

# Comparison of Physical and Biochemical Properties Between Pure and Adulterated Blossom honeys

## Saf ve Hileli Ballar Arasında Fiziksel ve Biyokimyasal Özelliklerin Karşılaştırılması

Research Article

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### ABSTRACT

The purpose of this study was to find new parameters permitting the identification of adulterated honeys. For this reason, different qualities of adulterated honeys were produced. Honey bee colonies fed with three different sugar syrups, high fructose corn syrup 55 (HFCS-55), sucrose syrup and invert sugar syrups at 3 L/day in one concentration (1:1.5 g/v) for 15 days. After harvest, some physico-chemical (<sup>13</sup>C/<sup>12</sup>C, C4%, pH, moisture, optical rotation, electrical conductivity, sugar analyses, proline and diastase) and biological (total phenolic contents, phenolic profile, and antioxidant capacities) properties of the honeys were analyzed and compared. Conductivity, proline content, total phenolic content, diastase activities and antioxidant properties of the honeys were found distinguished parameters. Antioxidant capacity was significantly decreased in adulterated honeys.

### Key Words

Pure honey, adulteration, physico-chemical parameters, antioxidant, phenolics.

### ÖZ

Bu çalışmanın amacı, sahte balların tanımlanması için yeni parametrelerin bulunmasıdır. Bu sebepten ötürü, farklı kalitede sahte ballar üretildi. Üç farklı şeker şurubu, yüksek fruktoz mısır şurubu 55 (HFCS-55), sukroz şurubu ve invert şeker şurubu 15 gün boyunca günde bir derişimde (1:1.5 g/v) günde 3 L olacak şekilde kolonilerde bal arıları beslendi. Hasat edildikten sonra, bazı fiziko-kimyasal (<sup>13</sup>C/<sup>12</sup>C, %C4, pH, nem, optik rotasyon, elektrik iletkenliği, şeker analizleri, prolin ve diastaz), biyolojik (toplam fenolik içeriği, fenolik profil ve antioksidan kapasiteleri) özellikleri ballarda karşılaştırılarak analiz edildi. Balların iletkenlik, prolin içeriği, toplam fenolik içeriği, diastaz aktiviteleri ve antioksidan özellikleri belirgin parametreler olarak bulundu. Antioksidan kapasiteleri sahte ballarda önemli ölçüde azalmıştır.

### Anahtar Kelimeler

Saf bal, hileli, fiziko-kimyasal parametreler, antioksidan, fenolikler.

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## INTRODUCTION

Honey is a high-energy, nutritious natural product used by man for hundreds of years because of its therapeutic properties and for protection against diseases. The adverse effects on human health of various chemicals are encouraging people to turn toward what is traditional and natural. There has been a shift toward bee products and honey in particular. In addition to the fact that honey is very popular and has a high economic value, concerns over making even greater profits leads to the production of adulterated honeys [1,2]. Honey is a natural compound, nearly 95% of the dry weight of which consists of fructose and glucose [3].

Various enzymes, organic acids, phenolic compounds, amino acids and minerals in its composition represent honey's biologically active compounds, and these vary depending on the floral characteristics of the region where it is produced [4,5]. Classical honey parameters set out in honey codices are no longer sufficient to determine the purity of honey [6,7]. These parameters are also unable to establish the true quality of honey, in other words, its biologically active value. New methods are therefore being sought to reveal adulteration in honey. Since honey is a mixture it has become one of the most highly adulterated products. Various means of adulteration are employed in order to increase productivity in bee colonies. The most commonly employed method is feeding bees with sugar syrups. Substances such as sucrose, starch-based sugars and invert sugar syrups significantly enhance honey production in the hive. All honeys produced using sugar syrups are adulterated, but it is difficult to determine the exact type and amount of adulteration [2,7,8]. There has been a considerable recent increase in the number of studies aimed at revealing adulteration in honey. Adulteration is tested by determining carbon isotope ( $d^{13}C$ ) levels in honey using elemental analyzer-isotope ratio mass spectrometer (EA-IRMS). However, this method is more appropriate for some types of sugar and is not recommended for beet sugars and corn sugars [1,2].

## MATERIALS and METHODS

### Chemicals

2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu's phenol reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), were purchased from Sigma Chemical Co. (St Louis, MO, USA).

