

# Comparing Biochemical Properties of Pure and Adulterated honeys Produced By Feeding Honeybees (*Apis mellifera* L.) Colonies with Different Levels of Industrial Commercial Sugars <sup>[1]</sup>

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## Abstract

In the present study, 100 pure and adulterated honey samples produced by feeding colonies with different levels (5, 20 and 100 L/colony) of various commercial industrial sugar syrups such as High Fructose Corn Syrup 85 (HFCS-85), High Fructose Corn Syrup 55 (HFCS-55), Glucose Monohydrate Sugar (GMS), Bee Feeding Syrup (BFS) and Sucrose Syrup (SS) were evaluated in terms of sugar ingredients, physicochemical, mineral matter, vitamin and enzyme contents. Proline, electrical conductivity, free acidity, vitamin, mineral and enzyme content of honey were significantly affected by sugar origin and syrup levels. Fructose and glucose content of honey were not reliable criteria for distinguishing adulterated honey obtained by overfeeding honey bees (100 L/colony syrup) with sugar having balanced monosaccharide fraction. For pure blossom honey having EC value below 0.20 mS/cm should be evaluated as indirect adulterated with commercial industrial sugars originating from both C3 and C4 plants. In addition, proline content of pure blossom honey below 300 mg/kg might be an indicator for overfeeding honey bee colonies with HFCS-85 and SS. It is clear that the important biochemical and biological degradation occurred in honey when the colonies overfed with industrial sugars syrup during the main nectar flow. The values of biochemical properties found in the present study are important references to be used for revision of national and international standards.

**Keywords:** Honey, Commercial sugars, Colony, Feeding, Adulterated, Biochemical properties

## Endüstriyel Ticari Şekerlerin Farklı Şerbet Seviyeleri İle Beslenen Bal Arısı (*Apis mellifera* L.) Kolonilerinden Üretilmiş Katkılı ve Saf Balların Biyokimyasal Özellikler Yönünden Karşılaştırılması

## Özet

Bu çalışmada, endüstriyel mısırdan üretilen ticari High Fructose Corn Syrup 85 (HFCS-85), High Fructose Corn Syrup 55 (HFCS-55) ve arı yemi (BFS) şurupları ile glukoz monohydrate (GMS) ve sukroz (SS) şekerlerinin 5, 20 ve 100 litre/koloni şerbet seviyeleri ile üretilmiş hileli ve saf (PBH) 100 bal örneğinin biyokimyasal özellikleri değerlendirilmiştir. 100 bal örneği su, fruktoz, glukoz, F/G, F+G, Glukoz/Su, maltoz, laktoz, sukroz, kül, serbest asitlik, viskozite, hydroxymethylfurfural (HMF), diyastaz, invertaz, α-glukosidaz, glukoz oksidaz, proline, elektriksel iletkenlik, Na, K, K/Na, vitamin B5 ve vitamin C yönünden karşılaştırılmıştır. Laktoz, maltoz ve glukosidaz enzimi dışındaki biyokimyasal özellikleri şeker çeşidi ve şerbet seviyesine göre önemli (P<0,001) farklılık göstermişlerdir. Arılar saf glukozmonohidrat şekerinden yararlanamamış fakat sukroz şekerini aşırı düzeyde severek tüketmişlerdir. Şeker içeriğinde monosakkarit yapıdaki fruktoz miktarı arttığında arı bunu izomerize edememiştir. Koloniye verilen şerbet miktarı arttıkça arılar daha fazla enzim üretememişlerdir. Şerbet miktarı arttıkça balın protein, enzim, vitamin, mineral madde ve şeker yapısı gibi çok önemli düzeyde biyokimyasal değişim ve biyolojik kayıp meydana gelmiştir. Prolin, asitlik, iletkenlik ve K/Na oranı aşırı besleme ile üretilen hileli balları tanımlamada önemli özelliklerdir. Biyokimyasal özelliklerin belirlenen değerleri mevcut ulusal ve uluslararası standartların düzenlenmesi için önemli referanslardır.

**Anahtar sözcükler:** Bal, Endüstriyel Şeker, Koloni, Besleme, Hile, Biyokimyasal özellikler



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## INTRODUCTION

In fact, as a human food, honey should be pure, hygienic and unadulterated [1-4]. However, adulteration has been performed directly via the addition of commercial sugar syrups to the honey [5,6] or indirectly via overfeeding honey bee colonies with these commercial industrial sugars during the main nectar flow period [3,7,8]. These industrial sugars used for adulteration are produced from sugar beet and mainly maize starch by heat, enzyme or acid treatment producing a substance known as High Fructose Corn Syrup (HFCS). HFCS is found in the market under different names according to the fructose content (for instance HFCS-42, HFCS-55, HFCS 85 and HFCS-92). Fructose is preferred by food industry and adulterated honey producers due to having many advantages such as high sweetness degree, having many similar features with honey such as late crystallization, low pH, high osmotic pressure, being liquid and cheap [9]. Thus consumption of fructose corn syrup increased markedly up to a share approximately 40% in the sweetener. On the other hand, almost all fructose syrup (90%) has been produced from GMO corn. In Turkey each year 900 thousand tons starch-based sugar is produced by five domestic and foreign production firms [10]. However it is well known that industrial fructose usage causes serious health problems in living organism. It is reported that fructose is taken into the body without being controlled by insulin, which creates dependency, causes cell aging, impairs spatial memory, metabolic syndrome, obesity and many pathologic illness [11-13].

Fraudulent and adulterated honey production is a problem all over the world. These types of honey are produced by feeding honey bee colonies with industrial sugars ranging from 50 to 300 litres per colony during main nectar flowing period. It is unfair and not ethical to sell these types of honey as a pure honey in the market [1,3,14,15].

