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Effects of honey enrichment with *Spirulina platensis* on phenolics, bioaccessibility, antioxidant capacity and fatty acids

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ABSTRACT

The honeybees have been fed by *Spirulina platensis* extract and algal honey with green color produced by the bees naturally was obtained the first time in order to enhance its medicinal properties, mainly antioxidant properties. Antioxidant capacity, bioaccessibility of phenolics, hydrolysable and extractable phenolics, sugar, phenolic and fatty acid profiles were determined in the *Spirulina* honey. Total phenolic content of the *Spirulina* honey was 14.37 mg GAE/100g and increased 28.53% with *Spirulina* enrichment. Antioxidant capacities of the *Spirulina* honey were determined as 6.31, 5.85 and 3.66 μmol Trolox/g by ABTS, CUPRAC and DPPH methods in bioaccessible extracts. No significant difference was observed between control and *Spirulina* honey samples in terms of bioaccessibility of the total phenolics ($p \leq 0.01$). Among the detected phenolic compounds (15 phenolics), acacetin (48.55%) and pinocembrine (47.13%) were the highest. Total phenolic content of the honey can be enriched up to 20% by feeding of the honey bees with 10% of *Spirulina*. Saccharose content in *Spirulina* honey was lower than the control honey as 4.42%.

1. Introduction

Today, the functional food products market is growing at an incredible rate. The reasons for this are; rapid information sharing in the media, increasing consumer awareness about healthy and nutritious food consumption, the relationship between many diseases and nutrition, and the indispensable demand of human beings for good and new.

As with many foods, honey has been tried to be enriched with functional ingredients and other functional foods in the past. This is because honey, which already has a natural and nutritious content, is enriched with other functional components that it does not contain, and its functional composition and health benefits are further increased.

There are three main difficulties in enriching the composition of honey naturally and within the natural life cycle of bees. First, the natural nutritional composition and sensory properties of honey should not deteriorate during the enrichment process. Honey is a preferred food because it is natural. Second, the rheological properties of honey are not suitable for every enrichment ingredient. Honey is a viscous liquid with non-newtonian flow properties and its water content is very low. Therefore, the mechanical mixing process and the dissolution of the added ingredient homogeneously in the honey is very difficult (Khosravi-Darani et al., 2013). Moreover, if the mixing process is done with powerful mixers; air bubbles are included in the thixotropic consistency of honey, which can lead to nutrient component and flavor losses due to oxidation.

In the method called herbal infusion, honey can be enriched with a limited number of ingredients completely unnaturally. In this method, some components accepted by the bees can be dissolved in sugar syrup and used in bee nutrition. In this case, feeding the bees with sugar syrup makes the naturalness of the honey produced questionable. In addition, bees do not consume every component as food, only if the food they consume is attractive and has an appetite (EFSA, 2017).

None of the studies carried out to date, including the studies with the herbal infusion method, have provided a complete solution to these three problems. A fully natural, fully functional honey produced within the bees' own life cycle, not sourced from sugar syrup and sucrose, and

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whose composition consists entirely of natural ingredients, has not yet been produced, completely.

Honey is produced by bees from the nectar of flowers; supersaturated solution of sugars, mainly fructose, glucose, and maltose-like sugars with traces of sucrose, glucose-oxidase, hydrogen peroxide, phenolics, flavonoids, terpenes etc. It is considered as a medicinal food alongside its nutritional potential and used for some diseases like ulcer (Hilary, Habib, Souka, Ibrahim, & Platat, 2017; Dżugan et al., 2018; Chotchoungchatchai, Krairit, Tragulpiankit, & Prathanturarug, 2020).

In recent years, it has been determined that there is a strong relationship between antioxidant components in foods and the prevention of some chronic diseases. Honey has defined as food having antioxidant, anti-inflammatory, anti-tumor, antibacterial, antiviral and antiradical (Yarom et al., 2020; Sicak, Sahin-Yaglıoglu, & Ozturk, 2021). Depending on factors such as climate, environmental conditions and bee population, honey may also contain bee products with strong antioxidant properties such as bee bread, royal jelly and propolis (Kruszewski, Jedlińska, Antczak, Lipińska, & Witrowa-Rajchert, 2014; Socha, Gałkowska, Bugaj, & Juszczak, 2015).

The antioxidant capacity of honey depends largely on the flora where the beehive is located, the botanical origin of the honey, season and environmental factors (Aljadi & Kamaruddin, 2004).

Phenolics and aromatic acids are identified as major antioxidant components available in the honey (Khosravi-Darani et al., 2013). Compounds with antioxidant character in honey are mainly flavonoids (chrysin, pinocembrin, pinobanksin, quercetin, kaempferol, luteolin, galangin, apigenin, hesperetin, myricetin), phenolic acids (caffeic, coumaric, ferrulic, ellagic, chlorogenic), ascorbic acid, catalase, peroxidase, carotenoids. The quantity of these components also varies widely according to the floral and geographical origin of honey (Zhang et al., 2021).

As the closest research subject to our study, in the past, enrichment of honey with herbs has been tried to increase the health benefits and functional properties of honey. Herbal honey is produced using honeybees feeding on plant extracts, fruit juices or herbal infusions in sugar solutions. Herb honey is a honey-like product produced by feeding of the honey bees with the solutions of bee-honeys and saccharose mixture containing fruit juices or herbal extracts (Dżugan, Sowa, Kwaśniewska, Wesołowska, & Czernicka, 2017; Socha, Juszczak, & Pietrzyk, 2009). It's an alternative bee-product that contains all biologically active substances of honey and fortified with supplemented food material. However, honey-like products or honey mixtures that are not natural and do not match the natural sensory properties of honey are not liked by consumers. In the past, as a raw material in the production of herbal honey; the use of some herbs, vegetables and fruits has been tried (Pohorecka, 2004). On the other hand, it should be taken into consideration that the components used as food for bees will affect the specific properties of herb honey (Socha, Juszczak, Pietrzyk, 2009).

