Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 1119–1125
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Determination of important biochemical properties of honey to discriminate pure and adulterated honey with sucrose (*Saccharum officinarum* L.) syrup

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Received 2 September 2006; received in revised form 13 February 2007; accepted 15 February 2007

Abstract

The aims of the present study were to determine biochemical properties of honey samples and to discriminate pure and adulterated honey produced by the standard bee feeding method (control honey), the shaking method (pure blossom honey), and overfeeding (100 kg/colony syrup) with sucrose syrup (adulterated honey). The biochemical properties evaluated were moisture, ash, acidity, hydroxymethylfurfural (HMF), specific sugars (i.e. fructose, glucose, fructose–glucose, sucrose, and maltose), diastase activity, $\delta^{13}C$ value (honey), $\delta^{13}C$ value (protein), electrical conductivity, potassium, vitamin C, and proline. Fifteen honey samples were analyzed by discriminant analysis stepwise method. Proline, electrical conductivity and sucrose were found as discriminative characters of samples. Based on these three properties 100% of original group cases (samples) correctly classified in their real group. We found that the honey produced by feeding with 100 kg sucrose syrup per colony contained the sucrose as low as pure blossom honey. Therefore, the sugar (sucrose, fructose and glucose) content of honey cannot be used to distinguish between adulterated (sucrose syrup) and pure blossom honey.
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Keywords: Honey; Pure; Feeding; Sucrose; Biochemical properties; Discriminant analysis

1. Introduction

The quality and biochemical properties of honey are related to honey maturity, production methods, climatic conditions, processing and storage conditions, as well as the nectar source of the honey (Bogdanov, 1999; Crane, 1979; Oddo & Bogdanov, 2004; White, 1978). However, quality and composition of honey are negatively affected by the other factors such as overfeeding with sucrose and other sucrose variants, harvesting prior to maturity, unhealthy storage conditions, and overused veterinary drugs (Bakan, 2002; Bogdanov et al., 2000; Oddo & Bogdanov, 2004; Sahinler, Sahinler, & Gul, 2004).

To recover more honey from hives, overfeeding of bees with sugar (Basoglu, Sorkun, Loker, Dogan, & Wetherilt, 1996; Sorkun et al., 2002; White, 1979) and other types of sucrose (Kerkvliet & Meijer, 2000) may be practiced. However, these approaches negatively affect the proline content (Basoglu et al., 1996; White, 1979), sugar content (Bogdanov et al., 2005; White, 1978), and mineral content (Ozcan, Arslan, & Ceylan, 2006; Rashed & Soltan, 2004; Sorkun et al., 2002) of honey. Another important issue is that worker bees convert disaccharide (sucrose) to monosaccharide (glucose and fructose) (Crane, 1979; White, 1978; Winston, 1987). However, it is not known how much sucrose is converted

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0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.02.024

into monosaccharide by worker bees. Nonetheless, some properties of honey can be evaluated to distinguish between adulterated honey with sugar and pure honey (Bogdanov et al., 2000; Codex Alimentarius, 2001; White & Winters, 1989). While Wetherilt, Basoglu, and Pala (1993), White (1978), and Basoglu et al. (1996) found that proline, potassium, and sodium contents of honey can be used to distinguish pure honey samples from the others (sucrose), Sorkun et al. (2002) showed that sucrose, proline, and mineral contents have been valuable for separating different types honey samples from one another. Further, Silici (2004) determined that sucrose and invert sugar are important parameters for the separation of honey samples. Also many researchers studied honey samples adulterated with different sugars (cane or corn) by carbon stable isotopic ratio analysis (SIRA) method (Martin, Macias, Sanchez, & Rivera, 1998; Padovan, De Jong, Rodrigues, & Marchini, 2003; White, Winters, Martin, & Rossmann, 1998). Most of these studies were carried out with honey samples found on the open market. But what amount and which types of sugar were used for feeding bees produced these honeys is not known exactly. Nevertheless, these studies showed that some chemical properties could be used to distinguish between honey samples. According to Anklam (1998), SIRA method is useful to discriminate C_4 sugars (cane and corn) instead of C_3 sugar (beet sugar). In addition the Commission of the EU is encouraging the development of harmonized analytical methods to permit the verification of compliance with the quality specification for the different honeys. So, some additional studies are needed to determine the effects of feeding with sucrose at varying quantities on the biochemical properties of honey produced during the main honey-producing season.