Many researchers reported that the use of excessive sugar with the intent of producing greater yields [5] adversely affected sugar content [3,5,7,9,10,16,17], mineral matter content and proline content of the honey [3-5,18]. In addition, sugar origin ( $C_3$  or  $C_4$ ) used for bee feeding affected the honey carbon isotope ratio and  $C_4\%$  [5,8,12]. Many findings on honey adulteration belong to studies where adulterations were performed by the addition of sugar or syrups directly to the honey [19-23]. Namely, sugar-added honey does not undergo any changes due to the fact that samples are not processed biologically by the bee. Furthermore, in these studies, many honey samples subjected to biochemical analysis were taken from the market, meaning that their production conditions were not known exactly. In a similar way, the production conditions of the honey characterised as natural or pure in many of these studies were not clearly known. Also such indirect adulteration is extremely difficult to detect [24]. Above all, we do not know how to distinguish adulterated honeys taken from the colonies that were

overfed with industrial commercial sugars from pure honeys. The standards should be revised by including new criteria for identification of indirectly adulterated honey [1,3,15]. We strongly believe that there is a need to assess different types and levels of industrial commercial sugars in feeding honey bee colonies on the field to address all of the questions discussed above.

We investigated the changes occurring in the biochemical properties of those honeys coming from honey bee colonies fed with different levels of various commercial sugars including HFCS-85), HFCS-55, glucose monohydrate (GMS), bee feeding syrup (BFS), sucrose syrups (SS) and Pure Blossom Honey (PBS) produced under the same environmental conditions. The aims of the study were: (1) to determine changes in the wide range of biochemical properties of pure and adulterated honeys, (2) to characterised these kind of honey biochemically and find which biochemical properties can be used for the identification of adulterated honeys and (3) to make a contribution to the current methods and standards.

## MATERIAL and METHODS

### *Honey Bee Colonies*

Honey samples were taken from colonies in the Apicultural Research and Application Unit of the Ondokuzmayis University, Samsun, Turkey. Colonies were retained in the vicinity of Gulacar Valley, near Gumushane province (40.274°N, 39.29°E), during the nectar flow period. The Gulacar valley is rich in nectar-producing plant species [8].

### *Sugar Sources*

Types, origins, compositions, forms and proportions of the industrial sugars used in the study are summarised in *Table 1*.

### *Maintenance and Preparation of The Colony*

The colonies with two- aged queen bees of the same genetic origin of Caucasian honey bee subspecies (*Apis mellifera caucasica* G.) were used in the study. All of the environmental factors such as frames covered with adult bees, frames covered with brood, foundation comb, drugs, transport and control procedures were standardised. Standard bee-feeding methods were applied in the early spring [9,25]. The shaking method was applied to the colonies [25].

### *Syrup Levels and Preparation*

Sugars and syrup levels were used for the first time as a bee food supplement during the main nectar flow period in this study. Syrups were prepared daily, mixed often, left for one day and were finally given to the colonies. Levels of 5, 20 and 100 L/colony of HFCS-85, HFCS-55, BFS and SS were used in the study. Syrup was given to the experimental colonies at different intervals (twice for the 5, eight times

**Table 1.** Types, origins, compositions, forms, proportions and supplied company names of the industrial sugars used in the experiment

Sugar Sources	Origin of Sugar	Form	Composition	Usage Proportion
HFCS.85	Corn ( <i>Zea mays</i> )	Liquid	84.9% fructose, 12.8% dextrose	1:3 (water : sugar, w:w)
HFCS.55	Corn ( <i>Zea mays</i> )	Liquid	55.6% fructose, 39.6% dextrose	1:3 (water : sugar, w:w)
GMS	Wheat ( <i>Triticum vulgare</i> )	Powder	99.0% glucose	70% water + 30% sugar
SS	Beet sugar ( <i>Beta vulgaris</i> )	Crystalline	99.5% sucrose	1:1.5 (water : sugar, w:w)
BFS	Beet sugar ( <i>Beta vulgaris</i> )	Liquid Pasteurised	30-36% sucrose, 27-30% glucose, 37-40% fructose	1:3 (water : BFS, w:w)

for the 20 and forty times for the 100 L/colony). Before the application of new syrup, the amount of unconsumed syrup (g/colony) was recorded on each colony's card.

### Honey Harvest and Honey Sample Preparation

The honey produced in this study was regarded as a polyfloral honey. Honey was harvested by centrifugation and filtered through a 0.2-mm sieve into lactin. Honey samples were taken from six colonies which were chosen randomly from all of the available groups, and a total of 100 (five industrial sugars \* three syrup levels \* six repetitions = 90 adulterated samples + 10 pure honey or control) samples were used.

### Analytical Methods

The following compositional properties were determined for pure blossom honey and adulterated honey samples: moisture (%), ash (%), free acidity (meq kg<sup>-1</sup>), viscosity (cP), hydroxymethylfurfural (HMF, mg kg<sup>-1</sup>), diastase number (DS, Schade scale value), invertase (u kg<sup>-1</sup>), α-glucosidase (u kg<sup>-1</sup>), proline (mg 100 g<sup>-1</sup>), electrical conductivity (mS cm<sup>-1</sup>), Na (mg 100 g<sup>-1</sup>), K (mg 100 g<sup>-1</sup>), fructose (g 100 g<sup>-1</sup>), glucose (g 100 g<sup>-1</sup>), sucrose (g 100 g<sup>-1</sup>), maltose (g 100 g<sup>-1</sup>), lactose (g 100 g<sup>-1</sup>), vitamin B<sub>5</sub> (mg 100 g<sup>-1</sup>) and vitamin C (mg 100 g<sup>-1</sup>).

Moisture was measured at 20°C by Abbe refractometer (Digital refractometer Atoga, Germany) by refractive methods [19]. Fructose, glucose, maltose, and sucrose (g 100 g<sup>-1</sup>) were identified and determined by high performance liquid chromatography (HPLC) according to AOAC [26] and DIN 10758 [27]. Hydroxymethylfurfural (HMF) was determined spectro-photometrically as outlined by the Harmonisation methods of International Honey Commission (IHC) [14]. The diastatic activity was based on starch hydrolysis [26] (method 958.09) at 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 (method 923.03) [26]. Potassium and sodium were determined using the Atomic Absorbance Spectrophotometer (AAS) according to [26] (AOAC, 1998 method 985.35). Proline was determined spectrophotometrically using ninhydrin in methyl cellosolve, and absorbance was read at 512 nm. A standard curve using pure proline was constructed according to [26] (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 the Harmonised Methods of the IHC [14]. Free acidity was determined photometrically by [26] (AOAC, 1998 method 962.19), and vitamin C and vitamin B<sub>5</sub> were determined by the R-Biopharm Vitafast Panthotenic Acid, Microbiological microtitre Plate Test to quantitate amounts present.