Spirulina is blue-green algae and non-toxic species of cyanobacteria. It is a supplement contains vitamins, minerals, essential fatty acids and antioxidants. The family of *Spirulina* naturally grows as microalgae in alkaline waters tropic areas and its industrial production is steadily increasing. It has been declared as a GRAS (Generally Recognized as Safe) product by FDA on 2011 and a great nutritional source as content of high protein with essential amino acids, vitamins (Vitamin B_{12}, B_6, B_2, A, E), minerals (Fe, Ca, P, Mg, Zn, Cu, Cr, Mn, Na, K, and Se), enzymes, essential fatty acids, and other nutrients (Gutiérrez-Salmeán, Fabila-Castillo, & Chamorro-Cevallos, 2015; Kent, Welladsen, Mangott, & Li, 2015). Due to its antioxidant enzyme activity, *Spirulina* prevents free radical scavenging, hindering lipid peroxidation, avoiding DNA damage (Abdelkhalek, Ghazy, & Abdel-Daim, 2015). *Spirulina* has a health benefits on inflammation, hypercholesterolemia, hyperlipidemia, carcinoma and some metabolic diseases (Deng & Chow, 2010). For taking advantage of this functional and nutritional potential, *Spirulina* has been added to different food products with different purposes moreover a large number of study conducted on fortification of food products included yoghurt (Guldas & Irkin, 2010).

It was aimed to feed the bee colony with *Spirulina* in its natural and usual life cycle and as a result of the application of a special feeding method, a new green-colored functional honey produced by bees containing *Spirulina* and sugar-free was obtained. The effect of natural enrichment with *Spirulina* on the antioxidant content of honey and the bioaccessibility of antioxidant components were investigated versus control honey. The contribution of *Spirulina*, functional component rich in lipid, to the fatty acid composition of honey was also compared.

2. Material and methods

2.1. Materials

The experimental honey productions were implemented in the Bursa Uludag University Campus (Bursa/Turkey) by management of Bursa Uludag University Beekeeping Development Application and Research Center (AGAM) between May–July 2016 and 2017. Honey bee (*Apis mellifera anatolica*) colonies were managed and checked regularly for parasites and diseases by AGAM.

The *Spirulina* in powdered/dried form was used and supplied from the company Holland & Barrett (Warwickshire, England). The 10% of *Spirulina* solution was prepared and presented to worker bees for feeding in natural way. It is not directly mixed into the honey. It is allowed to feed the worker bees in natural way by passing through into their digestion systems.In every four days, 300 mL of the *Spirulina* solution (10%) was used in each hive for the bee feeding. The feeding was continued for three months from May to July in the years of 2016 and 2017. The feeding of the colonies and the production of enriched honeys were implemented from two colonies versus two control hives in 2016. The experimental honey production was repeated with the selected hives in 2017.

The end of the feeding period, approximately 4 kg of enriched honey (with *Spirulina*) and the honey from control colonies were produced from each hive, bottled into the 850 gr of the glass jars, sealed with metal screw cap and stored in 15–20 °C until they were used for the analyses.

2.2. Methods

2.2.1. Physico-chemical analyses

Brix (Total Soluble Solids), total titratable acidity (TTA) and pH were determined according to the methods proposed by AOAC (2002).

2.2.2. Sugar analyses

The sugar profiles of the honey samples were determined by the HPLC (Thermo Fisher Scientific Inc., Waltham, MA, USA) analysis (Dżugan et al., 2017). The detected sugars by the HPLC analysis were fructose, glucose and saccharose. The detectors used for the separation were aerosol detector (CAD) and ultraviolet-diode array detector (UV/DAD). For the chromatographic analysis, Shodex Asahipak NH2P-504E column (C_{18}, 250 nm × 4.6 nm) and mixture of acetonitrile: water (78:22, v/v) were used. The flow rate of mobile phase was 1 mL/min. The temperature, the injection volume and determination time, were 35 °C, 10 μL and 30 min, respectively. For the sugar quantification, following sample preparation method was used. For the analysis, 0.5 g of honey sample was weighted and dissolved in 70% of ethanol solution (50 mL). All samples were heated at 35–40 °C in a water bath (Thermo Fisher Scientific Inc., Waltham, MA, USA). The honey samples were filtered by PTFE filters (0.22-μm), diluted 10-times and injected. The acetonitrile and carbohydrate standards were supplied from Sigma-Aldrich (St. Louis, USA). The honey sugars were determined from the retention times and relative peak areas of the identified sample curves.

2.2.3. Total phenolics and antioxidant capacity

The Total Phenolic Content (TPC) and antioxidant capacity (AC) of honey samples evaluated in terms of extractable, hydrolysable, bio-accessible fractions of phenolics (Vitali, Dragojević, & Šebečić, 2009).

2.2.3.1. Extraction of phenolic compounds. For the determination of the extractable phenolics, at first, 2 g of sample was mixed with 20 mL of HCl/methanol/water (1:80:10, v/v) solution. Then, the mixture was shaken in a water bath (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 250 rpm for 2 h at 20 °C. Afterwards extracts were centrifuged (3 K 30, Sigma, Germany) at 3500 rpm for 10 min at 4 °C. The residue of extractable phenolics was mixed 20 mL of methanol/H_2SO_4 (10:1) and kept in a shaking water-bath at 85 °C for 20 h to obtain hydrolysable phenolics. Following, the extracts were cooled in the room temperature and centrifuged at 3500 rpm for 10 min at 4 °C (3 K 30, Sigma, Germany). For extraction of bioaccessible phenolics, *in vitro* digestion enzymatic extraction method was used. 2 g of sample was mixed with pepsin (40 mg/mL, 0.1 M HCl) and incubated for 2 h in a shaking water bath (250 rpm) at 37 °C. Then, pancreatin and bile (2 mg/mL) mixture (12 mg/mL), were added, incubated for 2 h more (37 °C, 250 rpm) and centrifuged (15 °C, 3500 rpm, 10 min). Three different extractions of samples were stored at −18 °C until analysis. The samples were prepared by the three different procedures of extraction and were stored at −18 °C until the time of analyzing. The *in vitro* digestion procedure was performed for each honey sample in triplicate.

2.2.3.2. Determination of total phenolic content. Total phenol content was determined according to the Folin-Ciocalteu method proposed by Apak, Guclu, Ozyurek, and Celik (2008). The results were calculated as mg Gallic acid equivalents (GAE) per 100 g weighted sample and presented as the mean ± SD for triplicates analyses of each sample extract.

2.2.3.3. Determination antioxidant capacity. The antioxidant capacity of the prepared extracts was determined according to three different methods as ABTS (2,20-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt), DPPH (2,2diphenyl-1-picrylhydrazyl) and CUPRAC (Cupric reducing antioxidant capacity) methods according to Apak et al. (2008) after slight modification. The results were calculated as μmol Trolox equivalent (TE) per g weight sample and presented as mean ± SD for triplicates.

