboratory in their original packaging, protected from sunlight, and immediately analyzed.

Determination of total phenolic content (TPC)

The TPC of royal jelly mixtures was specified using the Folin-Ciocalteu procedure (Singleton & Rossi, 1965).

Firstly, 12 μL of each royal jelly mixture was mixed with methanol and was added to 60 μL of 2.5 mL of 0.2 mol/L Folin-Ciocalteu solution for 10 minutes. Afterwards, 48 μL Na_2CO_3 solution (7.5% w/v) was supplemented with this reaction and incubated for 30 minutes at room temperature. The absorbance measurements were performed at 760 nm. Results were read as gallic acid equivalent (μM Gallic acid/g dry weight).

Determination of Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was altered from the method analyzed by Chang et al (2002). The royal jelly mixture sample was mixed with methanol (10 μL) and then added with 3 μL NaNO_2 solution (5% w/v) with 40 μL of distilled water. 5 minutes later, 3 μL of 10% (w/v) aluminum chloride solution was added. In the final stage, 20 μL of NaOH was reacted after 5 minutes. The absorbance was measured at 415 nm after 15 minutes of incubation at room temperature. Results were stated as quercetin equivalent (μM Quercetin/g dry weight).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

Heretofore, different procedures have been applied to survey the antioxidant activity of royal jelly samples. The antioxidant activity of royal jelly was estimated by the determination of DPPH (2,2-diphenyl-1-picrylhydrazyl), by the method of Liu et al. (2008) and Takım, 2010. The stock solution of 0.6 mM DPPH in methanol was equipped. The working solution was acquired by diluting the stock solution with methanol to get an absorbance of 1.1 at 517 nm. The reagent mixtures in the 96-well plates occurred of a sample (7.5 μL) and DPPH stock (150 μL) was dissolved in methanol and protected in a dark place for 30 minutes at room temperature. The absorbance was read at 517 nm against methanol as blank. The results were expressed as Trolox equivalent as antioxidant capacity (TEAC) (mg TE/mL dry weight).

Extraction Stage of LC-MS/MS Analysis

The royal jelly mixtures extraction for LC-MS/MS analysis was carried out using the method of Necip et al. (2021). 25 g of royal jelly mixture was diluted with 5X of acidic water (pH 2 with HCl) and filtered. The filtrate was then poured into a glass column loaded with Amberlit XAD-4 rosins. Therefore, phenolic compounds stayed in the column, and other polar compounds were washed with a hydrated solvent. Then the washing step was carried out. The sediment was melted in 5 mL of water and extracted with diethyl ether. The extracts were concentrated in degraded pressure at 30°C in a rotary evaporator. The dried residue was reacted with 0.5 mL of methanol and passed through a 0.45 μm membrane filter ready for LC-MS / MS analysis.

Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) Analysis of Phenolic Compounds

Analysis of the phenolic compounds was done with a UHPLC apparatus connected to a dual MS device. 15 different phenolic compounds were analyzed in the royal jelly mixtures. These compounds were epicatechin, caffeic acid, t-cinnamic acid, apigenin, resveratrol, luteolin, quercetin, chrysin, hesperetin, rhamnetin, pinocembrin, caffeic acid phenethyl ester, p-hydroxybenzoic acid, p-coumaric acid, ferulic acid (Necip et al., 2021).

Extraction Stage of LC-MS/MS Analysis

All data were analyzed statistically using SPSS 24.0 (SPSS Inc., Chicago, IL, USA) software. Results are given as mean \pm standard deviation (SPSS, 2017).

Results and Discussion

Antioxidant capacity, TPC, and TFC results are given in Table 1. and Figure 1. TPC ranged from 0.6 to 38.2 mg GAE/100 g, and the mean value was determined as 9.2 mg GAE/100 g. Flavonoids, a significant subclass of polyphenols, were also analyzed entirely in the royal jelly mixtures, the results ranged from 0.1 to 30.06 mg QE/100 g and a mean value of 11.08 mg QE/100 g. DPPH ranged from 4.4 to 93.0 mg TE/mL, and a mean value of 29.11 mg TE/mL (Table 1).