The aims of this study were to determine the biochemical properties of honey samples produced by three different methods and to be able to make use of these properties to distinguish between the adulterated (sucrose syrup) and pure blossom honey samples.

2. Materials and methods

2.1. Materials

This study was carried out at the Research and Application Farm of the Agriculture Faculty of Ondokuzmayıs University. Honey samples were taken from colonies on this farm. Colonies were retained in Samsun (41.2°N, 36.20°E) in the Black Sea Region during the winter and spring, and in the vicinity of Karagoller, near Gumushane (40.274°N, 39.29°E), during the nectar flow period (June, July and August). The Karagoller area is very rich in plant species. The main plant resources are thyme (*Satureja thybra* L.), white dead nettle (*Lamium album*), clover (*Trifilium ambiguum*), sage (*Salvia forskahler* L.), and tragacanth (*Astragalus microcehalus*) (Baytop, 1994).

2.2. Methods

2.2.1. Honey samples

For this experimental study, honey samples were produced from colonies with different feeding methods. These methods were standard bee feeding (control honey), the shaking method (pure honey) used for the first time in this study to produce pure blossom honey that will be a good example for standard, and bee feeding with dense sucrose syrup (adulterated honey).

2.2.1.1. Control honey. For the control honey sample generation, the colonies were settled in empty beehives with bees, brood and honey frames. Standard bee feeding methods were applied (Johansson & Johansson, 1978; Sammatora & Avitabile, 1998). Sixteen kilograms of syrup (1:1.5 w/w, water:sugar) was given to each colony in March and April to ensure the growth and strength the worker bee population for main nectar flow season. After bees settled in the hives, cake and syrup were not further provided to the colonies. Foundation comb was given to the colonies when needed.

2.2.1.2. Pure blossom honey. Pure honey was produced by the shaking method. Feeding, development, and maintenance of bee colonies were conducted up to a predetermined stage in the spring season, and after that point, queen and worker bees together were shaken to the empty hives. Only frames with attached wire mesh screen were provided to the hives in this group. Approximately 1 cm width honeycomb from pure beeswax was adhered to the upper part of the frame. After this process, no syrup, cake or comb foundations were given to the colonies of this group.

2.2.1.3. Adulterated honey with sucrose syrup. For adulterated honey, colonies (including both adult and young bees) were moved into empty hives along with honey. After this step, a total of 100 kg sucrose syrup composed of 1:1.5 (water:sugar, w:w) ratio was given to each colony during June and July 2004. Syrup was prepared every two days as given ratio. Cake was not given these colonies, but foundation honeycomb was given when needed.

Transporting and shaking processes were done with unpainted hives, and veterinary drugs were not used for any honey bee diseases. The processes were applied to all groups on the same day. After a week, colonies were transported to (470 km) another region in order to obtain rich and unpolluted nectar sources. Honey was harvested at the end of August 2004 by centrifuge and filtered with a 0.2 mm sieve into to lactin. After being retained in a resting tank for 10 days, the honey was collected in glass jars.

Honey samples were taken from five colonies, which were chosen randomly from among all available groups,

and total of 15 samples ($3 \times 5 = 15$) analyzed using the following analytical methods.

2.2.2. Analytical methods

All honey samples were analyzed using the same methods during the same time period to ensure uniform conditions and comparability. The following compositional properties were determined for control honey, pure blossom honey, and adulterated (sucrose) honey: moisture, ash, free acidity, hydroxymethyfurfural (HMF), diastase number, proline, electrical conductivity, $\delta^{13}C$ value (honey), $\delta^{13}C$ value (protein), fructose, glucose, sucrose, maltose, vitamin C, and potassium.

