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Araştırma Makalesi/Research Article

Some Physicochemical Properties and Sugar Composition of Multifloral Honeys from Different Regions of Turkey

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Abstract: In the present study, the physicochemical characteristics and sugar compositions of 14 different honey samples obtained from two different regions of Turkey were analyzed by using multivariate analysis methods. pH, acidity, moisture content (%), total soluble solids (Brix), diastase activity, total phenolic content, HMF and Lugol's reaction analyses of the samples were performed using the physicochemical parameters. Moreover, the fructose, glucose, sucrose, maltose, fructose/glucose, and fructose + glucose values of the samples were determined using HPLC-RI system. It was determined that 1 of 14 samples has been adulterated with maltose syrup and 2 of 14 samples have HMF content higher than the limit set by the law. The honey samples were classified based on their physicochemical cluster analysis. Moreover, the factors affecting the quality of honey were determined and the relationships between these factors were shown.

Keywords: Adulteration, chemometrics, honey, hierarchical cluster analysis, principle component analysis.

Türkiye'nin Farklı Bölgelerinden Multifloral Balların Bazı Fizikokimyasal Özellikleri ve Şeker Bileşimi

Öz Bu çalışmada farklı Türkiye'nin iki farklı coğrafik bölgesinden elde edilen 14 farklı bal örneğinin fiziko-kimyasal özellikleri ve şeker içerikleri çok değişkenli teknikleri kullanılarak incelenmiştir. Örneklerin pH, asitlik, %nem, toplam suda çözünür kuru madde (brix), diastaz aktivitesi, toplam fenolik içeriği, HMF ve lügol reaksiyon analizleri fiziko-kimyasal paramatreler olarak analiz edilmiştir. Ayrıca örneklerin früktoz, glukoz, sukroz, maltoz, früktoz/glukoz, früktoz+glukoz değerleri HPLC-RI sistemi ile tespit edilmiştir. 14 örneğin 1 adedinin maltoz şurubu ile tağşiş edildiği, 2 adedinin diyastaz aktivitesinin yasalarda belirtilen limitlerden daha düşük, 2 adedinin ise yasalarda belirtilen limitlerden daha yüksek HMF içerdiği belirlenmiştir. Çalışmada, bal örnekleri, temel bileşen analizi ve hiyerarşik küme analizi kullanılarak ilgili fizyokimyasal özelliklerine ve şeker kompozisyonuna göre sınıflandırılmıştır. Ayrıca balın kalitesine etkileyen faktörler belirlenerek, bu faktörlerin arasındaki ilişkiler ortaya konmuştur.

Anahtar Kelimeler: Bal, hiyerarşik kümeleme analizi, kemometri, tağşiş, temel bileşenler analizi

1. Introduction

Honey is a natural product produced by honeybees making use of complex and variable components such as plant nectars and honeydew (Li et al. 2012). Differing from the sugars such as glucose, fructose and sucrose constituting 65– 75% of total soluble solids, the honey contains various substances such as enzymes, vitamins, phenolic compounds, proteins, amino acids and minerals (Bilandžić et al. 2011). Dilution by water addition, extension quantity of honey with sucrose and other types of sugar syrups (e.g. corn syrup, high fructose corn syrup, maltose syrup), and feeding bees to sugars and syrups are the main adulteration techniques used by the producers in order to achieve more commercial gains (Anklam, 1998; Yıllmaz et al. 2014). Having rich plant cover, suitable ecological conditions, nectar resources suitable for beekeeping, and colony stocks, Turkey has an important place in the beekeeping sector. According to the data of FAO, China was the largest producer with 318,650 tons of honey production, followed by the USA with 81,480 tons and Turkey with 77,603 tons. (FAO, 2017).

The principal component analysis (PCA) and hierarchical cluster analysis (HCA) are two main approaches used in chemometrics and they are widely used in classification in food studies. In the previous studies, it was reported that the multivariate analysis methods such as PCA and discriminant analysis might provide predictability for the physiochemical and chemical parameters of honey such as HMF, moisture content, free acidity, electrical conductivity, total monosaccharides, diastase activity, proline content, fructose, glucose, and raffinose (Kıvrak et al. 2017).

The main objective of the present study is to investigate the physicochemical and sugar compositions of specific multifloral honeys collected from different regions in Turkey. Simple analysis methods were applied in order to identify potential adulterations of different honey samples. Moreover, in addition to the PCA, the linkage method and Pearson's correlation measurement method was implemented in order to reveal the natural grouping of quality parameters of data cluster and the grouping of samples in HCA.