### Statistical Analysis

The study was carried out according to the Randomised Factorial Plot Design and a Pairwise Permutation Test was used for comparison. Homogeneity of variance was performed using the Levene Variance Homogeneity Test and One-Sample Kolmogorov-Smirnov Test [28]. The variance analysis was performed by using the SAS packet programme and Duncan test was used for the comparison of averages [29].

## RESULTS

There were statistically significant differences ( $P < 0.001$ ) in all of the investigated biochemical properties except for lactose and maltose, which were not found in any of the honey samples (Table 2, 3, 4). In addition, interaction between the sugar type and sugar level was significant ( $P < 0.001$ ).

### Water

The mean water content of honey samples are presented in Table 2. The water values ranged between 15.72±0.10% and 19.20±0.03%.

### Sugar Ingredients

Sugar content of the honey samples were evaluated by using fructose, glucose and sucrose content, Fructose/Glucose ratio (F/G), sum of fructose and glucose (F+G), and glucose and water ratio (G/W) (Table 2). All of these parameters were significantly affected by sugar types and syrup levels ( $P < 0.000$ ).

### Physicochemical Properties

There were significant differences ( $P < 0.000$ ) among treatments in Hydroxymethylfurfural (HMF) content of the honey (Table 2). The HMF content ranged from 2.63±0.53 mg/kg to 10.93±0.74 mg/kg. Viscosity, electrical conductivity

**Table 2.** Means (X) and standard errors ( $\pm$ Sx) related to water, fructose, glucose, sucrose (g/100 g), F/G (ratio), F+G, glucose/water (ratio) and HMF (mg/100 g) contents of pure and adulterated honeys

Sugar Sources	SL	Water (Moister)	Fructose	Glucose	Sucrose	F/G	F + G	Glucose/Water	HMF
HFCS-85	5	17.42 $\pm$ 0.07 h	39.05 $\pm$ 0.83 cd	29.72 $\pm$ 0.80 ef	0 $\pm$ 0 b	1.32 $\pm$ 0.01 cd	68.77 $\pm$ 1.62 b-e	1.707 $\pm$ 0.049 bcd	2.63 $\pm$ 0.53 c
	20	19.20 $\pm$ 0.03 a	44.02 $\pm$ 0.59 b	24.00 $\pm$ 0.39 g	0 $\pm$ 0 b	1.84 $\pm$ 0.02 b	68.02 $\pm$ 0.95 b-e	1.250 $\pm$ 0.020 f	6.27 $\pm$ 1.16 b
	100	16.77 $\pm$ 0.05 k	57.07 $\pm$ 1.11 a	17.08 $\pm$ 0.53 h	0 $\pm$ 0 b	3.35 $\pm$ 0.07 a	74.15 $\pm$ 1.52 a	1.019 $\pm$ 0.032 g	10.68 $\pm$ 1.68 a
HFCS-55	5	18.60 $\pm$ 0.00 c	37.40 $\pm$ 0.96 def	29.87 $\pm$ 0.83 def	0 $\pm$ 0 b	1.25 $\pm$ 0.01 def	67.27 $\pm$ 1.79 cde	1.606 $\pm$ 0.044 cde	5.43 $\pm$ 1.64 bc
	20	17.07 $\pm$ 0.05 l	37.78 $\pm$ 0.49 c-f	29.35 $\pm$ 0.61 ef	0 $\pm$ 0 b	1.29 $\pm$ 0.01 cd	67.13 $\pm$ 1.09 cde	1.719 $\pm$ 0.035 bc	6.27 $\pm$ 1.19 b
	100	18.40 $\pm$ 0.00 d	37.20 $\pm$ 0.23 def	27.85 $\pm$ 0.27 f	0 $\pm$ 0 b	1.34 $\pm$ 0.01 c	65.05 $\pm$ 0.46 e	1.514 $\pm$ 0.015 e	10.93 $\pm$ 0.74 a
GMS	5	19.17 $\pm$ 0.02 a	37.45 $\pm$ 1.35 def	29.60 $\pm$ 1.41 ef	0 $\pm$ 0 b	1.27 $\pm$ 0.02 cde	67.05 $\pm$ 2.73 cde	1.545 $\pm$ 0.075 e	2.80 $\pm$ 0.59 bc
	20	19.20 $\pm$ 0.00 a	40.08 $\pm$ 0.47 c	33.43 $\pm$ 0.45 ab	0 $\pm$ 0 b	1.20 $\pm$ 0.01 fg	73.52 $\pm$ 0.86 a	1.741 $\pm$ 0.015 bc	2.93 $\pm$ 0.39 bc
	100	19.03 $\pm$ 0.05 b	36.42 $\pm$ 0.30 ef	34.23 $\pm$ 0.32 a	0 $\pm$ 0 b	1.06 $\pm$ 0.00 h	70.65 $\pm$ 0.62 a-d	1.798 $\pm$ 0.015 b	3.73 $\pm$ 0.68 bc
BFS	5	18.02 $\pm$ 0.02 fg	39.95 $\pm$ 0.57 c	31.77 $\pm$ 0.49 bcd	0 $\pm$ 0 b	1.26 $\pm$ 0.01 def	71.72 $\pm$ 1.02 ab	1.763 $\pm$ 0.027 bc	4.78 $\pm$ 1.13 bc
	20	18.98 $\pm$ 0.04 b	39.20 $\pm$ 0.67 cd	32.22 $\pm$ 0.63 bc	0 $\pm$ 0 b	1.22 $\pm$ 0.02 efg	71.42 $\pm$ 1.20 abc	1.697 $\pm$ 0.033 bcd	5.08 $\pm$ 1.02 bc
	100	18.12 $\pm$ 0.02 ef	38.57 $\pm$ 0.79 cde	32.37 $\pm$ 0.81 ab	0 $\pm$ 0 b	1.19 $\pm$ 0.01 fg	70.93 $\pm$ 1.57 abc	1.787 $\pm$ 0.045 b	4.50 $\pm$ 1.19 bc
SS	5	18.55 $\pm$ 0.07 c	36.98 $\pm$ 0.35 def	28.97 $\pm$ 0.3 ef	0 $\pm$ 0 b	1.28 $\pm$ 0.01 cde	65.95 $\pm$ 0.63 e	1.542 $\pm$ 0.015 de	3.67 $\pm$ 1.34 bc
	20	18.20 $\pm$ 0.06 e	38.95 $\pm$ 1.07 cd	30.27 $\pm$ 0.79 cde	0 $\pm$ 0 b	1.29 $\pm$ 0.00 cd	69.22 $\pm$ 1.86 b-e	1.663 $\pm$ 0.045 cde	4.67 $\pm$ 0.85 bc
	100	15.72 $\pm$ 0.10 m	36.07 $\pm$ 0.43 f	30.40 $\pm$ 0.41 cde	3.05 $\pm$ 0.08	1.19 $\pm$ 0.02 fg	66.47 $\pm$ 0.50 de	1.934 $\pm$ 0.023 a	4.68 $\pm$ 0.43 bc
PBH	-	17.93 $\pm$ 0.03 g	35.49 $\pm$ 0.68 f	30.35 $\pm$ 0.31 cde	0 $\pm$ 0 b	1.17 $\pm$ 0.01 g	65.84 $\pm$ 0.97 e	1.693 $\pm$ 0.017 bcd	3.71 $\pm$ 0.93 bc
Significance		<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000
Codex Standard		<20%	Sum of both >60 g/100 g		<5 g/100 g		>60 g/100 g		<40 mg/kg
European Union		<20%	Sum of both >60 g/100 g		<5 g/100 g		>60 g/100 g		<40 mg/kg