All chemical standards were HPLC-grade pure. The common phenolic compounds (gallic, protocatechuic acid, *p*-OH benzoic acid, catechin, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, rutin, *t*-cinnamic acid, luteolin) and sugars (ribose, arabinose, fructose, glucose, galactose, maltose, trehalose, melibiose, melezitose) were obtained from Sigma-Aldrich (Munich, Germany). Of the HPLC grade organic reagents, acetonitrile was supplied by Sigma-Aldrich Co. (St. Louis, MO, USA) and methanol was by Merck KGaA, Darmstadt, Germany). HPLC syringe filters (RC-membrane, 0.45  $\mu$ m) were obtained from Sartorius Minisart RC 15, Sartorius (Germany).

### Honey Samples

The characteristics of the sugar syrups used in the study and the firms from which they were purchased are summarized in Table 1. This study involved four different hive groups, each of the groups containing eight hive colonies, for each honey group. The hives were located in the same area at distances of 50 m apart. The study was performed in 2015 in the province of Erzurum in Turkey's East Anatolia region, in the honey season, June-July. The lower levels of the hives were left empty, and hives were given a single daily 3 L/day concentration of sugar syrup over 15 days. A sucrose syrup mixture of 1:1.5 (g/g) was prepared by us on a daily basis. Colonies were fed with direct high fructose corn syrups and high invert sugar syrups, they were supplied from Companies. Combs were removed from the hive and brought to the laboratory for investigation.

### Analytical Determinations

$d^{13}C$  isotope analyses of the pure and the adulterated honeys were performed once the specimen had been completely carbondioxidized using

**Table 1.** Composition of sugar syrups used in the experiment.

Honey	Sugar type	Origin	Composition
1	High Fructose Corn Syrups (HFCS-55)	Zea mays	Fructose 55%, Dextrose 55%
2	Sucrose Syrup (SS)	Beet sugar	Sucrose 99.5%
3	Invert sugar syrups (ISS)	Beet sugar	Fructose 39%, Sucrose 33%, Glucose 28%

elemental analysis-isotope ratio mass spectrometry (EA-IRMS) [9]. C4% sugar analysis in honey was calculated from the difference between  $d^{13}C$  isotopes in protein and honey as shown below [1,2,10].

$$C4\% = \delta^{13}C_{\text{protein}} - \delta^{13}C_{\text{honey}} / \delta^{13}C_{\text{protein}} - (-9.7).$$

Color value was measured using a Hunter spectrometer (CR-400, Minolta, Osaka, Japan), moisture using a refractometer (Atago, Tokyo, Japan), electrical conductivities using a conductometer (WTW inoLab Cond/720, Germany) and optical activity or rotation using a polarimeter (Beta PPP7 England). The physical characteristics of the honeys were in agreement with EU legislation [11].

Sugar analysis of the samples was performed using a refractive detector (RID) with HPLC (Elite LaChrom, Hitachi, Japan) using nucleosil column (200/4.6 Nucleosil 100-5  $NH_2$ ). All standards of sugars and honey samples were dissolved with deionized water and filtered with HPLC syringe filters (0.45  $\mu m$ ). The calibration curve of the sugars was calculated using five different concentrations of the standards (from 1.25 to 20 mg/mL). Calibration curves were determined at between 0.994 and 1.000. Seventy-nine percent acetonitrile and 21% water were used as mobile phase in programmed isocratic elution. Injection of the sample volume was 25 mL, column temperature 30°C and flow rate 1.5 mL/min. [3].

Proline content was measured using spectrometric assay [12]. Diastase activity was determined following the spectrophotometric method using a buffered solution of soluble starch and honey incubated in a thermostatic bath at 40°C [6]. Diastase units were expressed as 1% starch hydrolyzed by an enzyme in 1 g of honey in 1 h.

### Honey Extraction for Biological Activities Tests

Methanolic extracts of the honeys were used for antioxidant analyses. Briefly, a 15 g sample of honey was placed in a falcon tube (50 mL), and 50 mL 99% methanol was added. The mixture was continuously shaken with a shaker (Heidolph ProMax 2020, Schwabach, Germany) at room temperature for 24 h. The extract was filtered to remove particles using Whatson filter paper and then concentrated in a rotary evaporator (IKA-Werke, Staufen-Germany) under reduced pressure at 40°C. The residue was divided into two parts. The first part was dissolved in methanol and used for antioxidant tests, and the second was used for phenolic analysis in HPLC.