2. Materials and Methods

2.1. Samples

14 multifloral honey samples harvested in 2014/2015 season were collected in October 2014 from 4 different provinces (Manisa, Uşak, Sivas, Konya) located in two different regions of Turkey. All of the samples were directly obtained from the honey beekeepers as filtrated honey in 1.5 kg glass jars. The samples were kept at room temperature for two weeks before analysis.

2.2. Determintion of Physicochemical Properties of Honey samples

pH, acidity, total soluble solids, diastase activity (DA), hydroxymethylfurfural (HMF) content of the honey samples were determined according to International Honey Commission (Bogdanov, 2009). pH of the samples was determined by using pH-meter (WTW Inolab 730, Germany). Acidity (AC) of the samples were measured volumetrically and expressed as meq/kg. Total soluble solids (TSS) of the samples were measured using a digital densitometer/refractometer (Antoon Paar, DMA 500, Austria) and the results were expressed in Brix (BX). Diastase activity was determined by using PGI T70 double beam spectrophotometer (Leicestershire, UK). The hydroxymethylfurfural (HMF) content of the honey samples was determined based on the colorimetric reaction between barbituric acid, ptoluidine, and HMF forming a red-colored complex. The moisture content (MC) of the samples was determined in accordance with the principles specified by Sanchez et al. (2010). The refractive indices of honey samples were measured using а digital densitometer/refractometer (Antoon Paar, DMA 500, Austria). The total phenolic content of the honey samples, the Folin-Ciocalteu method was utilized (Meda et al. 2005) and the results was expressed as gallic acid equivalent (GAE)/100g honey. Lugol's reaction (LR) test was performed in accordance with the principles specified by Almeida-Muradian et al. (2013).

2.3. Sugars

Analysis of sugars (glucose, fructose, sucrose, and maltose) was made by using HPLC (Shimadzu, Tokyo, Japan) fitted with refractive index detector (Shimadzu RID-10A, Tokyo, Japan) at 30° C. An ACE ODS (100×4.6 mm; 5 µm) column was used with a mobile phase of acetonitrile/water (80:20, v/v) solution at a flow rate of 1.3 ml min⁻¹. The honey samples were prepared in accordance with IHC (Bogdanov, 2009). 5g honey sample was dissolved in 40 ml ultra-pure water. 25 ml methanol (HPLC grade, Merck, Darmstadt, Germany) was put into a 100 ml volumetric flask and the honey solution was transferred to the volumetric flask quantitatively. Then, the flask was filled with water to the marked level. The samples prepared were filtered through the 0.45 µm disposable filters (Sartorius, Göttingen, Germany) prior to the HPLC analysis. The quantification was performed using the external calibration method and the calibration curves and results were expressed as grams of sugar type in 100g of honey.

2.4. Statistics

All results results were given the means of triplicate measurements (n=3). The statistical analyses were performed by using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used in comparing the pH, acidity, water %, total soluble solids (Brix), diastase number, HMF, total phenolic compounds and sugar analysis results of samples obtained from different locations. Probability (p) values < 0.05 were considered to be significant. The chemometric evaluation was performed using two different multivariate analysis methods as PCA and HCA and the component matrix by making use of SPSS Version 22.0.