◆=For each syrup level n=6, HFCS= High Fructose Corn Syrup, GMS=Glucose Monohydrate Sugar, BFS=Bee Feeding Syrup, SS=Sucrose Sugar, PBH=Pure Blossom Honey (n=10), SL=Syrup levels (L/colony), \* Values within rows with different letters differ significantly (P<0.001)

(EC) and free acidity of the honeys showed significant differences ( $P<0.000$ ) depending on sugar types and syrup levels (Table 3). EC and free acidity values of the honey were within the range reported by Codex Alimentarius [2] and EU Council [30], Turkish Food Codex, Honey Notification [31].

#### Mineral Matter Content

There were significant differences among treatments in terms of these parameters ( $P<0.001$ ). The Na content of honey decreased rapidly in the 100 L/colony of HFCS-85 and HFCS-55, it was increased in the 100 L/colony of BFS and SS (Table 3). Potassium content of honey decreased with increasing syrup level of all sugar types except for GMS.

#### Proline Content

The proline content of pure and adulterated honey ranged from 772.83 $\pm$ 17.56 to 249.33 $\pm$ 6.27 mg/kg. Except for GMS, the proline content of honey decreased considerably with increasing syrup level of all sugar types. The decrease in proline was more than 2.5-3.0-fold. The highest proline was found in the pure honey, whereas there was a considerable decline in the honey taken from the 100 L/colony of HFCS, BFS and SS (Table 4).

#### Vitamin and Enzyme Content

Sugar types and sugar levels had significant effect ( $P<0.001$ ) on vitamin (vitamin C and B<sub>5</sub>) and enzyme content of honey samples. There were great decline in vitamins content of honeys taken from the 100 L/colony of all sugar types except for GMS. In the present study diastase number did not change with sugar types originating from C<sub>3</sub> or C<sub>4</sub> plants. Enzyme invertase content of honeys decreased with increasing syrup levels of all sugar types. There was no consistency in the enzyme  $\alpha$ -glucosidase content of honeys. It increased with increasing sugar levels of HFCS-85 and BFS; whereas it decreased when the syrup levels of SS and GMS were increased (Table 4). In addition we found significant positive and negative correlations between investigated biochemical characteristics (Table 5).

## DISCUSSION

The water values ranged between 15.72 $\pm$ 0.10% and 19.20 $\pm$ 0.03%, indicating that all values were below the standard value (20-21%) reported by Codex Alimentarius [2], the International Honey Commission [17], the EU Council [30] and the Turkish Honey Codex [31] for all pure and adulterated honeys. This might be resulted from the fact that all honeys were taken from colonies when completely ripening [14].

**Table 3.** Means ( $\bar{X}$ ) and standard errors ( $\pm S_x$ ) related to viscosity (cP), free acidity (meq/kg) electrical conductivity (mS/cm), ash (mg/100 g), Na (mg/100 g), K (mg/100 g) and K/Na (ratio) contents of pure and adulterated honeys