### Extraction Procedure for Phenolic Analysis

The second methanolic residue was dissolved in 10 mL distilled water at pH 2 and stirred (Heidolph shaker ProMax 2020, GmbH Co, KG) for 1h. The mixture was extracted with 3 mL of diethyl ether at least three times, stirred for 15 min and then shaken. The upper phase was placed into a 50 mL flask and extracted three times with 3 mL of ethyl acetate for 15 min. [13]. The upper phase, consisting of organic solvent, was evaporated in a rotary evaporator under reduced pressure at 40°C. The residue was weighed and dissolved in methanol for HPLC analysis for identification of phenolic compounds.

### Analysis of Phenolic Compounds by HPLC

HPLC analyses of phenolic profiles were carried out on Elite LaChrom Hitachi, Japan HPLC with UV-Vis detector. C18 column (150 mm x4.6 mm, 5 $\mu m$ ; Fortis) was used. Gradient elution was used for HPLC analyses modifying the method developed by De Villiers [14]. The mobile phase consisted of (A) 2% acetic acid in water and (B) acetonitrile:

water (70:30). The samples injection volume was 25  $\mu\text{L}$ , column temperature at 30°C and flow rate at 0.75 mL/min. The fourteen standards were recorded at 280 nm. The programmed solvent used began with a linear gradient held at 95% A for three minutes, decreasing to 80% A at 10 min, 60% A at 20 min, 20% A at 30 min and finally 95% A at 50 min. [3].

#### Determination of Total Phenolic Content (TPC)

Total phenolic contents of the methanolic extracts were determined following the Folin-Ciocalteu method using gallic acid as standard [15]. The Folin assay based on all phenolic contents including phenolic acids, flavonoids, and anthocyanins in an aquatic solution gives a blue color complex whose maximum absorbance can be read at 760 nm. Briefly, 680  $\mu\text{L}$  distilled water, 20  $\mu\text{L}$  methanolic honey extracts and 400  $\mu\text{L}$  of 0.5 N Folin-Ciocalteu reagents were mixed in a tube and vortexed for 2 min. Next, 400  $\mu\text{L}$   $\text{Na}_2\text{CO}_3$  (10%) was added and incubated for 2 h at room temperature. The absorbance was measured at 760 nm at the end of the incubation period. All measurements were performed in triplicate. TPC was expressed as mg of gallic acid equivalents per 100 g honey samples.

#### Determination of Total Antioxidant Capacity

The reducing ability of ferric tripyridyltriazine (Fe-III-TPTZ) complex was used for total antioxidant capacity assay [16]. Working FRAP reagent was prepared as required by mixing 25 mL of 300 mM acetate buffer, pH 3.6, with 2.5 mL of 10 mM TPTZ solution in 40 mM HCl and 2.5 mL 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution. Next, 3 mL freshly prepared FRAP reagent and 100  $\mu\text{L}$  of the samples was mixed and incubated in 4 min at 37°C and the absorbance was read at 593 nm against reagent blank containing distilled water. Trolox was used a positive control to construct a reference curve (62.5-1000  $\mu\text{M}$ ), FRAP values were expressed as  $\mu\text{M}$  Trolox equivalent of g.

#### Determination of Free Radical Scavenging Activity

The scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was used to determine the radical scavenging activity of the methanolic honey samples. The colorimetric test was assayed us-

ing the Molyneux method [17]. DPPH radical has a purple color which decays in the presence of antioxidant agents. The change in absorbance is monitored at 517 nm. For each sample, 0.75 mL of methanol extract solution was mixed with 0.75 mL of 0.1 mM DPPH (dissolved in methanol), vortexed and incubated for 50 min in the dark at room temperature. The blank contained DPPH solution with no sample. The results were expressed as  $\text{SC}_{50}$  (mg/mL), which was calculated from the curves by plotting absorbance values, with the  $\text{SC}_{50}$  values representing the concentration of extract (mg/mL) required to inhibit 50% of the radicals.

## RESULTS and DISCUSSION

The value of  $\text{d}^{13}\text{C}$  of the pure honeys and the adulterated honeys with sugar syrup are summarized in Table 2. This method is based on the  $^{13}\text{C}/^{12}\text{C}$  ratio of carbon atoms inside  $\text{CO}_2$  gas resulting from total combustion of pure honey and protein solution obtained from that honey, using a mass spectrometer. The percentage C4 sugar level is calculated from that value.  $\text{d}^{13}\text{C}\%$  isotope values of the pure honey and the adulterated honey were found below -25.00. According to the Honey Codes [6] the difference of  $\delta^{13}\text{C}$  between protein and raw honey must be -1 or more positive in blossom honeys and the level of C4 sugars calculated from the values  $\delta^{13}\text{C}$  of protein and raw honey must not exceed 7%.