2.2.4. Phenolic composition

2.2.4.1. Sample preparation. The dry *Spirulina* samples were prepared according to method of Yousef et al. (2013) with some modifications. The powdered *Spirulina* samples (2.5–3.0 g) were extracted with 0.3% acetic acid (30 mL) in MeOH/H_2O (70:30, v/v) using a high-speed homogenizer (IKA Ultra-Turrax) for 2 min. The contents were then transferred into 50 mL tubes and vortexed for 3–4 s. Then, they were centrifuged at 4000 rpm for 15 min at 20 °C. The collected supernatants were decanted into volumetric flasks (100 mL). Acetic acid solution (20 mL and 0.3%) was added on to the precipitated part in the flask and waited for 15 min at room temperature. During the period, the mixture was vortexed three times with 5 min of time intervals. The supernatant phases were collected into flask and the final volume was adjusted to 100 mL with the extraction solvent identified. The extracted phases were also mixed to obtain completely homogenized sample. The sample (3 mL) was filtered into the HPLC vials using 0.2 μm PTFE syringe filters (Fisher Scientific, New Hampshire, USA). The sample phase from each vial, a 10 μL volume was injected into the HPLC. If the sample was not used freshly, was stored at −20 °C.

2.2.4.2. HPLC analysis of the honey samples. The method of Wahdan (1998) was used for the extraction of phenolics. Phenolic composition of the honey samples was determined according to the method proposed by Socha, Juszczak, & Pietrzyk, (2009), with some minor modifications. At first, the honey samples were weight (5 g) and dissolved in 0.2 M of HCl solution (pH:2). The prepared solution was saturated with NaCl and extracted with ethyl acetate. Ethyl acetate (Merck; Darmstadt, Germany) was used for the extraction of the phenolic compounds (Juszczak, Socha, Rożnowski, Fortuna, T., & Nalepka, 2009). The solutions (20%, w/v) containing honey samples were acidified with HCl solution until level of pH 2 was provided. After that, the solutions were saturated with approximately 10 mL of sodium chloride (Merck, Darmstadt, Germany) solution (30% w/v). The final solutions were extracted with the pre-prepared ethyl acetate solutions at the three stages (At first, 50 mL and the followed two times of 25 mL). Ethyl acetate was vacuum-evaporated from the collected extracts at approximately 40 °C. The residue obtained was dissolved in 5 mL of methanol (Merck, Darmstadt, Germany) and stored at −18 °C. The extracts were filtered by means of Millex-LCR syringe filters (PTFE) for the HPLC (High--performance liquid chromatography) measurement (Thermo Scientific™ Dionex™ UltiMat e™ 3000 RSLC system Waltham, Massachusetts, USA). The recovery rate of phenolic compounds was tested for the selected phenolic standards. The recovery ratios were 88% for chlorogenic acid and 101% for gallic acid for the extraction procedure applied.

2.2.4.3. Recovery rate. To determine the efficacy of the extraction method, the standard phenolic acids (1 mg) were added into the samples analyzed in terms of composition of phenolics. Ten different samples were used for spiking and 10 μL of each sample extract was injected into the HPLC instrument. Comparison of the spiked samples was made versus the samples extracted without the addition of commercial standards. The recovery of standard compounds was estimated by evaluating the differences between the sample with or without the commercial standards. For each compound, the recovery was calculated with the following formulae: (TCA - CA)/TCA × 100), where CA is compound amount in the sample extract (μg) without spiking and TCA is the total compound amount (μg) in spiked sample extract.

Phenolic acids such as caffeic, chlorogenic, neochlorogenic and gallic acid were detected at 280 nm absorbance value, some flavonoids such as apigenin, pinocembrin and acecetin were measured at 290 nm and while the other flavonoids (luteolin, quercetin, pinoquercetin, 3.3-dimethyl-quercetin, sakuranetin, taxifolin, methyl quercetin and kaempferol) were determined at 330 nm. Gradient elution was conducted at a flow rate of 1 mL/min using a solution of acetic acid (Sigma-Aldrich, St. Louis, USA) and 2.5 g/100 mL of acetonitrile (Merck, Darmstadt, Germany) as a mobile phase. The solvent system was increased according to the linear gradient as following: It was initiated with 3% of acetonitrile and then increased to 8% for 10 min, 15% at 20 min, 20% at 30 min, 30% at 40 min and 40% at 50 min, respectively. Finally, the column was eluted isocratically with acetonitrile before the next injection. The compounds studied were separated on a RP18 Lichrosorb column (25 cm 4.5 mm) (Merck, Darmstadt, Germany) at a temperature of 25 °C. Quantification of individual phenolic acids and flavonoids was based on comparison with standards obtained from Sigma-Aldrich (St. Louis, USA) and Fluka Chemie AG (Buchs, Switzerland).

2.2.5. Fatty acids composition (GC-FID)

The lipid content of honey samples was determined according to AOAC (2005) for providing sample for fatty acid composition. The fatty acids were converted to regarded fatty acids methyl esters (FAME) by the method proposed by Matos et al. (2016). FAME of the related fatty acids were determined by gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan). Gas chromatograph was equipped with split injection unit (SPL-2014), flame-ionization detector (FID-2014) and restek capillary column (RTX) which has 105 m long and 0.25 mm internal diameter. The column coated with 0.25 μm of 10% cyanopropylphenyl and 90% bis(cyanopropyl)siloxane was used. The injector and detector temperatures were at 260 °C. The oven temperature was initially set at

140 °C and kept constant for 5 min. Then, it was increased to 260 °C with elevated temperature steps which were 2.5 °C per min. At the end, it was held at this final temperature for 30 min. The injection volume was 1 μL, and the split ratio was 10:1. The carrier gas was nitrogen (flow rate was 2.2 mL per min) at a constant pressure (130.3 kPa). Fatty acid methyl esters were identified by comparison with the retention time of individual standards (Sigma, St. Louis, USA). The fatty acids were identified by comparing their retention times with the selected standards (Sigma, USA). The relative amounts of fatty acids were calculated from the chromatographic peak areas. The data are expressed as the mean with standard deviation of three independent samples.

2.2.6. Chemicals

All reagents used were analytical-grade purity. High quality water, obtained using a Milli-Q system (Millipore, Bedford, MA, USA) was used exclusively. Ethanol and methanol used in overall analysis purchased from Merck (Darmstadt, Germany). Carbohydrate (glucose, fructose, saccharose) standards used were supplied from Sigma-Aldrich (St. Louis, USA). Quantification of individual phenolic acids (caffeic, chlorogenic, neochlorogenic and gallic acids) and flavonoids (kaempferol, quercetin, luteolin, apigenin, pinocembrin, pinoquercetin, 3,3-dimethylquercetin, sakuranetin, taxifolin, methyl quercetin and acecetin) was based on comparison with standards from Sigma-Aldrich (St. Louis, USA) and Fluka Chemie AG (Buchs, Switzerland). Antioxidant and phenolic compound analysis chemicals purchased from Sigma-Aldrich (St. Louis, USA; Trolox®, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, DPPH: 1,1-Diphenyl-2-picrylhydrazine, pepsin, pancreatin), Merck (Darmstadt, Germany; gallic acid, neocuproine) and Thermo Scientific (Waltham, Massachusetts, USA; bile salt).