Sugar Sources	SL	Viscosity	Free Acidity	Electrical Conductivity	Ash	Na	K	K/Na
HFCS-85	5	12405.5 $\pm$ 340.81 d	16.2 $\pm$ 0.02 b	0.211 $\pm$ 0.001 bcd	0.121 $\pm$ 0.011 a	0.783 $\pm$ 0.019 ab	17.15 $\pm$ 0.10 cde	21.97 $\pm$ 0.47 de
	20	5111.17 $\pm$ 52.1 h	14.7 $\pm$ 0.02 d	0.201 $\pm$ 0.000 de	0.113 $\pm$ 0.01 ab	0.792 $\pm$ 0.021 ab	16.88 $\pm$ 0.10 cde	19.69 $\pm$ 1.98 ef
	100	14605.42 $\pm$ 280.05 c	9.2 $\pm$ 0.02 g	0.117 $\pm$ 0.001 h	0.056 $\pm$ 0.007 g	0.585 $\pm$ 0.093 c-f	6.81 $\pm$ 0.10 f	12.87 $\pm$ 1.55 gh
HFCS-55	5	7005.58 $\pm$ 96.57 g	17.3 $\pm$ 0.02 a	0.216 $\pm$ 0.001 abc	0.088 $\pm$ 0.006def	0.730 $\pm$ 0.076 a-d	16.52 $\pm$ 0.19 de	23.96 $\pm$ 2.40 de
	20	15650.08 $\pm$ 609.63 b	16.0 $\pm$ 0.03 b	0.203 $\pm$ 0.001 cde	0.098 $\pm$ 0.007b-e	0.809 $\pm$ 0.140 a	20.9 $\pm$ 2.32 ab	26.67 $\pm$ 3.94 cd
	100	7611.08 $\pm$ 390.73 g	11.0 $\pm$ 0.00 f	0.138 $\pm$ 0.000 fg	0.055 $\pm$ 0.004g	0.465 $\pm$ 0.016 f	7.6 $\pm$ 0.09 f	16.35 $\pm$ 0.19 fg
GMS	5	5527.67 $\pm$ 135.97 h	16.2 $\pm$ 0.02 b	0.211 $\pm$ 0.001 a-d	0.083 $\pm$ 0.004ef	0.724 $\pm$ 0.078 a-d	22.49 $\pm$ 2.57 a	30.87 $\pm$ 0.74 bc
	20	5394.42 $\pm$ 172.62 h	15.8 $\pm$ 0.02 bc	0.211 $\pm$ 0.003 a-d	0.097 $\pm$ 0.011 b-e	0.558 $\pm$ 0.049 def	17.13 $\pm$ 0.17 cde	32.76 $\pm$ 2.24 ab
	100	11361.08 $\pm$ 588.55 e	16.3 $\pm$ 0.02 b	0.224 $\pm$ 0.001 a	0.124 $\pm$ 0.002 a	0.610 $\pm$ 0.066 c-f	21.81 $\pm$ 0.86 a	37.16 $\pm$ 2.41 a
BFS	5	9600 $\pm$ 456.83 f	17.0 $\pm$ 0.00 a	0.215 $\pm$ 0.001 abc	0.105 $\pm$ 0.006 a-d	0.598 $\pm$ 0.011 c-f	18.16 $\pm$ 0.11 cd	30.41 $\pm$ 0.59 bc
	20	6038.83 $\pm$ 204.56 h	17.0 $\pm$ 0.00 a	0.216 $\pm$ 0.001 abc	0.091 $\pm$ 0.007 cde	0.528 $\pm$ 0.016 ef	17.4 $\pm$ 0.08 cde	33.12 $\pm$ 0.96 ab
	100	9116.67 $\pm$ 373.24 f	11.5 $\pm$ 0.02 e	0.146 $\pm$ 0.001 f	0.082 $\pm$ 0.004 ef	0.622 $\pm$ 0.038 b-f	9.28 $\pm$ 0.08 f	15.15 $\pm$ 0.79 fg
SS	5	8955.5 $\pm$ 158.3 f	16.9 $\pm$ 0.01 a	0.221 $\pm$ 0.001 ab	0.090 $\pm$ 0.006 c-f	0.635 $\pm$ 0.034 b-f	19.25 $\pm$ 0.23 bc	30.86 $\pm$ 1.53 bc
	20	8888.83 $\pm$ 174.34 f	15.5 $\pm$ 0.02 c	0.195 $\pm$ 0.000 e	0.082 $\pm$ 0.005 ef	0.699 $\pm$ 0.019 a-e	15.05 $\pm$ 0.13 e	21.60 $\pm$ 0.46 de
	100	33111.0 $\pm$ 359.39 a	8.0 $\pm$ 0.00 h	0.130 $\pm$ 0.040 g	0.070 $\pm$ 0.005 fg	0.746 $\pm$ 0.016 abc	7.34 $\pm$ 0.05 f	9.86 $\pm$ 0.18 h
PBH	-	10773.2 $\pm$ 318.81 e	16.8 $\pm$ 0.02 a	0.213 $\pm$ 0.001 a-d	0.109 $\pm$ 0.004 abc	0.603 $\pm$ 0.020 c-f	18.11 $\pm$ 0.18 cd	30.75 $\pm$ 1.59 bc
Significance		<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000
Codex Standard			<50 mEq/kg	<0.8 mS/cm				
European Union			<50 mEq/kg	<0.8 mS/cm				

◆=For each syrup level n=6, HFCS=High Fructose Corn Syrup, GMS=Glucose Monohydrate Sugar, BFS=Bee Feeding Syrup, SS=Sucrose Sugar, PBH=Pure Blossom Honey (n=10), SL=Syrup levels (L/colony), \*Values within rows with different letters differ significantly (P<0.001)

In the 20 and 100 L/colony levels of HFCS-85, honey bees stored a high amount of fructose in honey as fructose. This might be resulted from high fructose content of the HFCS-85 compared to the other sugars (Table 1). This result was compatible with the result of Ruiz-Matute et al.<sup>[7]</sup> who used HFCS-42, HFCS-55, HFCS-75, HFCS+SS and SS sugars for feeding the honey bees. In our study, the honeys taken from the 20 and 100 L/colony of HFCS-85 evaluated as fraudulent due to the fact that they showed significant deviations from standard values<sup>[2,30]</sup>. The result also indicated that as being in the BFS, if the monosaccharide fraction of sugar is arranged in a balanced way (for instance 40-45 g/100 g fructose and 30-35 g/100 g glucose), honey bees can use them efficiently. This may explain why USA beekeepers use HFCS-42 syrup to feed their colonies<sup>[9]</sup>.

Honeys taken from the 20 and 100 L/colony of HFCS-85 and honey taken from the 100 L/colony of GMS were accepted as fraudulent because of the lower and higher glucose content, respectively, when compared to the standards<sup>[2,30]</sup>. These differences might be attributed to the glucose proportion of the used sugars; namely the HFCS-85 has no glucose, GMS has the highest glucose content (99.0%). It can be inferred from these results that if fructose content of used sugar is high, honey bees could not isomerise it efficiently to acceptable level of glucose in the honey. Of the 100 honey samples, only 12 samples were

identified as fraudulent when the fructose contents of the honeys were taken into consideration. On the other hand, 18 samples were classified as fraudulent when the glucose contents of honeys were taken into account. Therefore it is obvious that fructose and glucose content are not reliable parameters in order to identify indirect adulteration<sup>[3,32]</sup>. Sucrose was only found in the honey taken from the 100 L/colony of SS (3.05 $\pm$ 0.08 g/100 g). PBH had no sucrose. Ozcan et al.<sup>[33]</sup> and Ruiz-Matute et al.<sup>[7]</sup> also found no sucrose in control honey. The source of sucrose in the pure honey, where sucrose was not used as a supplemental food in this group, is nectar<sup>[5,32-34]</sup>.