This analytical technique especially used to identify adulteration in honeys produced by the external addition of C4 sugars or obtained from bees fed on C4 sugar.  $\delta^{13}\text{C}$  analysis is an isotopic technique relies on differences in isotope levels between C3 and C4, naturally present in plants due to photosynthesis [1,2,6,18] White and Anklam [5] reported limits for adulterated honeys (corn or sugar cane) of 21.5% and 23.5%. According to Padovanet al. (2007), the  $^{13}\text{C}/^{12}\text{C}$  ratio or  $\delta^{13}\text{C}$  honey values in the C3 cycle range between -22% and -33%, while those in the C4 cycle range between 10% and 20%. In agreement with the findings in our study, another study reported that this isotope determination technique is insufficient to reveal adulteration in honeys performed with sugar beet [1,2].

The physical characteristics of the honeys are summarized in Table 2. pH values of the honey

**Table 2.** Physical and chemical properties of honey samples.

Samples	PureHoney	HFCS-55	Sucrose Syrup	Invert Sugar Syrup	
Physical Parameters					
$\delta^{13}\text{C}_{\text{bal}}$ (%)	- 25.49	- 25.27	- 25.43	- 25.17	
$\delta^{12}\text{C}_{\text{protein}}$ (%)	- 25.26	- 25.40	- 25.77	- 23.93	
$\Delta\delta^{13}\text{C}(\text{protein-honey})$	0.23	- 0.13	- 0.34	1.24	
C4 (%)	- 1.48	0.83	2.12	- 8.71	
pH	3.82±0.01	4.05±0.02	3.86±0.01	3.95±0.03	
Moisture(%)	16.6±0.20 <sup>a</sup>	15.4±0.25 <sup>a</sup>	16.8±0.18 <sup>a</sup>	15.6±0.30 <sup>a</sup>	
Ash(%)	0.12±0.03 <sup>a</sup>	0.03±0.01 <sup>b</sup>	0.08±0.02 <sup>c</sup>	0.07±0.02 <sup>c</sup>	
Conductivity (mS/cm)	0.29±0.01 <sup>a</sup>	0.15±0.01 <sup>b</sup>	0.20±0.01 <sup>a</sup>	0.16±0.00 <sup>b</sup>	
Optic rotation	-2.03±0.07 <sup>a</sup>	0.61±0.02 <sup>b</sup>	1.52±0.03 <sup>c</sup>	1.00±0.02 <sup>d</sup>	
Colour	L*	54.53±5.20 <sup>a</sup>	66.95±3.50 <sup>b</sup>	86.65±6.21 <sup>c</sup>	68.98±2.05 <sup>d</sup>
	a*	1.65±0.50 <sup>a</sup>	4.27±0.62 <sup>b</sup>	5.53±0.39 <sup>c</sup>	8.82±0.72 <sup>d</sup>
	b*	31.56±2.10 <sup>a</sup>	14.99±3.00 <sup>b</sup>	13.15±1.56 <sup>b</sup>	24.27±3.60 <sup>c</sup>

Means ±standard deviations; Different letters (a-d) in the same lines are significantly different at the 5% level ( $p < 0.05$ ).

samples ranged from 3.82 to 4.05. No significant difference was determined between the pH of pure and adulterated honeys in this study. Due to the presence of the various amino acids and organic acids contained in honey, its pH value is generally shows acidic character, between 3.5 and 4.5 [5,11,19]. Gluconic acid at a major level in honey samples, and succinic, citric, malic, formic, oxalic and butyric acids, were present a minor levels. Amino acid of proline is present in high amount and but other nineteen are also lower in honey. Due to their weak acidic and weak basic characteristics, both organic acids and amino acids provide honey buffering at acidic pH [5,20]. Honeys' moisture values ranged between 15.4 and 16.8, and the differences were not significant. The moisture content of both the pure and adulterated honeys being below 20% reveals compatibility with the honey codices. The moisture content of honey depends mostly on the type of production associated with degree of maturity, and to a lesser extent on flora and climatic conditions [5,21-23]. Honeys harvested before full maturation have high moisture content, leading to honey fermentation. Some yeasts (osmophilic yeasts) are able to remain alive in honeys containing a high moisture level, and these cause the honey to

spoil. In contrast, the moisture content of mature honey is too low to permit any micro-organism to develop [7].