The honey enriched with *Spirulina* was extracted and analyzed using to the methods identified below.

2.2.7. Statistical analysis

All statistical analyses were carried out using SPSS statistical package (SPSS 16.0, Chicago, IL). The differences between means were considered according to % 0.05 level of statistical significance ($p < 0.05$). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used as statistical comparison methods.

3. Results and discussions

3.1. Physico-chemical properties

As seen in Table 1, the measurements obtained from the honey with *Spirulina* and the control honey were similar to the data obtained from the herb honeys in the researches made by Juszczak et al. (2009) and Socha, Juszczak, & Pietrzyk, (2009). Sugar contents in the honey can be changed according to the bee metabolism and the bee metabolism can be affected by the various external conditions. In this context, temperature and humidity of the weather are among the significant factors that can affect the metabolism of the bee. In the control honey versus the honey with *Spirulina*, saccharose decreased from 2.42 g/100g to 1.03 g/100g, while glucose and fructose increased from 38.25 g/100g to 39.61 g/100g and 41.60 g/100g to 42.20 g/100g. The differences observed in the contents of brix, pH, TTA, fructose and glucose were not significant between the honey types, statistically ($p > 0.05$). The slight decrease in the brix content from 81.90 to 79.40% can be related to the contents of the sugars which tend to decrease from the control honey versus the functional honey with *Spirulina* (Table 1).

Table 1
Physico-chemical properties of the honey samples.

	Control Honey	*Spirulina* Honey
Brix (g/100g)	81.90 ± 0.01[a]	79.40 ± 0.06[a]
pH	4.20 ± 0.01[a]	4.26 ± 0.12[a]
Total Titrable Acidity[a]	5.83 ± 0.45[a]	5.55 ± 0.34[a]
Fructose (g/100g)	41.60 ± 1.12[a]	42.20 ± 1.31[a]
Glucose (g/100g)	38.25 ± 1.45[a]	39.61 ± 1.55[a]
Saccharose (g/100g)	2.42 ± 0.23[a]	1.03 ± 0.19[b]
F/G Ratio	1.09 ± 0.02[a]	1.07 ± 0.01[a]
Crude Oil (g/100g)	0.26 ± 0.01[a]	0.78 ± 0.01[b]

Mean values ± standard deviation (N = 3 × 2) with different superscripts in the same row are significantly different (p < 0.05).
[a] Total Titrable Acidity expressed as g/100g malic acid equivalent.

The content of crude oil increased significantly due to the enrichment of the honey with *Spirulina* ($p > 0.05$). The crude oil content increased to 0.78 from 0.26% by the enrichment of *Spirulina* (Table 1). These were probably caused by the high contents of crude oil available in the *Spirulina* enriched.

3.2. Total phenolic content, antioxidant capacity and bioaccessibility

The extraction of phenolic compounds is mainly influenced by chemical structure of the phenolics, extraction method, particle size of the samples and presence of interfering substances in foods. Therefore, it is difficult to identify a standard/uniform procedure that is suitable for extraction of all phenolics in various food matrixes. Some compounds available in food structure could be extracted more easily, but some compounds that presents in glycosylated and/or ester forms, needed to be hydrolyzed by intestinal enzymes (Porrini & Riso, 2008). For this reason, different solvents and procedures were used for the extraction of extractable, hydrolysable and bioaccessible phenols in the honey samples.

The antioxidant capacity of foods arises from the substances with different chemical properties. There are various determination methods based on different principles for the measurement of antioxidant capacity. The reason of why different methods have been used for determination of antioxidant capacity is that foods are the mixture of many nutrients and chemicals, which show different single and/or complex chemical reactions and thus antioxidant effects. On the other hand, some interactions are able to occur among the supplement added, food component and the active reagent available in the assay used for measurement of antioxidant capacity. DPPH, ABTS and CUPRAC are the selected methods in the honey samples among the most common antioxidant capacity measurement methods (Seraglio et al., 2017; do Nascimento et al., 2018).

In general, when the results regarding total phenolic content and antioxidant capacity were considered (Table 2), the contents of hydrolysable extracts were higher than the extractable extracts for both samples. The extractable total phenolic content of the samples was between 13.76 and 13.95 mg GAE/g, whereas the hydrolysable ones only changed between 21.75 and 22.63 mg GAE/g. Statistically, the differences between the means of the control honey and the *Spirulina* honey were not significant for the extractable and the hydrolysable total phenolics ($p > 0.05$). The highest bioaccessibility values belonged to the total phenolic contents were 14.37% and 11.18% for the honey with *Spirulina* and the control honey.

The levels of antioxidant capacity belonged to the extractable extracts measured by the assays of CUPRAC, ABTS and DPPH were 1.57, 1.31 and 1.75 μmol Trolox/g for the control samples (Table 2). The antioxidant capacity of the hydrolysable extracts belonged to the control honey varied, between 4.58 and 7.46 μmol Trolox/g.

Antioxidant capacities of the samples with *Spirulina* were found higher than control samples if the all methods were considered. For the *Spirulina* honey, the values regarding ABTS, DPPH and CUPRAC ranged from 1.34 to 6.46, 2.01 to 9.40 and 1.79–8.60 μmol Trolox/g. The previous studies that were made *in vitro* and *in vivo* conditions indicate that *Spirulina* species had a great potential on reducing the oxidative stress. This potential was explained due to the phenolic compounds such as phycocyanins, carotenoids (especially β-carotene), PUFA's, polysaccharides, vitamins and minerals (Wu et al., 2016).

It is thought that CUPRAC method is the most suitable antioxidant

Table 2
Total phenolic content, antioxidant capacity of the honey samples.