3. Results and Discussion

3.1. Physicochemical Properties

The physicochemical properties of the samples obtained from different provinces of Turkey are presented in Table 1. The highest pH value was found in N4, followed by K2 and N3, respectively. As stated in the other studies, the acidic character of honey is mostly attributed to the presence of organic acids and the pH value of the honey varied between 3.2 and 5.5 (Bogdanov et al. 2004). This acidic character of honey protects the products from microbial spoilage. It was determined that none of the samples was found to have AC value higher than 50 meq. kg⁻¹ specified as the upper limit in Turkish Food Codex and directive of EU Commission (EU Council, 2002; TGK, 2012). The mean moisture content (MC%) percentage of honey samples was found to be 16.73±1.74. According to the EU Commission's directive on honey and regulations of Turkish Food Codex regarding the honey, the MC% of any type of honey other than baker's honey shall not exceed beyond 20%. The MC% values of all the honey samples were lower than this level (EU Council, 2002; TGK, 2012). The honeys having BX value of 81.4 or higher are accepted to be highgrade ones (A and B), whereas those having BX values between 80 and 81.3 were accepted to be Class C (low grade) honeys (USDA, 1985). From this aspect, all of the honey samples except for U4 and S2 were found to be in highgrade honey class. It was determined that 2 of the samples (S1, N4) do not meet the minimum DA value of 8 set by honey regulation of Turkish Food Codex and honey directive of EU Commission (EU Council, 2002; TGK, 2012). It was found that the HMF content of 2 samples had HMF more than 40 mg.kg⁻¹ set in the honey directive of Turkish Food Codex and EU Commission's directive on honey (EU Council 2002; TGK, 2012). Moreover, given the DA of these two samples, it was determined that the values were much lower than the limit set by the law. The DA values obtained for these samples were in corroboration with the data obtained for HMF. It is believed that the HMF value of sample S1 increased and DA decreased because of overheating the sample or feeding the bees artificially to the sugar syrup. Given the Lugol's test results or sugar analysis results of sample N4 together, it is believed that this sample is adulterated honey mixed with MAL syrup. The mean TFC contents of the multifloral honey samples were found to be similar to those reported by Bertoncelj et al. (2007) for multifloral honeys and lower than the values reported Kıvrak et al. (2017). LR analysis is based on the reaction between iodine and potassium iodure in presence of glucose, and the color of the solution changes from red-purple to blue. The intensity of color depends on the dextrin amount of glucose. When the dyed solution becomes blue, the reaction is considered positive (Almeida-Muradian et al. 2013). Except for sample N4, the LR results of all the samples were found to be negative. The positive result obtained from this test indicates that starch-based sugar has been added to the sample N4. The sugar analysis of the samples corroborates this finding.

Greige 1. Turkiye de jarkii lokasyonlardan elde edilen dal ornekterinin jizikokimyasal özetlikleri								
Sample	pН	AC	MC%	BX	DA	HMF	TFC	LR
Code								
K1	3.62 ^{bc}	11.25 ^a	15.33 ^{ab}	82.70 ^{cd}	19.10 ^g	9.70 ^{ab}	30.52 ^{ab}	-
K2	3.72 ^c	23.50 ^c	16.83 ^{bc}	81.37 ^{bc}	17.90^{f}	15.55 ^{bcd}	28.53 ^{ab}	-
K3	3.38 ^{abc}	13.00 ^a	16.80 ^{bc}	81.47 ^{bc}	13.90 ^e	11.81 ^{abc}	26.64 ^{ab}	-
U1	3.35 ^{ab}	30.50 ^d	16.43 ^{bc}	81.77 ^{bc}	11.90 ^d	23.62 ^{de}	43.89°	-
U2	3.42 ^{abc}	24.50 ^{cd}	16.93 ^{bc}	81.37 ^{bc}	10.90 ^{cd}	24.29 ^e	35.66 ^b	-
U3	3.38 ^{abc}	25.50 ^{cd}	17.87 ^{cd}	81.30 ^{bc}	8.30 ^b	23.81 ^{de}	34.87 ^b	-
U4	3.60 ^{bc}	21.00 ^{bc}	19.17 ^d	79.10 ^a	38.50 ^h	12.48 ^{abc}	26.96 ^{ab}	-
S1	3.60 ^{bc}	15.50 ^{ab}	15.48 ^{ab}	82.73 ^{cd}	1.00 ^a	57.12 ^f	22.68 ^a	-
S2	3.35 ^{ab}	16.01 ^{ab}	19.03 ^d	79.17 ^a	10.10 ^c	17.18 ^{bcd}	30.75 ^{ab}	-
S 3	3.43 ^{abc}	15.00 ^{ab}	16.83 ^{bc}	81.43 ^{bc}	13.90 ^e	12.58 ^{abc}	27.82 ^{ab}	-
N1	3.46 ^{abc}	12.50 ^a	13.60 ^a	84.57 ^d	17.90^{f}	7.10 ^a	23.55 ^a	-
N2	3.34 ^{ab}	22.50 ^c	15.53 ^{ab}	82.67 ^{cd}	10.90 ^{cd}	19.2 ^{cde}	35.26 ^b	-
N3	3.21 ^a	15.00 ^{ab}	17.70 ^{cd}	81.60 ^{ab}	8.30 ^b	17.28 ^{bcde}	34.39 ^b	-
*N4	4.08	17.00	16.73	81.50	2.50	45.60	24.19	+
Min.	3.21	11.25	13.60	79.17	1.00	7.10	22.68	
Max.	4.08	30.50	19.17	84.57	38.50	57.60	43.89	
MEAN	3.50	18.90	16.73	81.55	14.05	19.36	30.89	
SD	0.26	6.50	1.74	1.62	8.57	12.80	6.92	