The total ash content of pure and adulterated honeys ranged between 0.03% and 0.12% Table 2. The pure honey had the highest ash content, while the adulterated honey produced with HFCS had the lowest level. Similar levels were detected in honeys involving feeding with sucrose and invert sugars. Honey's ash content varies in accordance with the flora used by the bees [24,25]. However, honeys with a high mineral content have also been reported to contain a high percentage of ash. In addition, a positive correlation has been reported between the ash or mineral content of honey and its color, and dark honeys are generally reported to have higher ash contents [8,21,22]. Dark-colored honeys with high ash contents are generally reported to taste bitter [2,20]. Ash levels of between 0.02% and 0.18% have been reported in Argentinean honeys [24] and of between 0.02% and 0.28% in floral honeys [21]. The values from our study are compatible with those in the literature and the honey food codex [21,24,25].

Electrical conductivity, another of the physical characteristics of honey, ranged between 0.15 mS/cm and 0.29 mS/cm Table 2. The pure honey had greater conductivity than the adulterated honeys ( $p < 0.05$ ). The honeys adulterated with sucrose syrup had higher conductivity than the two other adulterated honeys. The conductivity of honey is directly proportional to its mineral level and associated ash content. A positive correlation was determined between honey's ash levels and conductivity ( $R^2$ : 0.86,  $p < 0.05$ ). One study involving Portuguese organic honeys reported that electrical conductivity varied between 0.09 mS/cm and 0.43 mS/cm, with a mean conductivity value of 0.26 mS/cm [26]. Electrical conductivity between 0.414 mS/cm and 1.748 mS/cm has been reported in pine honeys [27]. On the basis of the values obtained in this study, conductivity is not a sufficient parameter in the detection of adulterated honeys.

Honeys' optical rotation angles range between -2.03 and 0.61. In this study, the pure honeys and those obtained from bees fed on sucrose syrup and invert sugar syrup had a negative angle of optical rotation, while honey from bees fed with HFCS sugar syrup had a positive angle (Table 2). Blossom honeys generally have negative optical rotation (or levorotatory) values while honeydew honeys have positive (or dextrorotatory) rotation values. This property has been used to distinguish honeydew honeys and adulterated honeys [8,28,29]. Although a positive optical rotation angle is a distinguishing parameter for honeydew honeys, it is interesting that the adulterated honey with HFCS syrup also had positive values. The positive rotation in blossom honeys may be regarded as an important parameter revealing adulteration. However, this applies to HFCS sugar syrups, while sucrose and invert sugar syrup have negative values. Therefore, while optical rotation value is an important factor showing adulteration, but the finding is not sufficient to confirm adulteration.

Honeys' color values were determined as Hunter  $L^*a^*b^*$ , and the values are shown in Table 2 [6]. Tristimulus color values for honeys were expressed as  $L^*$  for darkness/lightness (0 black, 100 white),  $a^*$  (-a greenness, +a redness), and  $b^*$  (-b blueness, +b yellowness). Honey's L values ranged from L 54.53 to 86.65,

with a low L value expressing darkness. The darkest honey was the pure honey, while the color was lighter in the adulterated honeys. The lightest-color was obtained in the sucrose syrup adulterated honey. In the same way as L values, the adulterated honey's  $a$  and  $b$  values also differ from those of the pure honey. Honey color is an important physical parameter and varies depending on the flora involved and on associated vitamin, pigment, phenolic substance and mineral contents. Chlorophyll, carotene and xanthophyll's type compounds bestow a yellow-green color [20]. In addition, Maillard products emerging with exposure to heat treatment also cause honey to darken. Since the colors of the honeys studied were measured immediately without exposure to heat treatment Maillard reaction products are not thought to affect color [25].

One study employing the same color measurement techniques in floral honeys reported  $L^*$  values between 43.02 and 81.21,  $a^*$  values between 2.91 and 63.40 and  $b^*$  values between 25.44 and 43.47 [30]. Slovenian honeys have been reported to exhibit  $L^*$  values between 42.12 and 64.60,  $a^*$  values between -3.41 and 10.14 and  $b^*$  values between 17.95 and 4.45 [31]. The values from this study for the pure honey are in agreement with those in the literature, but color parameters singly cannot be used to determine honey adulteration.