Moisture was measured at 20 °C by Abbe refractometer (Digital refractometer Atoga, Germany) by refractive methods AOAC (1998). Method 969.38B Fructose, glucose, maltose, and sucrose were identified and determined by high performance liquid chromatography (HPLC) according to DIN 10758 (1997). Hydroxymethylfurfural (HMF) was determined spectrophotometrically as outlined by Harmonization methods of International Honey Commission (IHC) (Bogdanov et al., 2000). The diastatic activity was based on starch hydrolysis (AOAC, 1998 method 958.09) as 300/time to a value of absorbance of 0.235 at 660 nm. A weighed sample was ignited in a muffle furnace at 550 °C to a constant weight for ash determination (AOAC, 1998 method 923.03). Potassium was determined by using the Atomic absorbance spectrophotometer (AAS) according to AOAC (1998) method 985.35. Proline was determined spectrophotometrically by using ninhydrin in methyl cellosolve, and absorbance was read at 512 nm. A standard curve using pure proline was constructed according to AOAC (1998) method 979.20. After calibrating the conductimeter, the electrical conductivity of each honey solution at 20% dry matter was measured at 20 °C by Harmonised methods of the IHC (Bogdanov et al., 2000). $\delta^{13}C$ values were determined by isotope ratio mass spectrometry after complete sample combustion to carbon dioxide, as prescribed by AOAC (1998) method 991.41. Free acidity was determined photometrically by AOAC (1998) method 962.19, and vitamin C was determined by AOAC (1998) method 967.21.

2.2.3. Statistical analysis

A stepwise-selection discriminant analysis technique (Cooley & Lohnes, 1971) was performed in attempt to classify honey samples group and to investigate linear combination of the biochemical properties that were formed and serve as the basis for assigning samples into one of the groups. Thirteen properties except for $D\delta^{13}CP$ (difference in $\delta^{13}C$ value between honey and its protein), invert sugar and fructose–glucose ratio were evaluated. At the end of analysis, the most important discriminative biochemical properties were determined (SPSSx, 1986). Duncan's multiple comparison test was applied for comparison of the averages.

3. Results and discussion

The mean results and basic statistics obtained from the biochemical analyses are given in Table 1. Statistical analysis revealed that no significant differences exist between any of the honey samples based on their ash, HMF, diastase activity and fructose–glucose ratio contents ($P > 0.05$). There were statistically significant differences at varying level between each of the honey sample in relation to electrical conductivity, and proline values ($P < 0.001$); $\delta^{13}C$ value (honey), $\delta^{13}C$ value (protein), fructose, sucrose, moisture, and maltose contents ($P < 0.01$); and glucose, vitamin C, and potassium ($P < 0.05$). The highest proline content was determined in the pure blossom honey samples. In addition, control honey samples had lower beet sugar (sucrose) content than adulterated honey. Pure blossom and control honey samples had similar values for biochemical properties such as moisture, acidity, electrical conductivity, $\delta^{13}C$ value (protein), fructose content, glucose content, and potassium content; however, the values for these properties in the pure blossom and control honey samples were higher than those of adulterated honey samples. Adulterated (sucrose) honey samples contained higher sucrose and maltose contents than the other two honey produced methods. The lowest moisture content was determined in adulterated honey samples. Control honey samples contained the highest level of vitamin C, while adulterated (sucrose) honey samples contained the lowest.

Biochemical properties of all honey samples were evaluated based on the discriminant analysis stepwise method. Table 2 shows the eigen values as well as the contributions and significance of the discriminant functions.

When 13 biochemical properties were analysed by discriminant step-wise method, three properties remained in the analysis with the discrimination steps as proline, electrical conductivity and sucrose, respectively. Proline was in the first step, electrical conductivity in the second, and sucrose in the third. The most important discriminating properties were proline and electrical conductivity, which are effective on the first discriminant function, while sucrose is effective on the second discriminant function. The first discriminant function was described 87% of the total variance with regard to the biochemical properties of honey samples, while the second discriminant function explained 13% of the total variance (Table 2).