The F/G values determined for the 20 and 100 L/colony of HFCS-85 were higher than the values (F/G 0.95 for *Brassica* honey to 1.61 for *Robinia* honey) reported by Piazza and Oddo<sup>[35]</sup> and Oddo et al.<sup>[36]</sup> for the 19 unifloral honey types generated from different plant sources. In their study total range of the F/G ratio of 6719 honey samples produced in 21 countries of the European geographical area was 1.03. Whereas, in our study difference in the F/G ratio of honey between the 20 and 100 L/colony of HFCS-85 was 1.51. These two groups were evaluated as fraudulent due to the fact that they showed important deviation from the standard. The sum of fructose and glucose (F+G) values corresponded with the value (>60 g/100 g) given by Codex Alimentarius<sup>[2]</sup>. Therefore, there was no adulterated

**Table 4.** Means ( $\bar{X}$ ) and standard errors ( $\pm S_x$ ) related to proline (mg/100 g), vitamin C (mg/100 g), vitamin B<sub>5</sub> (mg/100 g), diastase (u/kg), Invertase (u/kg) and  $\alpha$ -glucosidase (u/kg) of pure and adulterated honeys

Sugar Sources	SL	Proline	Vit C	Vit B <sub>5</sub>	Diastase	Invertase	$\alpha$ -glucosidase
HFCS-85	5*	709.33±7.57 bc	2.54±0.01 ef	0.105±0.001 a	8.33±0.00 a	66.23±0.10 b	22.88±0.63 c
	20	530.00±3.85 f	1.89±0.02 g	0.077±0.001 g	7.70±0.00 b	58.15±0.63 fgh	27.43±0.72 b
	100	279.17±2.83 h	0.20±0.01 k	0.062±0.003 i	7.70±0.00 b	57.65±0.19 ghi	27.64±0.87 b
HFCS-55	5	707.50±16.12 bc	2.76±0.06 cd	0.096±0.002 b	7.14±0.00 c	71.08±0.56 a	30.78±0.57 a
	20	618.17±13.73 e	3.70±0.08 a	0.084±0.001 ef	7.14±0.00 c	70.20±0.33 a	31.23±0.50 a
	100	348.67±6.42 g	0.34±0.02 k	0.059±0.001 i	7.70±0.00 b	62.57±0.35 c	26.94±0.92 b
GMS	5	673.17±9.25 cd	3.44±0.07 b	0.081±0.003 fg	7.70±0.00 b	62.65±0.35 c	28.02±0.47 b
	20	646.33±26.25 de	2.45±0.15 f	0.090±0.001 cd	8.33±0.00 a	55.94±0.75 l	23.25±0.62 c
	100	719.67±10.73 b	3.65±0.16 a	0.097±0.001 b	8.33±0.00 a	57.33±1.76 ghi	22.62±0.78 c
BFS	5	702.33±18.37 bc	1.57±0.07 h	0.091±0.002 bcd	8.33±0.00 a	56.73±0.37 hi	24.08±0.35 c
	20	709.00±10.39 bc	2.72±0.08 de	0.092±0.001 bc	7.70±0.00 b	62.15±0.33 cd	27.86±0.78 b
	100	372.83±3.61 g	0.26±0.01 k	0.069±0.001 h	7.70±0.00 b	61.27±0.24 cde	26.67±0.98 b
SS	5	772.83±17.56 a	2.83±0.04 cd	0.092±0.001 bc	7.70±0.00 b	61.38±0.74 cde	27.70±0.74 b
	20	704.50±9.67 bc	1.30±0.02 i	0.086±0.001 de	7.70±0.00 b	60.23±1.02 def	26.40±0.98 b
	100	249.33±6.27 h	0.19±0.01 k	0.050±0.001 k	7.70±0.00 b	58.53±0.83 fgh	22.38±0.59 c
PBH		768.20±13.06 a	2.94±0.02 c	0.094±0.003 bc	7.70±0.00 b	59.33±0.73 efg	27.36±1.24 b
P		<0.000	<0.000	<0.000	<0.000	<0.000	<0.000
Codex Standard		>180 mm/kg			>8 u/kg		
European Union					>8 u/kg		

◆=For each syrup level n=6, HFCS=High Fructose Corn Syrup, GMS=Glucose Monohydrate Sugar, BFS=Bee Feeding Syrup, SS=Sucrose Sugar, PBH=Pure Blossom Honey (n=10), SL=Syrup levels (L/colony), \*Values within rows with different letters differ significantly (P<0.001)

**Table 5.** Correlation coefficients in terms of some biochemical properties of pure and adulterated honeys**Tablo 5.** Saf ve katkılı balların bazı biyokimyasal özellikleri arasındaki ilişkiyi ifade eden korelasyon katsayılarına (r) ilişkin değerler

Characteristics	G	S	F/G	F+G	P	HMF	W	Ac	EC	K/Na	Vit B <sub>5</sub>	Vit C	I	Dyz	Gls
F	-0.636**	-0.159	0.908**	0.629**	-0.416	0.399	-0.180	-0.394	-0.432	0.262	-0.267**	-0.390**	-0.243*	0.026	0.056
G		0.055	-0.877**	0.199	0.450	-0.430	0.286	0.433	0.471	-0.396	0.444	0.341	0.332	0.283**	-0.299**
S			-0.101	-0.146	-0.503	-0.034	-0.644	-0.587**	-0.436	0.590	-0.513	-0.369	-0.138	-0.060	-0.311**
F/G				0.269	-0.486	0.461	-0.287	-0.477	-0.518	0.398	-0.365**	-0.410**	-0.195	-0.107	0.165
F+G					-0.075	0.073	0.060	-0.064	-0.074	-0.066	0.049	-0.134	-0.264**	-0.319**	-0.221*
P						-0.438	0.515	0.954**	0.914**	-0.770**	0.866	0.823	0.428	0.104	0.094
HMF							-0.162	-0.400	-0.457	0.346	-0.332**	-0.412**	0.044	-0.244*	-0.250*
W								0.602**	0.584**	-0.675**	0.432**	0.442**	-0.063	0.186	0.074
Ac									0.935**	-0.808**	0.854	0.835	0.429	0.103	0.169
EC										-0.781**	0.788	0.833	0.431	0.177	0.023
K/Na											0.652**	0.745**	-0.051	0.269**	-0.080
Vit B <sub>5</sub>												0.713**	0.223*	0.249*	0.030
Vit C													0.320**	-0.014	0.174
I														-0.593**	0.521**
Dyz															-0.741**