Samples	Extractable Phenolics				Hydrolysable Phenolics				Bioaccessible Phenolics			
	Total Phenolic Content (mg GAE/100g)	Antioxidant Capacity (μmol Trolox/g)			Total Phenolic Content (mg GAE/100g)	Antioxidant Capacity (μmol Trolox/g)			Total Phenolic Content (mg GAE/100g)	Antioxidant Capacity (μmol Trolox/g)		
		ABTS	CUPRAC	DPPH		ABTS	CUPRAC	DPPH		ABTS	CUPRAC	DPPH
Control Honey	13.76 ± 0.02[a]	1.31 ± 0.05[a]	1.57 ± 0.28[a]	1.75 ± 0.26[a]	21.75 ± 0.63[a]	4.58 ± 0.57[a]	6.42 ± 0.03[a]	7.46 ± 1.83[a]	11.18 ± 0.22[a]	4.29 ± 0.36[a]	4.53 ± 0.02[a]	3.94 ± 0.02[a]
***Spirulina* Honey**	13.95 ± 0.35[a]	1.34 ± 0.03[a]	1.79 ± 0.24[b]	2.01 ± 0.08[b]	22.63 ± 1.56[a]	6.46 ± 0.03[b]	8.60 ± 0.09[b]	9.40 ± 0.43[b]	14.37 ± 0.54[b]	5.85 ± 0.19[b]	6.31 ± 0.54[b]	3.66 ± 0.01[b]

Mean values ± standard deviation (N = 3 × 2) with different superscripts (a-b) in the same row are significantly different ($p < 0.05$) in terms of total phenolic content and antioxidant capacity.

assay for extractable and hydrolysable extracts of the *Spirulina* honey. Because, there was found a strong correlation between the high phenolic contents and the results of CUPRAC antioxidant assay in our research. This correlation has also been observed in detail by the previous researchers (Zhang et al., 2021). It has been detected that CUPRAC capacities of herbal teas were found to correlate strongly with the high phenolic content (Alavi & Golmakani, 2017). The phenolics that have been determined by Apak et al. (2008) were similar to the ones that were detected in our research and the high antioxidant capacities measured by CUPRAC assay were due to the major phenolic compounds in the *Spirulina* honey as acacetin, pinochembrin, kaempferol, luteolin and sakuranetin (Table 3). Antioxidant capacity measured by the CUPRAC method ranged between 1.57-1.79 and 6.42–8.60 μmol Trolox/g for the control honey and the *Spirulina* honey belonged to the extractable and the hydrolysable extracts. Kaygusuz et al. (2016) have found that CUPRAC values ranged from 23.8 to 6.92 μmol Trolox/g for oak and chestnut honey, whereas they were changed 1.1–9.1 μmol Trolox/g for heather, pine, *Astragalus*, acacia and lavender honey. A strong linear correlation was found between the antioxidant capacity and the total phenolic content (Table 2); the samples with higher phenolic contents also had greater antioxidant capacities as mentioned by Khosravi-Darani et al. (2013).

Antioxidant capacity values decreased significantly after *in vitro* gastrointestinal digestion in comparison with the initial values ($p < 0.05$). These results were similar to *Mimosa scabrella* Bentham honeydew honeys (Seraglio et al., 2017). Antioxidant capacity of the bioaccessible phenolics reduced approximately 10–81% depending on the antioxidant capacity assay used. Antioxidant capacity values of bioaccessible phenolics were higher with CUPRAC compared to ABTS and DPPH methods. The bioaccessibility values of phenolics in the control and *Spirulina* honey were changed 74 and 80% according to the CUPRAC method.

Table 3
Reverse Phase-HPLC analysis of the honey phenolics.

Phenolics	RT (min)/λ (nm)	Control[a] (μg/mL)	*Spirulina* Honey[a] (μg/mL)
Neochlorogenic acid	5/285	0.02 ± 0.01[a]	0.02 ± 0.01[a]
Chlorogenic	6/285	0.01 ± 0.01[a]	0.02 ± 0.01[a]
Luteolin	7/330	0.58 ± 0.09[a]	1.26 ± 0.12[b]
Caffeic acid	8/285	0.02 ± 0.01[a]	0.04 ± 0.02[b]
Quercetin	9/330	0.26 ± 0.04[a]	0.25 ± 0.05[a]
Apigenin	10/290	0.25 ± 0.06[a]	0.35 ± 0.08[b]
Pinocembrin	12/290	47.13 ± 0.23[a]	47.34 ± 0.12[a]
Gallic acid	13/285	0.03 ± 0.01[a]	0.04 ± 0.01[a]
Pinoquercetin	17/330	0.09 ± 0.03[a]	0.07 ± 0.02[a]
3.3-dimethyl quercetin	23/330	0.12 ± 0.03[a]	0.11 ± 0.04[a]
Sakuranetin	24/330	1.78 ± 0.09[a]	1.85 ± 0.11[b]
Taxifolin	28/330	0.09 ± 0.02[a]	0.22 ± 0.05[b]
Methylquercetin	43/330	0.49 ± 0.07[a]	0.59 ± 0.04[b]
Kaempherol	45/330	0.59 ± 0.04[a]	1.10 ± 0.03[b]
Acacetin	47/290	46.73 ± 0.91[a]	48.55 ± 0.22[b]

[a] Mean values ± standard deviation with different superscripts in the same row are significantly different ($p < 0.05$).

The changes observed between the content of extractable or hydrolysable extracts and the bioaccessibility were probably due to their complex chemical nature, the interactions among those components available, and some processes such as heating (Porrini & Riso, 2008; Abourashed, 2013; Zhang et al., 2021). For instance; the structural changes of polyphenols cause the loss of antioxidant capacity depending on the free phenols are higher than the glycosides, and iron-phenol chelates and/or the phenolic acids inter-react with the other molecules in the food matrix (Kasote, Katyare, Hegde, & Bae, 2015; Safafar, Van Wagenen, Møller, & Jacobsen, 2015).

3.3. Phenolic profiles

When the phenolic compositions of the samples were considered (Table 3), 15 different phenolic compounds were detected comparing to their standard compounds. The two most abundant phenolic compounds in the control honey were pinocembrin (47.13 μg/mL) and acacetin (46.73 μg/mL), while the highest ones in the honey with *Spirulina* were acacetin (48.55 μg/mL) and pinocembrin (47.34 μg/mL).

Honey with *Spirulina* versus the control honey caused significant increases in the amounts of luteolin, caffeic acid, apigenin, sakuranetin, taxifolin, methylquercetin, kaempferol and acacetin as 117.24, 100, 40, 3.93, 144.44, 20.41, 86.44 and 3.89% ($p < 0.05$).