Table 3 shows that Fisher's linear discriminant classification functions were related to proline, electrical conductivity, and sucrose, as they are properties that demonstrate differences between the honey samples types and determine their positions in the coordinate system. Based on the proline, electrical conductivity, and sucrose properties, 100% of original grouped cases (samples) correctly classified, and as shown in Fig. 1, no overlapping occurred between the samples of represent any group. Nonetheless, honey samples of overfeeding groups (sucrose) scattered in the narrow area when compared to other two groups samples

Table 1
Mean and standard error for biochemical contents of honey samples produced as control, pure, or sucrose feeding

Properties	Unit	Honey		
		Control	Pure	Adulterated
Moisture	g per 100 g	$16.10 \pm 0.12a^{**}$	$16.40 \pm 0.01a$	$15.57 \pm 0.09b$
Ash	g per 100 g	0.059 ± 0.009	0.052 ± 0.006	0.039 ± 0.004 NS
Acidity	meq kg^{-1}	$27.84 \pm 2.32a^{*}$	$28.58 \pm 0.89a$	$24.46 \pm 0.51b$
HMF	mg kg^{-1}	6.27 ± 0.13	6.18 ± 0.27	6.91 ± 0.51 NS
Diastase activity	SScale	16.50 ± 0.28	18.12 ± 1.04	17.08 ± 0.56 NS
Proline	mg 100 g^{-1}	$50.16 \pm 0.47b^{**}$	$63.00 \pm 1.92a$	$41.64 \pm 0.88c$
EC	mS cm^{-1}	$0.224 \pm 0.007a^{**}$	$0.230 \pm 0.001a$	$0.176 \pm 0.000b$
δ^{13}C value (honey)	‰	$-25.58 \pm 0.054a^{*}$	$-25.20 \pm 0.027b$	$-25.72 \pm 0.072b$
δ^{13}C value (protein)	‰	$-25.34 \pm 0.077b^{*}$	$-25.39 \pm 0.102b$	$-25.74 \pm 0.026a$
Dδ^{13}CP	Difference	+0.243	−0.195	−0.040
Fructose	g 100 g^{-1}	$40.06 \pm 0.20a^{**}$	$40.22 \pm 0.13a$	$38.97 \pm 0.08b$
Glucose	g 100 g^{-1}	$33.95 \pm 0.22a^{*}$	$33.40 \pm 0.16a$	$32.48 \pm 0.53b$
Fructose–glucose	Ratio	1.182 ± 0.004	1.201 ± 0.004	1.198 ± 0.004 NS
Sucrose	g 100 g^{-1}	$3.84 \pm 0.16c^{**}$	$4.29 \pm 0.14b$	$4.75 \pm 0.05a$
IS	g 100 g^{-1}	$74.00 \pm 0.42a^{**}$	$73.62 \pm 0.18a$	$71.45 \pm 0.46b$
Maltose	g 100 g^{-1}	$2.81 \pm 0.29b^{**}$	$2.35 \pm 0.05c$	$3.60 \pm 0.12a$
Vitamin C	mg 100 g^{-1}	33.92 ± 1.92 a*	$19.29 \pm 0.53b$	$11.23 \pm 1.88c$
Potassium	mg 100 g^{-1}	$18.22 \pm 2.19a^{*}$	$16.22 \pm 0.48ab$	$12.42 \pm 0.42c$

Dδ^{13}CP, the difference in δ^{13}C value between honey and its protein; EC, electrical conductivity; IS, invert sugar (fructose + glucose). Values within rows with different letters differ significantly (* $P < 0.05$; ** $P < 0.01$; NS, non significant).

Table 2
The eigen values and the contributions and significance of the discriminant functions

Function number	Eigen value	Variance (%)	Cumulative (%)	Canonical correlation	Wilks' Lambda	*P*
1	47.61	87.00	87.00	0.99	0.003	0.001
2	7.14	13.00	100.00	0.93	0.123	0.001

Table 3
Fisher's linear discriminant classification functions and the related coefficients for each honey sample groups

Parameters	Honey samples			Tolerance level	F to remove	Wilks' Lambda
	Control	Pure blossom	Adulterated (sucrose)			
Proline	18.82	23.31	16.85	1.000	22.77	0.014
EC	7897.11	8372.45	6634.97	0.998	6.79	0.006
Sucrose	214.77	241.82	231.99	0.929	4.69	0.005
Constant	−1757.32	−2228.87	−1490.00			

EC, electrical conductivity.