G=glucose, S=sucrose, F=fructose, P=proline, Ac=acidity, EC=electrical conductivity, W=water, Dyz=diastase, I=invertase, GlS=glucosidase, HMF: hydroxymethylfurfural, \*, \*\* shows significance at P<0.05 and P<0.01 levels.

honey when the sum of F+G was used as a criterion. These results indicated that the F+G was not a good parameter for identification of indirect adulteration [32]. Of the 100 honey samples, 12 honeys from the 20 and 100 L/colony of HFCS-85 were classified as fraudulent when the F/G ratio was taken into consideration. This result indicated that F/G ratio over 1.84 can be an indicator of overfeeding honey bees with HFCS-85.

The rate of G/W did not change with the source of the sugar ( $C_3$  and  $C_4$  plant). The highest changes occurred for different levels of HFCS-85. The rate of change was about 65-70%. The G/W ratio decreased with increasing levels of HFCS-85. Oddo et al. [36] reported that the G/W showed differences according to the plant sources of honey. The reason for the higher rate of G/W in the 100 L/colony of SS is that honey bees used it efficiently. While the fructose and glucose content of the given sucrose were equal (50%), these rates changed to  $36.07 \pm 0.43\%$  fructose and  $30.35 \pm 0.41\%$  glucose after being processed by the honey bees. Namely, even in the adulterated honey produced by feeding the honey bees with the 100 L/colony of sucrose syrup, the fructose and glucose levels of honeys were similar to those of pure honeys. In fact, we expected the highest G/W ratio for the GMS due to its high glucose content, but this expectation did not realise, because the water content of honey in the GMS was higher, as the honey bees did not ripening the GMS syrup properly. The G/W ratio is a criterion for crystallisation [34,35]. If the G/W ratio of honey is lower than 1.70, it does not crystallise and remains liquid; if this rate is over 2.10, the honey crystallises in a short time. In our study, all honeys crystallised, even the pure honey, with the exception of the 100 L/colony of HFCS-85. Although the G/W ratio of some of them were lower than 1.70, they crystallised (Table 2).

HMF is a criterion that shows whether honey was stored under good conditions and also whether the honey was treated with heat or not [1,34,37]. The low HMF content of the honeys in the present study was attributed to the fact that they were newly harvested honeys (not stored for a long duration), not treated with heat, and stored under proper conditions before sending for analysis. These results supported by Visquert et al. [38] who found low HMF (<10 mg/kg) in all honey samples. Therefore, HMF should not be used for the identification of adulterated honey produced by overfeeding the honey bees with industrial commercial sugars.

In all sugar types except for GMS, electrical conductivity, free acidity and ash content of the honey decreased with increasing level of sugar syrups. In the highest syrup level (100 L/colony) of these sugars decrease in EC, free acidity and ash content was dramatic, which indicated that honey underwent important biochemical change. Significant correlations between EC and free acidity ( $r=0.935$ ,  $P<0.001$ ), proline ( $r=0.914$ ,  $P<0.001$ ), vitamin C ( $r=0.833$ ,  $P<0.001$ ) and  $B_5$  ( $r=0.788$ ,  $P<0.001$ ) support this result (Table 5).

Free acidity shows great variation depending on the plant sources. Terrab et al. [39] reported the range of free acidity as 10.3-102 meq/kg for Moroccan unifloral honeys. Oddo et al. [36] gave the total range of free acidity as 49.1 meq/kg for 19 European unifloral honeys. Decrease in free acidity of honey produced by feeding honey bees with industrial commercial sugar syrup reported also by Ozcan et al. [33] and Guler et al. [3]. According to our result, the free acidity of honey is an important criterion for the determination of adulteration made by HFCS and SS and it should be at least 15-16 meq/kg in pure blossom honey. Although EC is the most important trait for determination of the botanic or floral source and ash content of the honey [4,34,36,40], it is also important for the identification of adulterated honey with industrial sugars via colony feeding. Guler et al. [3] reported that EC is the second biochemical characters following proline for discriminating pure and adulterated honeys produced by sucrose feeding. According to the Codex Alimentarius [2] EC should not be more than 0.8 mS/cm. According to us for blossom honeys having EC value under 0.20 mS/cm should be evaluated as indirect adulterated with commercial sugars originating from both  $C_3$  and  $C_4$  plants. This supported by the results of Guler et al. [3] who found that while EC value of control and pure honey over 0.20 mS/cm (0.224 mS/cm for control and 0.230 mS/cm for pure honey) and it was below 0.20 mS/cm for sucrose adulterated honey (0.176 mS/cm).

The Na content of honey decreased rapidly in the 100 L/colony of HFCS-85 and HFCS-55, it increased in the 100 L/colony of BFS and SS. These differences might be attributed to origin of the sugar used. While the origin of the first two sugars (HFCS-85 and HFCS-55) is  $C_4$  plant, the origin of SS is  $C_3$  plant, of BFS is composed of  $C_3$  and  $C_4$  plant. The finding of high Na content of SS honey compared to pure blossom honey is incompatible with the result of Ozcan et al. [33] who reported slightly decrease in Na content for sucrose syrup honey and inverted sucrose syrup honey. Differences might be resulted from differences in given amount of sugar syrup to the bee colonies and differences in sugar composition. They heated sucrose syrup, added HCl solution for pH adjustment and added  $Na_2CO_3$  for neutralization.

In the present study the K content of the honey in the 100 L/colony of SS ( $7.34 \pm 0.05$  mg/100 g) was lower than the value reported by Guler et al. [3] for sucrose-adulterated honey with the 100 L/colony of sucrose. Potassium, an essential nutrient for nerve cell functioning in honeybees, is used for the definition of plant sources of honey or the identification of honeydew and blossom honey [32,34]. It is also an important tool for the identification of adulteration. According to our results, the K content of honey should be over 16 mg/100 g.