TPC, TFC and DPPH activities were highest in S6 and S7 samples, and lowest in S1 and S2 samples. The highest TPC, TFC and DPPH activities in the 6th and 7th samples may be due to the high propolis content and the carob molasses content. Although the 3rd sample contains a higher percentage of honey and propolis than the S5 sample, it was observed that the antioxidant

Table 1. The number of bees per m² determined at different counting times of Hungarian vetch varieties (units)

No	Sample content	Total flavonoid (mg QE/100 g dry weight)	Total phenolic (mg GAE/100 g dry weight)	DPPH (mg TE/mL dry weight)
S1	royal jelly(3%) honey(95.5%) propolis(1.5%)	0.1 \pm 0.1	0.9 \pm 0.3	2.1 \pm 0.4
S2	royal jelly(1.5%) honey (97.5%) propolis (1%)	0.1 \pm 0.1	0.6 \pm 0.2	1.6 \pm 0.9
S3	royal jelly (3%) honey (95.5%) propolis (1.5%)	2.9 \pm 0.8	3.7 \pm 0.3	7.4 \pm 1.4
S4	royal jelly (1.5%) honey (97.5%) propolis (1%)	0.8 \pm 0.1	2.4 \pm 0.1	4.9 \pm 0.9
S5	royal jelly (5%) honey (89 %) propolis (1%) ginseng (5%)	1.7 \pm 0.2	10.9 \pm 0.1	26.7 \pm 1.0
S6	royal jelly (12%) harnup molasses (30%) propolis (18%) polen (30%) cacao (10%)	30.6 \pm 0.2	20.9 \pm 7.4	68.1 \pm 2.1
S7	royal jelly (24%) harnup molasses (15%) propolis (36%) polen (25%)	28.2 \pm 14.1	38.2 \pm 5.1	93.0 \pm 8.2

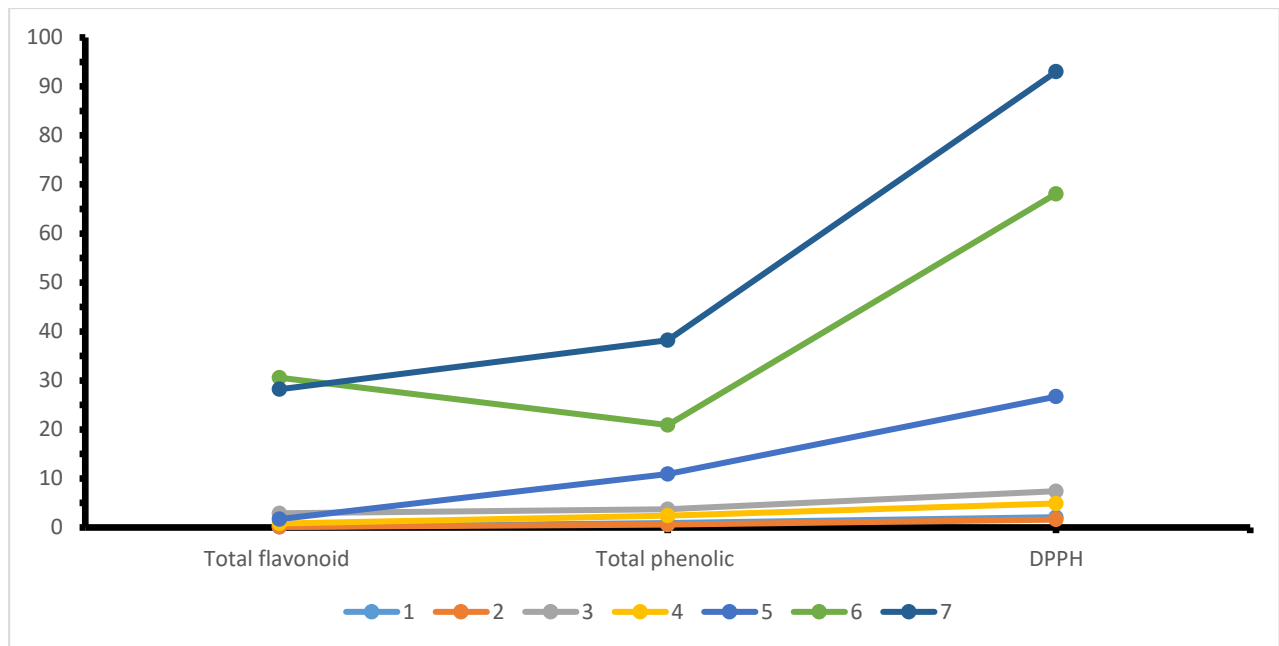


Figure 1. Total phenolic, total flavonoid and DPPH values of the samples

activity was very high in the S5 sample. This may be due to the ginseng (5%) contained in the 5th sample. It was determined that as TPC and TFC amounts of all samples increased, DPPH activities also increased.

The phenolic compound profile and amounts of the samples are given in Table 2. Caffeic acid phenethyl ester was the highest yielding phenolic compound in all samples, and Luteolin was the lowest phenolic compound. The highest phenolic compound contents were determined in the S6 and S7 samples. The phenolic

compound contents of the samples were in harmony with the total phenolic and total flavonoid contents, and the total phenolic and total flavonoid contents of the samples with high phenolic content were also high. In addition, phenolic compound content was compatible with DPPH activity, and samples with high phenolic content were also found to have high DPPH activities.