(Fig. 1). This might be attributed to behavior of forage worker bees and their plant preferences. In other words, while the worker bees of pure and control groups colonies may be needed to collect nectar from outside of hive (from different plant species), the worker bees of sucrose fed group (adulterated) may not be needed to collect nectar from forage due to finding food as sugar syrup for their usage.

The correlations matrix of the all biochemical properties of the honey samples calculated via discriminant analysis are shown in Table 4.

While the most positively correlated characteristics were identified as potassium and electrical conductivity ($r = 0.754$). Glucose ($r = 0.635$), potassium ($r = 0.616$), and electrical conductivity ($r = 0.551$) were also highly significantly correlated with water. Vitamin C was also correlated with fructose at a high level of significance ($r = 0.534$). The most negatively correlated factors were sucrose and acidity ($r = -0.731$). Maltose was also negatively correlated at a high level of significance with potassium ($r = -0.585$), water ($r = -0.563$), and glucose ($r = -0.534$).

This study indicated that the most important biochemical parameters for discriminating honey samples are proline, electrical conductivity, and sucrose. These properties were evaluated elaborately below.

3.1. Proline

Proline, an amino acid in honey, was identified as the most important biochemical property at the end of the discriminant analysis stepwise method. It was determined that this distinction is resulting from its relation with the

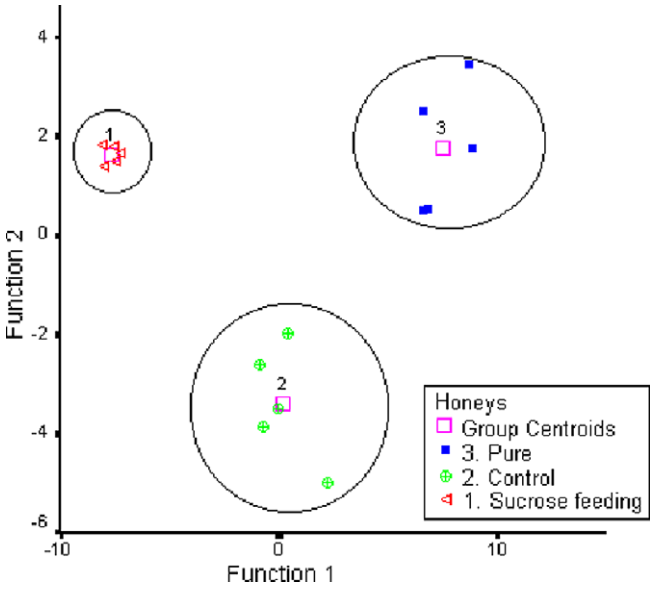

Fig. 1. Discriminant analysis of honey samples produced by different methods. Horizontal axis: canonical function 1, vertical axis: canonical function 2. Each number symbolizes the center of one honey sample group, and each point represents an individual honey sample.

first discriminate function. The first discriminant function defined 87% of the differences between honey samples based on 13 biochemical properties. The control group contained 10 mg per 100 g more proline than adulterated (sucrose) honey sample. In addition, pure blossom honey groups had 22 mg per 100 g more proline than adulterated (sucrose) honey sample. The importance of proline has been emphasized to discriminate natural and artificial honey samples in previous studies (Basoglu et al., 1996; Sorkun et al., 2002; White, 1979). Sorkun et al. (2002) determined the proline content of natural honey produced in Turkey as 59.80 mg per 100 g honey, which is similar with the result of present study. However, they also determined the proline content of artificial (beet sugar) honey as 30 mg per 100 g honey, which is lower than the values obtained in our study (41.64 mg per 100 g). These differences might be attributed to beekeeping conditions (feeding with more syrup), plant species and environmental factors (White, 1979). Oddo et al. (2004) and Piazza and Oddo (2004) investigated 6719 honey samples from 21 countries of European geography area and found that proline content of the samples ranged from 222 (acacia) to 956 (thyme) mg/kg depending on their plant species.

The proline content of honey changes according to its floral type (Oddo et al., 2004), and also worker bees add proline to honey (White, 1979). Proline is the second important quality parameters of the honey in Codex Alimentarius and the reference value for proline is given as 18 mg per 100 g honey (Bogdanov et al., 2000). Our study showed that 100 g pure honey contained 63 mg proline, while the same amount of adulterated honey with sucrose syrup had 41 mg proline. This study indicated that standard value for proline suggested by Codex Alimentarius is very low; therefore we suggest that standard value for proline should be examined with more honey samples.