Table 1. Physicochemical properties of honey samples obtained different locations in Turkey

 Cizelge 1. Türkive'de farklı lokasyonlardan elde edilen bal örneklerinin fizikokimvasal özellikleri

Different letters in each column correspond to significantly different values. (p < 0.05)

SD: Standart deviations of the samples belonged to each column

*: Adulterated honey sample was neglected from statistical analysis, minimum, maximum, mean and Standart Deviation (SD) calculations.

3.2. Sugar Composition

The FRU, GLU, SUC, and MAL contents of the samples and mean invert sugar (F+G) and Fructose/Glucose (F/G) ratios are presented in Table 2. The sample N4 having a positive result from Lugol reaction test was not included in statistical analyses for sugar composition of the samples. FRU and GLU were observed in all the honey samples, whereas MAL was not seen in sample K1 and SUC in samples U3 and U4. FRU, which is responsible for most of the physical and nutritional characteristics of the honey, is accepted to be the basic sugar with the highest percentage in the honey (Krell, 1996). The mean FRU content of the samples (37.92 g.100g⁻¹) was lower than reported for multifloral honeys by Can et al. (2015) for Turkey and similar to those reported for multifloral honeys by Koç Uçak et al. (2017) for Turkey and by Abdulkhaliq et al. (2017) for Palestine. In the literature on beekeeping, it is specified that the risk of crystallization increases when GLU concentration passes beyond 30% (Zhelyazkova and Lazarov, 2017). Except for the samples U1, U2, U4, and N4, the GLU contents of all the samples were higher than 30 g.100g⁻¹ Given the GLU contents of the samples, it can be seen that there was crystallization risk in 10 samples and crystallization was detected in 5 samples. The SUC content of none of the samples was higher than 5 g.100g⁻¹ set to be upper limit by the honey directive of Turkish Food Codex and the European Commission's Directive on Honey (EU Council, 2002; TGK, 2012). SUC content of the nectar is conveyed to the hive by the honeybees and then transformed to GLU and FRU there; this process is called maturation (Pryce-Jones, 1950). Thus, SUC constitutes approx. 1% of the dry-weight of honeys. However, if the beekeepers overfeed the hives to sugar syrup or if the honeys are harvested before the maturation, then the SUC content of honey increases (Wang et al. 2015). It was reported that the MAL content of natural honeys should be lower than 30 mg g^{-1} (3.0%) (Preedy, 2012) but it may be higher than 50 mg g^{-1} (5%) in case of the presence of specific plant species in the environment (Ahmed et al. 2012). The MAL contents higher than 5% indicate the adulteration by using hydrolyzed starch syrup. Except for the sample N4, the MAL contents of all the honey samples were lower than 50 mg g^{-1} .

It is known that the adulteration has been made by adding hydrolyzed starch syrup, which has lower commercial value, into the honey in order to make more gain. Fujita (2012) analyzed the samples of 5 kinds of honey sold in the Japanese market and they found that one of the samples had MAL content of 17.153 g dL⁻¹. The MAL content of sample N4 was 33.63% and this sample was found to be added with MAL syrup. Except for the sample N4 found to be adulterated, it was determined that all the honey samples met the minimum F+G of 60 g.100g⁻¹ set by the honey regulation of Turkish Food Codex and honey directive of EU Commission (EU Council 2002; TGK, 2012). F/G ratio is considered as an indicator of crystallization capability of honey and it is also used in revealing the origin of honey. F/G ratios higher than 1.3 cause slow crystallization, whereas the values lower than 1.0 cause accelerated reaction (Buba et al. 2013). Except for the adulterated honey sample N4 and the samples K1, K3 and N3, the F/G ratios of all the samples were higher than 1.0. F/G ratios of 3 of the samples, in which the crystallization was observed, were higher than 1 and that of 3 samples had F/G ratio close to 1.0. Besides the F/G ratio, the GLU content is an important parameter used in evaluating the crystallization of honey. The honeys having GLU percentage higher than 35% and F/G ratio close to 1.0 significantly tend to crystallization. Our results are complied with the finding of Rybak-Chmielewska and Szczęsna (2003) in Poland (n=18, range= 1.05-1.30, mean= 1.15), Can et al. (2015) (n=7, mean= 1.29) and Cetin et al. (2011) (n=50, range= 1.01-1.85, mean= 1.22) in Turkey and Mendes et al. (1998) (n=15, range= 1.03-1.33, mean= 1.17) in Portugal.