The phenolics in the herb honeys (aloe, black chokeberry, hawthorn, mint, pine, thyme etc.) were found as caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, gallic acid, gentisic acid, sinapic acid and syringic acid by Socha, Juszczak, & Pietrzyk, (2009). The phenolics determined in the microalgae and the cyanobacteria by Safafar et al. (2015) and Jerez-Martel et al. (2017) were the phenolic acids such as gallic acid, caffeic acid, ferulic acid, *p*-coumaric acid, cinnamic acid, syringic acid, protocatechuic acid and chlorogenic acid with the flavonoids such as catechin and epicatechin.

When the phenolic composition of honey is compared with *Spirulina* and control honey (Table 3), it is seen that it is a successful enrichment product and has a strong antioxidant composition consisting of a mixture of phenolic compounds found in control honey and *Spirulina* supplement.

3.4. Fatty acids composition

It is clear that enrichment of honey with *Spirulina* causes a significant increase in the overall fatty acid content of honey derived from *Spirulina* (Table 4), significantly ($p \leq 0.05$). It can be proposed that *Spirulina* supplementation into the honey by the natural way which was made by the honey bees (not incorporating *Spirulina* into the honey) is an effective way to increase amount of significant fatty acids such as ω-3 and ω-6.

In the previous studies focused on the determination of fatty acid profile of *Spirulina*, different results have been reported by the researchers (Colla, Bertolin, & Costa, 2004; Diraman, Koru, & Dibeklioglu, 2009; Ronda et al., 2014; Al-Dhabi & Valan Arasu, 2016; Matos et al.,

Table 4
The comparison of fatty acid profiles of the control honey, *Spirulina* powder and the honey enriched with *Spirulina*.

	Spirulina platensis	*Spirulina* Honey	Control Honey
Saturated Fatty Acids (SFA)			
C12:0	87.35 ± 3.11 [b]	0.92 ± 0.03 [a]	ND
C14:0	94.27 ± 4.26 [b]	0.84 ± 0.04 [a]	ND
C16:0	3380.17 ± 5.41 [c]	37.18 ± 3.10 [b]	7.83 ± 0.01 [a]
C18:0	31.94 ± 4.23 [b]	0.33 ± 0.02 [a]	ND
Other SFA′ s	323.33 ± 11.06 [b]	1.27 ± 0.09 [a]	ND
Total	***3916.89* ± *36.12* [c]**	***40.54* ± *82* [b]**	***7.83* ± *0.01* [a]**
Monounsaturated Fatty Acids (MFA)			
C15:1	378.45 ± 11.32 [b]	3.61 ± 0.88 [a]	ND
C16:1	546.14 ± 9.32 [b]	6.41 ± 0.61[a]	ND
C18:1	69.25 ± 2.45 [c]	30.43 0.43 ± 0.03[b]	10.40 ± 0.02[a]
Other MFA′ s	97.66 ± 6.29 [b]	0.83 ± 0.04 [a]	ND
Total	***1091.50* ± *13.11*[c]**	***41.28 11.28* ± *0.86* [b]**	***10.40* ± *0.02* [a]**
Polyunsaturated Fatty Acids (PFA)			
***ω*-3**			
C16:4	36.14 ± 3.24 [b]	0.35 ± 0.04 [a]	ND
C18:3 (ALA, *α*-Linolenic acid)	144.76 ± 4.76 [b]	1.60 ± 0.06 [a]	ND
C20:5 (EPA, Eicosapentaenoic acid)	32.65 ± 2.71 [b]	0.29 ± 0.03 [a]	ND
C22:6 (DHA, Docosahexaenoic acid)	67.49 ± 3.33 [b]	0.72 ± 0.05 [a]	ND
Total	***281.04* ± *5.77* [b]**	***2.96* ± *0.28* [a]**	***ND***
***ω*-6**			
C18:2 (LA, Linoleic acid)	144.81 ± 18.76 [c]	70.61 ± 0.08 [b]	49.40 ± 0.09 [a]
C18:3 (GLA, *γ*-linolenic acid)	1866.27 ± 37.37 [b]	20.75 ± 4.62 [a]	ND
C20:4 (AA, Arachidonic acid)	54.86 ± 4.91 [b]	0.62 ± 0.04 [a]	ND
C22:5	46.19 ± 5.54 [b]	0.52 ± 0.09 [a]	ND
Total	***2112.13* ± *34.29* [c]**	***92.50* ± *2.87* [b]**	***49.40* ± *0.09* [a]**

Other SFA′ s (Tridecanoic acid C13:0, Pentadecanoic acid C15:0, Behenic acid C22:0) and other MUFA′ s (Margaroleic acid C17:1, Erucic acid C22:1, Nervonic acid C24:1) were not considered due to minor purities. The values given in the table are as mg/100 g ± SD (n = 3), ND: Not detected.
The mean values with different superscripts are significantly different in the same row, statistically ($p \leq 0.05$).

2016). In some of the previous researches, different extraction methods have been used to determine the fatty acid profile. For example, hexane solvent in the fatty acid extraction different from others has been used by Diraman et al. (2009). Colla et al. (2004) used methanol/chloroform solvent for the lipid extraction. Therefore, the solvent used in the extraction can also be one of the discriminative factors to obtain different results. In our research; AOAC (2005) method in lipid extraction, then FAME methylation procedure and GC determination with FID detector were used successfully to determine fatty acid content in the experimental samples as proposed by Matos et al. (2016). We found that the fatty acid profile classified in our research is consistent with the fatty acid profile established by Matos et al. (2016). On the other hand, the differences determined in the quantity of fatty acids can arise from the climate and growth conditions of the *Spirulina* provided versus to the *Spirulina* samples used by Matos et al. (2016).

Honey is a very poor food in terms of lipid content and fatty acids. Therefore, trace amounts of lipid compounds could not be detected in the samples (Kanbur, Yuksek, Atamov, & Ozcelik, 2021; Swears & Manley-Harris, 2021). In our research, the only fatty acids determined in the control honey samples were palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2) and that were found in trace amounts. As given in Table 4, the supplementation of the honey with *Spirulina* increased the total fatty acid content significantly ($p \leq 0.05$) and enriched fatty acid profile of the honey (Table 4).

The rich fatty acid profile of *Spirulina* has been transferred into the honey as a result of supplementation of the honey with powdered *Spirulina* by means of bee feeding. Approximately, the saturated and monosaturated fatty acid contents increased to 40 and 11 mg/100g from 0.03 to 0.04 mg/100g while polyunsaturated fatty acids such as *ω*-3 and *ω*-6 which are the important food components for human nutrition and health, increased to 3 and 24 mg/100g from the level of "not detected" and 0.19 mg/100g (Table 4).