3.2. Electrical conductivity (EC)

Electrical conductivity was the second most important parameter after proline with regard to discriminate honey samples. In Codex Alimentarius the minimum electrical conductivity value of pure floral honey is given as 0.8 mS cm^{-1} (Bogdanov et al., 2000). Electrical conductivity values of all the samples investigated in this study are in agreement with the value reported in Codex Alimentarius. However, adulterated (sucrose) honey samples had a lower EC value (0.176 mS cm^{-1}) than pure blossom honey and control honey samples (0.230 ± 0.001 mS cm^{-1} and 0.224 ± 0.007 mS cm^{-1}, respectively). For these circumstances, EC should be considered determining the floral source of honey instead of the honey quality. This is recommended because sugar used for preparing adulterated honey samples is produced by sucrose syrup and is the only floral-related source. Furthermore, the other honey sample types are originated from nectar that is collected from many more flower species. In this case it was shown as the most useful quality parameter for classification of unifloral honeys by Bogdanov, Ruoff, and Oddo (2004).

The EC of honey had a strong positive correlation with potassium ($r = 0.754$). Potassium and EC contents of pure blossom and control honey samples were higher than those of the adulterated (sucrose) honey samples. The study showed that when the potassium content increases in honey, the EC content also increases.

Table 4
Correlation coefficients among characteristics of honeys produced as control, pure blossom, and sucrose syrup feeding

Parameters	K	Glucose	EC	Vit. C	Maltose	Sucrose	Acidity	Water	Fructose
K	1	0.354	0.754	0.282	−0.585	−0.014	−0.017	0.616	0.179
Glucose	0.354	1	0.327	0.056	−0.534	0.175	−0.110	0.635	−0.082
EC	0.754	0.327	1	0.550	−0.408	−0.177	−0.106	0.551	0.436
Vit. C	0.282	0.056	0.550	1	−0.130	−0.176	−0.393	0.207	0.534
Maltose	−0.585	−0.534	−0.408	−0.130	1	−0.268	0.156	−0.563	−0.236
Sucrose	−0.014	0.175	−0.177	−0.176	−0.268	1	−0.731	0.047	−0.163

K, potassium; EC, electrical conductivity.

3.3. Sucrose

Sucrose was the third most important parameter following proline and EC for discriminating honey samples. It was found that the sucrose content of honey was not an effective property for distinguishing pure blossom honey from adulterated (sucrose) honey. Although there was significant difference between the sucrose content of pure blossom and adulterated honey produced by feeding with sucrose syrup (100 kg), this difference was not very high. Because while 100 g of honey produced by over feeding with 100 kg syrup contained 4.75 ± 0.05 g of sucrose, the sucrose content of pure blossom honey (no feeding with sucrose) was 4.29 ± 0.14 g. This result was further confirmed with respect to the total invert sugar content of honey samples. While pure blossom honey contained 73.62 g of total invert sugar (fructose + glucose), the artificial honey (produced by over feeding sucrose syrup) had 71.45 g of total invert sugar. It seems that pure blossom honey and adulterated honey (sucrose) samples had nearly the same amount of total invert sugar. The sucrose content in honey samples produced without feeding with sugar syrup may be attributed to their nectar origin (White, 1979). Codex Alimentarius (2001) and many other standards (IHC, and Turkish Food Codex, 2000) accept the maximum sucrose amount in honey as 5% (Bogdanov et al., 2000). In this study, honey produced by overfeeding with sugar (sucrose) syrup contained less than this standard maximum value. This result might be explained that sucrose was inverted to fructose and glucose by an invertase enzyme, which was secreted from the hypopharyngeal glands of worker bees (Bogdanov, 1999; Crane, 1979; White, 1978; Winston, 1987). When the findings of the preceding researchers and the results of this study are evaluated together, it appears that worker bees convert nearly all available sucrose to fructose and glucose (i.e. 95% conversion). This result indicates that sucrose is not a reliable indicator for distinguishing pure honey from adulterated (sucrose syrup) honey, as being supported by White (1979) and Bogdanov et al. (2005). Bogdanov (1999) provided a more complex explanation for this situation. Nonetheless, more study is needed to be able to fully explain the sucrose syrup correlation with the honey sugar contents (fructose and glucose) and their relation to worker bees. The sucrose content of honey produced by feeding with sucrose syrup in this study was found lower than the values published in other literature (Basoglu et al., 1996; Silici, 2004; and Sorkun et al., 2002). Differences might be resulted from differences in feeding and producing methods used.