According to the results of the study, TPC, TFC, DPPH activity and phenolic content were found to be variable among the samples. In fact, this difference and

Table 2. Phenolic compound profile of the samples

Phenolic compound	S1	S2	S3	S4	S5	S6	S7
Epicatechin	4.9±0.1	5.6±1.2	12.8±1.4	6.5±1.3	36.2±2.6	91.2±3.6	130.1±2.3
Caffeic acid	21.2±0.6	25.7±2.4	46.4±3.2	29.8±2.1	109.5±3.7	312.7±8.7	528.7±8.9
t-Cinnamic acid	1.2±0.1	1.3±0.3	2.6±0.2	1.4±0.4	9.6±1.1	29.6±2.3	37.7±1.5
Apigenin	9.1±0.3	9.8±0.7	11.6±1.2	10.6±0.8	59.3±3.0	184.2±6.4	218.0±5.8
Resveratrol	0.8±0.4	1.1±0.1	1.6±0.3	1.4±0.2	5.7±0.2	16.9±2.3	27.9±2.1
Luteolin	0.7±0.1	0.9±0.1	1.2±0.1	1.1±0.1	4.1±0.4	12.5±1.2	14.6±1.1
Quercetin	2.9±0.3	3.8±0.8	6.6±0.9	4.0±0.1	27.0±1.7	67.5±2.3	85.9±2.1
Chrysin	165.1±2.9	179.8±6.4	207.2±5.8	187.0±3.7	742.3±11.2	1613.5±20.2	2431.6±12.5
Hesperetin	7.7±0.8	8.2±1.0	11.8±1.4	8.9±1.4	41.2±4.2	161.0±4.3	194.8±5.3
Rhamnetin	8.2±0.4	12.0±1.2	13.9±1.5	9.7±1.0	53.2±4.8	150.4±4.8	186.1±4.1
Pinocembrin	22.2±1.2	23.1±2.8	35.9±2.2	25.9±2.1	123.0±5.8	323.8±6.5	409.4±4.8
Caffeic acid phenethyl ester	246.5±4.7	254.4±6.3	424.7±8.4	260.9±4.9	1131.0±19.1	4753.1±38.6	5501.1±15.5
p-Hydroxybenzoic acid	2.2±0.2	2.8±0.8	5.4±1.2	3.0±0.2	12.8±1.1	40.9±2.4	60.3±1.2
p-Coumaric acid	15.4±1.3	16.1±1.2	16.4±1.0	16.2±0.8	55.4±2.3	151.9±4.3	206.6±2.2
Ferulic acid	20.0±1.1	20.4±2.3	32.3±2.6	21.3±1.1	171.5±4.9	337.4±3.8	431.5±3.8

variability is an expected result. Because the types and proportions of bee products contained in the samples are different. It is already known that the level of antioxidant capacity of bee products is closely related to their chemical composition. It was determined that TPC, TFC, DPPH activity and phenolic content increased as the ratio of royal jelly increased in the samples. In addition, it was determined that the 6th and 7th samples containing pollen had higher TPC, TFC, DPPH activity and phenolic content than the samples without pollen. Similar to this result we found, Ulusoy (2010) reported in a study that pollen contains 10-20 times more total phenolic substances than honey. Based on the literature data, it is reported that propolis has the strongest antioxidant property among all examined bee products, and also contains high amounts of TPC and TFC. Propolis is followed by bee pollen and royal jelly, respectively (Kocot, 2018). In our results, it was determined that the samples with high propolis content had increased TPC, TFC, DPPH activity and phenolic content. It can be said that the TPC, TFC, DPPH activity and phenolic content of the samples are variable, as well as the geographical location, climate and vegetation of the region where the bee product used in the samples was collected (Bakkaloğlu & Arıcı, 2019).

As a result of the literature research, no studies have been found on products similar to the one we have done. However, there are studies related to TPC, TFC, DPPH activity and phenolic contents of bee products. The results we found in the samples are similar to the results found in studies on bee products. Saroğlu (2018), showed that the total phenolic content of 9 different types of honey, pollen, and propolis samples from the Bayburt region for honey, pollen and propolis, respectively; 6.32-18.21, 769.4±17.7-547.64±15.43, 1564±178-7206±120.8 mg GAE/100 mg. Ülgen (2017) reported that the total phenolic content of honey samples was 70.60-212.06 GAE/100 g, and antioxidant activity of 38.30-138.28 mg AAG/g in the study he conducted to determine the bioactive properties of medicinal honey produced in Türkiye. Kalelioğlu Akbulut (2019) reported that the total phenolic content of various honey extracts is between 28.067-84.400 mg GAE/g, the phenolic content of pollen extracts is 471.567 and 1.151 mg GAE/g, the phenolic content of royal jelly extracts is 48.233-113, 40 mg GAE/g. Özdal (2017) reported that the total phenolic amount of propolis is between 6.18±0.36-157.25±12.2 mg GAE/g EEP and the total flavonoid amount is between 10.24±0.33-261.61±13.6 mg QE/g. Tabatabaei (2017) reported that the total phenolic content of pollen is between 21.23-27.66 (mg GAE/g), the flavonoid amount is between 03.72 and 4.97 (mg QE/g), DPPH activity is between 77.93 and 69.49% inhibition.