The K/Na ratio also decreased greatly with increasing syrup level of all sugar types except for GMS. The K/Na ratio of honey is one of the biochemical properties negatively

affected by adulteration following vitamin C and proline content of honey. Therefore, it is an important characteristic for the identification of adulterated honey produced by overfeeding honey bee colonies with industrial commercial sugar. We suggest that the K/Na rate of honey should be considered in standards. If the K/Na rate of blossom honey is less than 20, it should be considered adulterated. Thus, in the standards, the K/Na rate should be evaluated as  $>20$ .

Potassium and sodium content of honey shows great variability depending on plant sources. Fernandez-Torres et al.<sup>[41]</sup> found that K content of Spanish honeys ranged between 43.4-193.5 mg/100 g and Na content ranged between 1.17-21.8 mg/100 g. Conti et al.<sup>[42]</sup> reported that K content of Argentina honeys ranged between 281.3-13.4 mg/100 g and Na content ranged between 10.5-1.31 mg/100 g.

The proline content of honey decreased considerably with increasing syrup level of all sugar types except for GMS. Oddo et al.<sup>[36]</sup> and Piazza and Oddo<sup>[35]</sup> reported that differences in proline stemmed from plant sources. However, in the present study, differences in proline resulted from excessive sugar syrup usage rather than plant source; because the colonies were placed in the same apiary and we know that the HFCS originated from corn ( $C_4$ ), the GMS from wheat ( $C_3$ ), the BFS from mixture of  $C_3$  and  $C_4$  plants, the SS from sugar beet ( $C_3$ ). Proline is accepted as an indicator of maturity level<sup>[34,36]</sup>. Therefore, we concluded that differences in proline did not stem from maturation or plant source but from excessive feeding of the colonies with industrial sugars. Previously, proline was reported by many authors as the most important biochemical component for monitoring adulteration<sup>[3,5,33,34,36]</sup>. In Codex Alimentarius<sup>[2]</sup>, proline is considered a quality criterion and should be greater than 180 mg/kg. When Codex standards were taken into account, none of our honey samples were identified as adulterated. However, we know that the honey samples produced by feeding the colonies with 100 L/colony of HFCS, GMS, BFS and SS were exactly adulterated. Therefore, we suggest that the amount of proline given as a standard by Codex Alimentarius is too low to discriminate adulterated honey. For that reason, in the present study, the proline amount of PBH ( $768.2 \pm 13.06$  mg/kg) should be an important quality criterion for the standards. In addition there were significant positive correlations ( $P < 0.001$ ) between proline and water, acidity, electrical conductivity, vitamin  $B_5$ , and vitamin C. Therefore, proline and EC parameters should be evaluated elaborately to rearrange their limits in order to use them as quality criteria for distinguishing pure and indirectly adulterated honeys with commercial sugars. For blossom honey proline content below 300 mg/kg might be an indicator for overfeeding honey bee colonies with HFCS-85 ( $C_4$ ) and SS ( $C_3$ ).

There were great decline in vitamins C and  $B_5$  content of honeys taken from the 100 L/colony of all sugar types

except for GMS, indicating a biological loss of adulterated honey. Significant correlations among these vitamins and proline, EC, acidity and the K/Na ratio support this result (Table 5).

In the present study diastase number did not change with sugar types originating from  $C_3$  or  $C_4$  plants. The diastase number of most samples, including pure honey (PBH), was slightly lower than the standard value ( $>8$  u/kg). The diastase number of honey changes with plant source, ranging from  $4.6 \pm 2.8$  to  $39.3 \pm 7.9$  u/kg<sup>[36]</sup>, and is an indicator of samples that have not been subjected to heat treatment<sup>[34,43,44]</sup>. As a matter of fact, a statement from the researcher who performed the analyses confirmed this hypothesis.

Studies showed that there were enormous differences in enzyme activity depending on the botanical origin, even though the enzymes are usually added by the bees<sup>[24]</sup>. Lichtenberg-Kraag<sup>[45]</sup> found that the correlation between the concentration of sucrose and invertase activity is highly significant in the process of honey ripening. In the present study we found negative correlation between invertase and fructose and sucrose (Table 5). It is known that honey bees use invertase to invert sucrose of the nectar into fructose and glucose<sup>[5,34]</sup>. Enzyme invertase content of honeys decreased with increasing syrup levels of all sugar types. A greater reduction in the invertase enzyme levels of honey from HFCS sugars might have resulted from the fact that they contain more fructose than other monosaccharide sugars used in the study. Ruiz-Matute et al.<sup>[7]</sup> reported that when the fructose proportion of the sugar increased, honey bees encounter difficulty to isomerize the sugar into fructose (39-40%) and glucose (28-30%) to a level that found in natural honey.

We found no consistency in the enzyme  $\alpha$ -glucosidase content of honeys. It increased with increasing syrup levels of HFCS-85 and BFS. Whereas there was a decline in the enzyme  $\alpha$ -glucosidase content of honeys when the syrup levels of SS and GMS were (both  $C_3$  plants) increased. Differences might be attributed to differences in the origin of the commercial sugars and proportion of fructose, glucose and sucrose in the used commercial sugars.

In conclusion, honey bees utilised commercial sugar syrups depending on the sugar origin ( $C_3$  and  $C_4$  plants), purity levels and amount provided to the colonies. It is not possible to define the adulterated honey by using its sugar constituents such as fructose, glucose, fructose/glucose and sucrose. Whereas it is possible to determine or monitor adulteration in honey when the monosaccharide fructose rate of industrial sugars syrup is increased ( $>50$  g/100 g), as honey bees do not sufficiently or efficiently utilize these types of sugars. When glucose and fructose proportions were balanced or close to each other (fructose 40-45 and glucose 30-35 g/100 g), they were used efficiently by honey bees to the honey. Important biological

degradation occurred in some properties of honey especially in proline, electrical conductivity, free acidity, vitamin, mineral and enzyme contents when the colonies were fed with commercial sugars (20 L/colony and over) in the main nectar flow period. So it is unfair and not ethical to produce and sell these types of honey as a pure honey in the market.

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