A negative correlation between sucrose and acidity values has been identified. When sucrose increases, the acidity value decreases. In fact, pure honey and control honey samples, which have lower sucrose content, actually have a higher acidity value. The pure and control honey samples had higher acidity than the adulterated honey samples. This result is in agreement with the result of Wetherilt et al. (1993).

3.4. Other biochemical properties

$\delta^{13}C$ values were determined in correlation with a honey and its protein fraction by elemental analyzer with isotopic ratio mass spectrometer (IRMS). The protein value can be used as an internal standard. The value below -1 indicates the addition of C_4 plant sugars (Anklam, 1998; White & Winters, 1989; White et al., 1998). The limit for the detection of adulteration by this property is 7%. $\delta^{13}C$ value of sample groups was significantly different from each other. The $\delta^{13}C$ value of control and adulterated (sucrose syrup) honey were significantly higher than those of pure honey. The $\delta^{13}C$ value of all groups samples were above -25.00. All these values were above the value of -21.5 and -23.5 reported by White (1979) and Anklam (1998) as limit (standard) values of adulterated (corn or cane) honey. Therefore, this study revealed that $\delta^{13}C$ value is not reliable method for discrimination adulterated honey obtained from feeding sucrose syrup that made of C_3 plants. This situation was also emphasized by White and Winters (1989) and Anklam (1998). For that reason, in present study groups honey samples were not evaluated based on the differentiation between the $\delta^{13}C$ value (honey) and the $\delta^{13}C$ value (protein). The difference in $\delta^{13}C$ values between honey and its protein ($D\delta^{13}CP$) were either above -1 or had positive values (Table 1).

The value of ash, HMF, diastase number, total invert sugar, glucose, fructose, EC, moisture, and proline contents of the honey samples were in agreement the standard values reported by IHC (Bogdanov et al., 2000) and Codex Alimentarius (2001). Further, it appeared that some honey samples produced in the study qualified for special quality classification based on their properties. Standard values of special quality honey in some countries such as Germany, Belgium, Spain, Italy, and Sweden are identified as the following: moisture (17.5–18.0%), invert sugar (65 g per 100 g honey), diastase number (min. 8), HMF (max. 15 mg/kg), and sucrose (max. 5 g/100 g). Our pure and control honey samples had the following values: 16.10–16.40% moisture, 73.62–74.00 g per 100 g invert sugar, 16.26–19.40 diastase number, 6.18–6.27 mg/kg HMF, and 3.84–4.29 g per 100 g sucrose, which indicated that the honey samples in the shaking and control groups were of high quality.

4. Conclusion

In present study, production methods-known honey samples were used to discriminate pure blossom honey from adulterated honey with sucrose syrup. The study indicated that adulterated honey sample could be satisfactorily discriminated from pure blossom honey sample by using the proline content and electrical conductivity. It was also determined that more than 95% of the sucrose given to bees for feeding was converted to honey sugars (fructose and glucose) and adulterated honey had sucrose content as low as pure blossom honey. Therefore, it is not possible to discriminate pure blossom honey from adulterated

(sucrose syrup) honey by sugar (sucrose, fructose and glucose) content. Additionally, $\delta^{13}C$ (honey) and $\delta^{13}C$ (protein) analysis could not be used for discrimination of pure blossom honey from the adulterated honey with sucrose syrup by elemental analyzer with isotopic ratio mass spectrometer (IRMS). The biochemical values of honey produced by shaking method might be the best example for pure blossom honey's standard.

Acknowledgements

This study was financially supported by Research Fund of Ondokuzmayis University. The authors are grateful to Semiha Guler for her contribution to the manuscript.

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