4. Conclusion

Honey produced in this method is completely different from the honey that is produced by direct component supplementation from outside into honey and then mixed. The supplement or component added honey is produced by adding nutrients rich in bioactive compounds, fruit juice, or coloring agent into honey. But, this kind of mixed honey is not processed by bees. Differently, the enrichment component (*Spirulina platensis* powder) in our method is processed in bee metabolism through its digestion system and its health and nutrient value is increased by natural way.

This research has two important contributions. A new algae honey has been produced by bees as a natural and new functional food. Antioxidant properties and fatty acid composition (PUFAs) of traditional honey have been significantly increased. This enrichment, unlike all applications and patents, is made within the scope of the natural feeding cycle and metabolism of bees. Secondly, *Spirulina* as bee feed will be used as a nutritious food source to meet the nutritional needs of bees when they cannot find food and are weak.

Spirulina has been defined as a GRAS (Generally recognized as safe); although, it can cause different reflections on the honey bee life, particularly in their metabolism or bee products, since *Spirulina* is not included in the honey bee diet, naturally. Therefore, further investigation is required to clear its effects on bee life.

Declaration of competing interestCOI

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

CRediT authorship contribution statement

M. Guldas: Design of the study, Visualization, writing. **O. Gurbuz:** Design of the study, revising. **I. Cakmak:** Field study, design of the study, writing. **E. Yildiz:** Data collection, writing, revising. **H. Sen:** Field study, data collection.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112461.

References

Abdelkhalek, N. K., Ghazy, E. W., & Abdel-Daim, M. M. (2015). Pharmacodynamic interaction of Spirulina platensis and deltamethrin in freshwater fish nile tilapia, Oreochromis niloticus: Impact on lipid peroxidation and oxidative stress. *Environmental Science and Pollution Research, 22*(4), 3023–3031. https://doi.org/10.1007/s11356-014-3578-0

Abourashed, E. A. (2013). Bioavailability of plant-derived antioxidants. *Antioxidants, 2*(4), 309–325. https://doi.org/10.3390/antiox2040309

Al-Dhabi, N. A., & Valan Arasu, M. (2016). Quantification of phytochemicals from commercial Spirulina products and their antioxidant activities. *Evidence-based Complementary and Alternative Medicine*, 13. https://doi.org/10.1155/2016/7631864. Article ID 7631864.

Alavi, N., & Golmakani, M. T. (2017). Improving oxidative stability of virgin olive oil by addition of microalga Chlorella vulgaris biomass. *Journal of Food Science & Technology, 54*(8), 2464–2473. https://doi.org/10.1007/s13197-017-2689-2

Aljadi, A. M., & Kamaruddin, M. Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry, 85*(4), 513–518. https://doi.org/10.1016/S0308-8146(02)00596-4

AOAC. (2002). Sugars and sugar products. In W. Horowitz (Ed.), *Official methods of analysis of the association of official analytical chemists* (17th ed., pp. 22–33). Gaithersburg, MD: AOAC International.

AOAC. (2005). *2005 official method of analysis, method 945.16, Oil in cereal adjuncts* (18th ed.). Gaithersburg, MD: AOAC International.

Apak, R., Guclu, K., Ozyurek, M., & Celik, S. E. (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchim. Acta, 160*(4), 413–419. https://doi.org/10.1007/s00604-007-0777-0

Chotchoungchatchai, S., Krairit, O., Tragulpiankit, P., & Prathanturarug, S. (2020). The efficacy of honey and a Thai Herbal Oil preparation in the treatment of pressure ulcers based on Thai traditional medicine wound diagnosis versus standard practice: An open-label randomized controlled trial. *Contemporary Clinical Trials Commun., 17*, 100538. https://doi.org/10.1016/j.conctc.2020.100538

Colla, L. M., Bertolin, T. E., & Costa, J. A. V. (2004). Fatty acids profile of Spirulina platensis grown under different temperatures and nitrogen concentrations. *Zeitschrift für Naturforschung C, 59*(1–2), 55–59. https://doi.org/10.1515/znc-2004-1-212

Deng, R., & Chow, T. J. (2010). Hypolipidemic, antioxidant, and anti-inflammatory activities of microalgae Spirulina. *Cardiovascular Therapeutics, 28*(4), e33–e45. https://doi.org/10.1111/j.1755-5922.2010.00200.x

Diraman, H., Koru, E., & Dibeklioglu, H. (2009). Fatty acid profile of Spirulina platensis used as a food supplement. *Israeli Journal of Aquaculture – Bamidgeh, 61*(2), 134–142.

Dżugan, M., Sowa, P., Kwaśniewska, M., Wesołowska, M., & Czernicka, M. (2017). Physicochemical parameters and antioxidant activity of bee honey enriched with herbs. *Plant Foods for Human Nutrition, 72*(1), 74–81. https://doi.org/10.1007/s11130-016-0593-y

Dżugan, M., Tomczyk, M., Sowa, P., & Grabek-Lejko, D. (2018). Antioxidant activity as biomarker of honey variety. *Molecules, 23*(8), 2069. https://doi.org/10.3390/molecules23082069

Guldas, M., & Irkin, R. (2010). Influence of Spirulina platensis powder on the microflora of yoghurt and acidophilus milk. *Mljekarstvo, 60*(4), 237.

Gutiérrez-Salmeán, G., Fabila-Castillo, L., & Chamorro-Cevallos, G. (2015). Nutritional and toxicological aspects of Spirulina (Arthrospira). *Nutricion Hospitalaria, 32*(1), 34–40.

Hilary, S., Habib, H., Souka, U., Ibrahim, W., & Platat, C. (2017). Bioactivity of arid region honey: An in vitro study. *BMC Complementary and Alternative Medicine, 17*(1), 1–10. https://doi.org/10.1186/s12906-017-1664-9

Jerez-Martel, I., García-Poza, S., Rodríguez-Martel, G., Rico, M., Afonso-Olivares, C., & Gómez-Pinchetti, J. L. (2017). Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. *Journal of Food Quality*, 8. https://doi.org/10.1155/2017/2924508. Article ID 2924508.