In the Table 2 are given the varying proline, diastase activity and sugar parameters in the studied honeys. Proline levels were widely ranged, between 269.79 mg/kg and 1132.45 mg/kg. The highest proline value was determined in the pure honey and the lowest in the adulterated honey with different sugar syrups. Although honey contains all amino acids, proline is interestingly present in the highest quantities. While proline level is a marker of honey purity, it also reflects the work performance of the bees [32]. Experimental studies have reported that honeys produced by bees fed on sugar syrup contain low levels of proline [8]. According to the European honey index, the amount of proline that should be present is below 150 mg/kg in honey, but the value is 300 mg/kg according to the Turkish Honey Codex (TSE). The level in quality honeys, however, is very much higher, and the honey codices are in

need of revision [6]. Proline values in the honeys produced by bees fed on sugar syrups were below 300 mg/kg, and also the proline levels in the honeys codices should be revised.

Diastase activities of the honey samples were also showed a widely ranged between 7.3 and 20.4. The pure honey also had the highest diastase activity, and HFCS sugar syrup and invert sugar syrup was the lowest. Diastase is a digestive enzyme that performs starch hydrolysis and is responsible for the freshness and quality of honey. A level below 8 DU is undesirable in honey codices TSE [6]. Diastase activity in honeys decreases over time or with exposure to heat treatment. Low diastase activity in these honeys, not exposed to any heat treatment, being low in HFCS and invert sugar syrup honeys indicates adulteration. These data indicate that diastase activity can be a differentiating marker in adulterated honeys made with sugar syrups, with the exception of sucrose syrup.

Honeys' fructose, glucose and sucrose contents were determined using HPLC-RID. Sucrose contents of the pure honeys and those adulterated with sugar syrups were below 5%, in line with the honey codex, the lowest sucrose content being in the pure honey TSE, [6]. Honey obtained from bees fed on sucrose syrup was expected to exhibit the highest sucrose level, but that was actually determined at 1.56%. The main reason for this is inversion of sucrose, a honeybee digestive enzyme, by invertase into fructose and glucose. A large part of the sucrose syrup collected by bees undergoes inversion by invertase. One study reported sucrose levels of 1.23% in adulterated honey, similar to our findings [8]. Honeys' fructose and glucose levels do not differ from those of floral honeys [19]. Sucrose values of the honeys produced by bees fed with sucrose syrup once a day being compatible with the honey codex shows the difficulty in determining adulteration in honey.

More important than physical and chemical properties, adulteration affects honey's biologically active value. This study investigated how adulteration affected the biologically active compounds and antioxidant properties of honey. Ta-

ble 3 shows the antioxidant activities associated with total polyphenol contents in the four different quality honeys studied. Honeys' total phenolic contents ranged between 6.13 and 98.00 mg GAE/100 g. The pure honey had the highest phenolic content, followed by honeys made with sucrose syrup, invert syrup and HFCS syrup. Although the total phenolic contents of honeys produced by bees fed on invert and sucrose syrups were lower than those of pure honey, they were higher than those of many pure honeys in the literature [20,23]. This shows that while being fed on sugar syrup, bees also bring nectar and pollen into the hive from outside. Total phenolic contents show that when bees were fed with HFCS nectar, transportation from outside was reduced to a minimum, while they did not completely cease bringing in pollen and nectar in the other sugar groups.

Honeys' antioxidant capacities were assayed using two different tests apart from total phenolic content. Both antioxidant techniques (FRAP and DPPH) are based on electron transfer and are the most commonly used methods of determining antioxidant capacity [22,20,4]. Honeys' FRAP values ranged between 105 and 285  $\mu\text{molTrolox}/100\text{ g}$  (Table3). In this method, a high FRAP value indicates high antioxidant capacity, and the antioxidant capacity of the pure honey was approximately 2.5 times greater than that of the other honeys adulterated with sugar syrups. The FRAP values of the adulterated honeys with sucrose syrup was lower than that of the pure honeys but also the values were higher than those of the other two groups. One study reported the highest FRAP values in forest honey at 498  $\mu\text{molTE}/100\text{g}$ , and values of 405  $\mu\text{molTE}/100\text{g}$  in honeydew honey, 67  $\mu\text{molTE}/100\text{ g}$  in wild flowers honey, 61  $\mu\text{molTE}/100\text{g}$  in linden honey, 26  $\mu\text{molTE}/100\text{g}$  in acacia honey and 4.00  $\mu\text{molTE}/100\text{g}$  in adulterated honey [33]. Another study of blossom and adulterated honeys reported FRAP values ranged from 27.0  $\mu\text{molTE}/100\text{ g}$  to 96.6  $\mu\text{molTE}/100\text{ g}$ , but was measured 6.1  $\mu\text{molTE}/100\text{ g}$  in false honey [34]. The results are proved that adulteration with some sugar syrups increase antioxidant capacity of honey.