Yıldız (2011) reported that the total phenolic content of pollen is between 13.68-28.87 mg GAE/g and its antioxidant capacity is between 68.04-82.31 mM TEAC/g. Balkanska et al. (2017) The total phenolic content of royal jelly is 11.66 – 36.73 mg GAE/g RJ, Pavel

et al. (2014) found that the total phenolic content of royal jelly was 14.56-39.90 mg GAE/g, Ceksteryte et al. (2016) reported that the total phenolic content of royal jelly varied between 10.7 ± 0.03 mg GAE/g.

As a result of our literature review, we could not find a study in which the types and amounts of phenolic compounds were analyzed in royal jelly mixtures or similar foods. However, many studies have been carried out to determine phenolic compounds in many bee products (Bayram et al., 2018; Coşkun et al., 2018; Kılıç Altun S., & Aydemir M.E., 2020). Kılıç Altun & Aydemir (2020) reported that they detected 21 phenolic compounds in propolis collected in different regions of Anatolia in their study and the compounds they detected the highest was Quercetin (14.49 ppm) and Hydroxycinnamic acid (16.85 ppm). According to Bayram et al. (2018) reported that 64 propolis samples from different places of Hakkari exhibited a wealthy chemical substance in flavonoids, including furocoumarins and coumarins. Sorucu (2019) reported that propolis is opulent in phenolic acids such as caffeic acid, ferulic acid, gallic acid, and flavonoids such as pinocembrin, galangin, rutin, and apigenin. On the other hand, Coşkun et al. (2018) reported that they detected phenolic compounds such as naringenin, galangin luteolin, hesperetin, apigenin in 86 different propolis samples collected from 25 provinces in Türkiye. In addition, the researchers concluded that pinocembrin, chrysin is mainly found in Turkish propolis. In our study, pinocembrin and chrysin were detected in all samples.

As a result of the literature review, it has been seen that the phenolic contents of bee products vary widely. It was reported that the major biologically dominant components of propolis are flavonoids (flavanones and flavones), phenolic acids, and their esters. It has been reported that red propolis is characterized by many flavonoids (liquiritigenin, formononetin, pinobanksin-3-acetate, rutin, pinocembrin, luteolin, quercetin, isoliquiritigenin and, daidzein) (Salatino & Salatino, 2018; Graikou et al., 2016). The major biologically dominant components of bee pollen vary. The compounds of bee pollen are benzoic acid derivatives - p -hydroxybenzoic acid, syringic acid, gallic acid, vanillic acid and protocatechic acid - as well as caffeic acid and their glycerol esters, cinnamic acid derivatives - p - coumaric acid, and ferulic acid. Other more complex derivatives such as rosmarinic acid, amide derivatives of hydroxycinnamic and ferulic acids, and dihexoside are also reported (de Florio Almeida et al., 2017; Mohdaly et al., 2015; Sun et al., 2017). It was reported that the major biologically dominant components of royal jelly were flavanones (naringenin, hesperetin, isosacuranetin), flavones (chrysin, luteolin glucoside acacetin, apigenin and its glycoside), flavonols (kaempferol glucosides and isorhamnetin) and isoflavones (troids) (López-Gutiérrez et al., 2014).

The majority of these phenolic compounds reported in bee products were also detected in our study. The fact that bee products contain such a variety

of compounds can be explained by the geographical location of the region where they are collected, the season in which they are collected, the climate and the different vegetation.

Conclusion

As a result, it has been observed that food products containing bee products are wealth in phenolic contents and have high antioxidant properties. TPC, TFC, DPPH activity and phenolic contents were found to change as the type and ratio of bee products in the food changed. The highest TPC, TFC, DPPH activity and phenolic contents were found in products containing propolis and pollen. According to the results of our study, it was concluded that foods containing bee products (honey, propolis, bee pollen and royal jelly) are natural agents that can counteract the effects of oxidative stress, which is the main factor underlying many diseases.

Ethical Statement

Not applicable.

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Author Contributions

Serap KILIÇ ALTUN: Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Project administration

Mehmet Emin AYDEMİR: Conceptualization, Formal analysis, Funding Acquisition, Investigation, Methodology

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