Table 2. Mean sugar composition of the samples obtained from different locations of Turkey.
 Çizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama şeker bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama bileşimi Cizelge 2.Türkiye'nin farklı lokasyonlarından alınan numunelerin ortalama bileşimi Cizelge 2.Türkiye'nin farklı bileşimi Cizelge 2.Türkiye'nin farklı bileşim

Sugars (g/100g)							
Sample Code	FRU	GLU	SUC	MAL	F+G	F/G	CR
K1	32.33 ^a	35.41 ^g	0.08 ^b	0.00 ^a	67.74 ^b	0.91ª	С
K2	40.43 ^j	37.88 ⁱ	0.14 ^c	2.57 ^g	78.31 ^f	1.07 ^d	С
K3	35.36 ^b	36.30 ^h	0.08^{b}	0.23 ^b	71.66 ^d	0.97 ^b	С
U1	41.24^{1}	26.66 ^a	0.04^{ab}	3.74 ¹	67.90 ^b	1.55 ⁱ	-
U2	39.95 ⁱ	28.08 ^b	0.02^{a}	1.59 ^e	68.03 ^b	1.42 ^h	-
U3	38.89 ^h	33.16 ^f	0.00^{a}	0.76^{d}	72.06 ^d	1.17^{f}	-
U4	40.72 ^k	29.00 ^c	0.00^{a}	0.39 ^c	69.72°	1.40 ^h	-
S1	36.98 ^e	35.00 ^g	2.06 ^g	3.14 ^h	71.98 ^d	1.06 ^d	-
S2	37.93 ^f	30.02 ^d	3.02 ^h	4.38 ^k	67.95 ^b	1.26 ^g	-
S3	36.03 ^d	31.93 ^e	1.31 ^f	4.14 ^j	67.96 ^b	1.13 ^e	-
N1	35.83°	30.72 ^d	4.13 ⁱ	3.87 ⁱ	66.55 ^a	1.17^{f}	-
N2	38.70 ^g	37.89 ⁱ	0.24 ^d	2.33 ^f	76.58 ^e	1.02 ^c	С
N3	38.62 ^g	40.48^{j}	0.88 ^e	2.72 ^g	79.10 ^f	0.95 ^b	С
*N4	12.33	20.71	0.24	33.63	33.04	0.60	-
Min	32.33	26.66	0.0	0.00	66.55	0.91	
Max	41.24	40.48	4.13	4.38	79.10	1.42	
MEAN	37.92	33.27	1.01	2.29	71.20	1.12	
SD	2.48	4.17	1.32	1.53	4.29	0.23	
Different latters in each column correspond to significantly different values $(n < 0.05)$							

Different letters in each column correspond to significantly different values. (p < 0.05)

SD: Standart deviations of the samples belonged to each column

C: Crystallized honey

*: Adulterated honey sample was neglected from statistical analysis, minimum, maximum, mean and SD calculations.

3.3. Multivariate Analysis

The Sample N4, which is the adulterated one, was excluded from the PCA, Correlation Matrix, and HCA analyses, which are applied to the quality parameters, in order to achieve more robust and more objective results. Moreover, in order to reveal the effects of adulterated honey on the clustering, the sample N4 was included in HCA applied to the samples.

The first 4 principle components (PCs) explain 74.36% of the total variance and the variances of PC1, PC2, PC3, and PC4 are