Juszczak, L., Socha, R., Rożnowski, J., Fortuna, T., & Nalepka, K. (2009). Physicochemical properties and quality parameters of herbhoneys. *Food Chemistry, 113*(2), 538–542. https://doi.org/10.1016/j.foodchem.2008.07.098

Kanbur, E. D., Yuksek, T., Atamov, V., & Ozcelik, A. E. (2021). A comparison of the physicochemical properties of chestnut and highland honey: The case of Senoz Valley in the Rize province of Turkey. *Food Chemistry, 345*, 128864. https://doi.org/10.1016/j.foodchem.2020.128864

Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences, 11*(8), 982. https://doi.org/10.7150/ijbs.12096

Kaygusuz, H., Tezcan, F., Erim, F. B., Yildiz, O., Sahin, H., Can, Z., et al. (2016). Characterization of Anatolian honeys based on minerals, bioactive components and principal component analysis. *LWT-Food Science and Technology, 68*, 273–279. https://doi.org/10.1016/j.lwt.2015.12.005

Kent, M., Welladsen, H. M., Mangott, A., & Li, Y. (2015). Nutritional evaluation of Australian microalgae as potential human health supplements. *PloS One, 10*(2), Article e0118985. https://doi.org/10.1371/journal.pone.0118985

Khosravi-Darani, K., Khaksar, R., Esmaeili, S., Seyed-Reihani, F., Zoghi, A., & Shahbazizadeh, S. (2013). Antifungal and anti-bacterial synergistic effects of mixture of honey and herbal extracts. *Zahedan Journal of Research in Medical Sciences, 15*(8), 30–33.

Kruszewski, B., Jedlińska, A., Antczak, M., Lipińska, E., & Witrowa-Rajchert, D. (2014). Novel method of producing honey powders and assessment of their biological activity. *Food. Science. Technology. Quality, 1*, 160–172.

Matos, Â. P., Feller, R., Moecke, E. H. S., de Oliveira, J. V., Junior, A. F., Derner, R. B., et al. (2016). Chemical characterization of six microalgae with potential utility for food application. *Journal of the American Oil Chemists' Society, 93*(7), 963–972. https://doi.org/10.1007/s11746-016-2849-y

do Nascimento, K. S., Sattler, J. A. G., Macedo, L. F. L., González, C. V. S., de Melo, I. L. P., da Silva Araújo, E., et al. (2018). Phenolic compounds, antioxidant capacity and physicochemical properties of Brazilian *Apis mellifera* honeys. *LWT-Food Science and Technology, 91*, 85–94. https://doi.org/10.1016/j.lwt.2018.01.016

Pohorecka, K. (2004). Effect of standardized plant herb extracts on general condition of the honeybee (*Apis mellifera* L.). *Bulletin of the Veterinary Institute in Pulawy, 48*(4), 415–419.

Porrini, M., & Riso, P. (2008). Factors influencing the bioavailability of antioxidants in foods: A critical appraisal. *Nutrition, Metabolism, and Cardiovascular Diseases, 18*(10), 647–650. https://doi.org/10.1016/j.numecd.2008.08.004

Ronda, S. R., Parupudi, P. L. C., Vemula, S., Tumma, S., Botlagunta, M., Settaluri, V. S., et al. (2014). Optimization of medium components using orthogonal arrays for γ-Linolenic acid production by Spirulina platensis. *Korean Journal of Chemical Engineering, 31*(10), 1839–1844. https://doi.org/10.1007/s11814-014-0082-7

Safafar, H., Van Wagenen, J., Møller, P., & Jacobsen, C. (2015). Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Marine Drugs, 13*(12), 7339–7356. https://doi.org/10.3390/md13127069

Seraglio, S. K. T., Valese, A. C., Daguer, H., Bergamo, G., Azevedo, M. S., Nehring, P., et al. (2017). Effect of in vitro gastrointestinal digestion on the bioaccessibility of phenolic compounds, minerals, and antioxidant capacity of Mimosa scabrella Bentham honeydew honeys. *Food Research International, 99*, 670–678. https://doi.org/10.1016/j.foodres.2017.06.024

Sicak, Y., Sahin-Yaglıoglu, A., & Ozturk, M. (2021). Bioactivities and phenolic constituents relationship of Mugla thyme and pine honey of Turkey with the chemometric approach. *Journal of Food Measurement and Characterization*, 1–14. https://doi.org/10.1007/s11694-021-00940-8

Socha, R., Gałkowska, D., Bugaj, M., & Juszczak, L. (2015). Phenolic composition and antioxidant activity of propolis from various regions of Poland. *Natural Product Research, 29*(5), 416–422. https://doi.org/10.1080/14786419.2014.949705

Socha, R., Juszczak, L., Pietrzyk, S., & Fortuna, T. (2009). Antioxidant activity and phenolic composition of herbhoneys. *Food Chemistry, 113*(2), 568–574. https://doi.org/10.1016/j.foodchem.2008.08.029

Swears, R. M., & Manley-Harris, M. (2021). Composition and potential as a prebiotic functional food of a Giant Willow Aphid (*Tuberolachnus salignus*) honeydew honey produced in New Zealand. *Food Chemistry, 345*, 128662. https://doi.org/10.1016/j.foodchem.2020.128662

Vitali, D., Dragojević, I. V., & Šebečić, B. (2009). Effects of incorporation of integral raw materials and dietary fibre on the selected nutritional and functional properties of biscuits. *Food Chemistry, 114*(4), 1462–1469. https://doi.org/10.1016/j.foodchem.2008.11.032

Wahdan, H. A. L. (1998). Causes of the antimicrobial activity of honey. *Infection, 26*(1), 26–31.

Wu, Q., Liu, L., Miron, A., Klímová, B., Wan, D., & Kuča, K. (2016). The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: An overview. *Archives of Toxicology, 90*(8), 1817–1840. https://doi.org/10.1007/s00204-016-1744-5

Yarom, N., Hovan, A., Bossi, P., Ariyawardana, A., Jensen, S. B., Gobbo, M., et al., Mucositis Study Group of the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO). (2020). Systematic review of natural and miscellaneous agents, for the management of oral mucositis in cancer patients and clinical practice guidelines-part 2: Honey, herbal compounds, saliva stimulants, probiotics, and miscellaneous agents. *Supportive Care in Cancer*, 1–16. https://doi.org/10.1007/s00520-019-04887-x

Yousef, G. G., Brown, A. F., Funakoshi, Y., Mbeunkui, F., Grace, M. H., Ballington, J. R., et al. (2013). Efficient quantification of the health-relevant anthocyanin and phenolic acid profiles in commercial cultivars and breeding selections of blueberries (Vaccinium spp.). *Journal of Agricultural and Food Chemistry, 61*(20), 4806–4815. https://doi.org/10.1021/jf400823s

Zhang, X. H., Qing, X. D., Mu, S. T., Wang, D., Zheng, J. J., Zhou, S. J., et al. (2021). Authentication of honey of different nectar sources and antioxidant property evaluation by phenolic composition analysis with chemometrics. *Food Control, 124*, 107900.