**Table 3.** Antioxidant parameters and phenolic compounds of honey samples.

Samples	Pure Honey	HFCS-55	Sucrose Syrup	Invert Sugar Syrup
Antioxidant Parameters*				
Total Polyphenol (mg GA/100 g)	98.00±2.00 <sup>a</sup>	6.13±0.30 <sup>b</sup>	53.30±3.00 <sup>c</sup>	34.60±1.00 <sup>d</sup>
FRAP (µmolTrolox/100 g)	284.10±18.00 <sup>a</sup>	105.00±15.20 <sup>b</sup>	183.70±8.10 <sup>c</sup>	106.40±12.00 <sup>b</sup>
DPPH SC50 (mg/mL)	13.70±0.11 <sup>a</sup>	82.36±0.70 <sup>b</sup>	33.63±0.12 <sup>c</sup>	64.57±0.43 <sup>d</sup>
Phenolic Contents (µg phenolics/g honey sample)*				
Gallicacid	1.84	1.20	0.91	0.82
Protocatechuic acid	Nd	0.52	n.d	0.52
<i>p</i> -OH benzoic acid	0.68	0.53	n.d	0.50
Vanillic acid	0.98	0.81	0.85	0.816
Caffeic acid	1.50	1.24	1.34	1.20
Syringic acid	0.69	n.d	n.d	0.60
<i>p</i> -Coumaric acid	0.60	0.55	0.59	0.55
Sinamik acid	0.57	0.55	0.63	0.52
Ferulic acid	0.96	0.81	0.84	0.80
Rutin	2.93	0.61	1.44	0.53
Catechin	2.35	1.71	1.41	1.51
Epicatechin	6.39	n.d	4.68	n.d
Luteolin	n.d	n.d	n.d	n.d

Means ±standard deviations; Different letters (a-d) in the same lines are significantly different at the 5% level ( $p < 0.05$ ) \* The experiments were performed in triplicate. n.d: Not Detected.

Honeys' free radical scavenging capacities were tested using commercial DPPH assay and values were expressed as  $SC_{50}$ . A high DPPH value indicates low radical scavenging ability. The lowest  $SC_{50}$  value was in pure honey, followed by sucrose syrup (Table 3). As with FRAP activity, the lowest DPPH scavenging property was determined in HFCS honey.

Honeys' phenolic compounds were analyzed using HPLC-UV, and the resulting data are summarized in Table 3. Thirteen different standards (phenolic compounds) were used in the analysis. While lutein was beneath detectable limits in all specimens from all honeys, the other standards were detected in honeys at varying levels. With the exception of protocatechuic acid and lutein, all the standard phenolic compounds investigated were present at higher concentrations in the

pure honey compared to the adulterated honeys. No significant findings that might reveal adulteration on the basis of phenolic compounds were identified, but adulteration with sugar syrup was observed to lead to a decrease or complete disappearance of phenolic compounds in honey. This increase was also cause a decrease in antioxidant capacity as well as other biological effects on the human such as anti-microbial, anti-viral, anti-inflammatory, and anti-tumoral activities of the adulterated honeys. In conclusion, adulterated honeys will have lower antioxidant capacities than those of pure honeys.

In the differentiation of adulteration in honeys produced by bees fed on various sugar honeys,  $\delta^{13}C$  isotope and  $C_4\%$  values are differentiating parameters for sugar cane honeys. The positive optical rotation angles of the adulterated honeys

produced by bees fed on high fructose content syrup emerged as a significant distinguishing parameter in this study. Diastase activity was observed to play an important role in the quality of honey, with the exception of freshness. Proline values are an important parameter in determining the purity of honey, and better quality honeys can be obtained by raising the proline values in the honey codices. Adulterated honeys produced with sucrose are closer to the pure honeys in terms of both physicochemical properties and other biological characteristics. Total phenolic content also emerged as an important marker in the detection of honey adulteration and as a value requiring measurement in classic honey analyses.

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#### CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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