28.30%, 19.61%, 15.74%, and 10.71%, respectively. The highest eigenvector in PC1 is explained by the acidity, fructose content, total phenolic compound, and sucrose content, whereas it is explained by glucose, F+G, and F/G in PC2, by diastase activity, maltose content, sucrose content, and HMF in PC3, and by moisture content, pH and BX in PC4 (Figure 1). Given the results of PCA applied to the quality parameters, it can be seen that the positive and negative correlations between the quality parameters constituting the PCs provide very important information about the factors directly affecting the quality of honey. As seen in Figure 1(a), PC1 is explained mainly by AC, FRU, and TP in the positive zone and SUC variations in negative zone. The positive correlation between TP and AC can be accepted as an important quality parameter. It is known that most of the TP compounds (e.g., flavonoids and phenolic acids) in the honey are acidic in character. To date, many phenolic compounds, which are acidic in character, such as gallic acid, chlorogenic acid, caffeic acid, pcoumaric acid, ferulic acid, and ellagic acid were determined in the honey (Pyrzynska and Biesaga, 2009). The acidity of honeys having a high level of TP content can be expected to be high. However, it should be noted that the phenolic content of the honey is significantly affected by the geographical region, climate conditions, and flora. Moreover, PC1 reveals the negative correlation between SUC and FRU. It can be seen that the parameters affected by the FRU and GLU, which are the main components of the honey, are clustered in PC2 (Figure 1(a)). PC2 mainly represents the positive variations in GLU and F+G and negative variations in F/G. Given these variations that were achieved from the analyses, for the samples with high GLU content, it can be stated that F+G can be high and F/G ratios can be low. PC3 contains mainly the quality parameters affected by the heat treatment (Fig 1(b)) and it can be clearly seen that HMF and MAL have a negative relationship with DA. As known, high-heat treatment applied to the honey causes an increase in HMF (Anklam, 1998) and decrease in DA. It can be stated that the quality of honey increases together with the increase in the negativity of the value of PC3. PC4 indicates that MC has a negative relationship with pH and BX values (Figure 1(c)). This can be interpreted in the way that the level of BX might be underdetermined for the honey samples with high MC values.



Figure 1. PCA loadings of quality properties; (a) PC1 versus PC2, (b) PC1 versus PC3, (c) PC1 versus PC4

Şekil 1. Kalite özelliklerinin Temel bileşen analizi (PCA) yüklemeleri; (a) PC1'e karşı PC2, (b) PC1'e karşı PC3, (c) PC1' ekarşı PC4

The dendrogram (Figure 2) of variables (quality factors) obtained from the HCA analysis shows that GLU, FPG (F+G), BX, and pH are in the same cluster. The results similar to

those obtained for PC2 loadings obtained from PCA were achieved in this group. SUC, MAL, and HMF, which were determined to be in the same cluster by using HCA, confirm the PC3 loadings. Similarly, AC, FRU, FGR and TP that are in the same cluster confirm PC1, whereas MC and DA confirm PC4 loadings.



Figure 1. Hierarchical cluster analysis (HCA) dendrogram of different quality parameters for honey samples

Şekil 2. Bal numuneleri için farklı kalite parametrelerinin hiyerarşik küme analizi (HCA) dendrogramı



Figure 2. Hierarchical cluster analysis (HCA) dendrogram of honey samples *Şekil 3.* Bal numunelerinin hiyerarşik küme analizi (HCA) dendrogramı

The dendrogram obtained from the HCA analysis performed in order to determine the clusters in the sample is presented in Figure 3. It can be seen that all the samples, except for the samples U4, S1, and N4, are grouped in two main clusters. The first main cluster consists of the samples K3, S3, K1, N1, N2, N3, S2, and K2, whereas the second cluster consists of samples U1, U2, and U3. The sample U4 has the highest DA, whereas the sample S1 has the highest HMF value. The sample N4 is the one, which was found to be mixed with maltose syrup. This result shows that the HCA is capable of effectively distinguishing the samples exhibiting characteristics different from the main cluster or clusters.

4. Conclusion

The honey is one of the most commonly demanded products because of the nutritional and medicinal characteristics attributed to different components of it. Because of its high commercial value, it is one of the food products exposed to adulteration at most. Simple analytical methods are enough for determining some of these adulterations, whereas some of the adulterations require more complex devices and analysis methods. The physicochemical characteristics are still important in determining the quality of honey, and the present study carried out on the multifloral honeys in Turkey corroborates this result. The results obtained indicate that the honeys have been adulterated despite all the legal precautions. Moreover, the present study also revealed that HMF and DA are important parameters in determining the quality of honey. The beekeepers in Turkey transport the hives to different regions in different seasons and this causes the honeybees be exposed to different floras and, to consequently, the multifloral honeys to have largely similar physicochemical characteristics. The results of the present study revealed that the sugar analyses performed using HPLC has an important role in determining the adulterations made by using the available sugar syrups. Furthermore, the multivariate data analyses methods such as PCA and HCA can be used in determining the relationships between the quality parameters of multifloral honeys, and they successfully distinguish the samples that do not meet the quality criteria (high HMF content, very high DA, adulterated product).

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