

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

# A CHEAPLY NON-DESTRUCTIVE TECHNIQUE TO ESTIMATE HONEY QUALITY: THERMAL IMAGING AND MACHINE LEARNING

Bal Kalitesini Tahmin Etmek İçin Ucuz, Tahribatsız Bir Teknik: Termal Görüntüleme ve Makine Öğrenimi

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### ABSTRACT

The aim of this study was to estimate honey quality based on proline and Brix content using a thermal imaging and machine learning algorithm. The proline, Brix and color properties of twenty honey samples were determined. Proline and Brix values were classified and estimated using the classification and regression tree (CART) algorithm. The mean proline and Brix content in honey samples was  $678.83 \pm 192.16$  mg/kg and  $83.2 \pm 0.79\%$ , respectively. CART analysis revealed that high proline honey samples had L values above 48.143 and b\* values below 35.416. In contrast, honey samples with low Brix values were characterized by L and a\* values below 55.860 and 53.660, respectively, and were identified as freshly harvested. The CART algorithm classified the proline and Brix values with an accuracy of 95% and 100%, respectively ( $p < 0.001$ ). As a result, whitish, bluish, blackish and greenish honeys are of higher quality due to high proline and low Brix content. However, to accurately assess honey quality based on its color traits, comprehensive studies with more honey samples and origin, are required.

**Keywords:** Adulteration, Proline, Brix, Honey quality, CART algorithm

### ÖZ

Bu çalışmanın amacı, termal görüntüleme ve makine öğrenmesi yaklaşımı kullanılarak baldaki prolin ve Brix içeriğine dayalı bal kalitesinin tahmin edilmesidir. 20 farklı bal örneğine ait prolin, Brix ve renk özellikleri belirlendi. Prolin ve Brix seviyeleri, sınıflandırma ve regresyon ağacı algoritması kullanılarak tahmin edildi ve sınıflandırıldı. Ballarda ortalama prolin ve Brix içeriği sırasıyla  $678,83 \pm 192,16$  mg/kg ve  $\%83,2 \pm 0,79$  olarak belirlendi. CART analizi ile yüksek prolinli balların L değerlerinin 48.143'ün üzerinde ve b\* değerlerinin ise 35.416'nın altında olduğu tespit edildi. Ancak, Brix değeri düşük olan balların ise sırasıyla 55.860 ve 53.660'ın altında L ve a\* değerlerine sahip olduğu ve yeni hasat edildiği bulunmuştur. CART algoritması ile prolin ve Brix seviyeleri sırasıyla %95 ve %100 doğrulukla sınıflandırdı ( $p < 0.001$ ). Sonuç olarak, beyazımsı, mavimsi, siyahımsı ve yeşilimsi balların yüksek prolin ve düşük Brix içeriği nedeniyle daha kaliteli olduğu belirlenmiştir. Ancak renk özelliklerine dayalı balın kalitesini doğru bir şekilde değerlendirmek için daha fazla ve farklı orijinli bal örnekleri ile kapsamlı çalışmalara ihtiyaç vardır.

**Anahtar kelimeler:** Tağşiş, Prolin, Brix, Balın kalitesi, CART algoritması

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### GENİŞLETİLMİŞ ÖZET

**Çalışmanın amacı:** Balda tağşiş, tüketicileri balın kalitesini ve piyasa değerini değerlendirme konusunda aldatılabilir. Piyasayı dengelemek ve balın besin değerini korumak için bal kalitesinin değerlendirilmesine yönelik basit ve uygun maliyetli yöntemler geliştirmek çok önemlidir. Balda yüksek prolin (mg/kg) ve düşük Brix (%) değerleri olgunluğun, saflığın ve optimal şeker-su dengesinin önemli göstergeleridir. Bu çalışmanın temel amacı, termal görüntüleme ve makine öğrenmesi yaklaşımı kullanılarak baldaki prolin ve Brix içeriğine dayalı bal kalitesinin tahmin edilmesidir.

**Gereç ve yöntem:** Bu araştırmada, Konya'nın Bozkır İlçesinde bulunan 13 arıcıdan 2020 ve 2021 yıllarına ait her biri bir kilogram ağırlığında yirmi benzersiz bal örneği kullanıldı. Bu bal örneklerinin prolin, Brix ve renk özellikleri sırasıyla IHC yöntemi, refraktometre ve ImageJ programı ile spektrofotometre kullanılarak belirlendi. Prolin ve brix (bağımlı değişkenler) ile renk özellikleri ve hasat yılı (bağımsız değişkenler) arasındaki ilişkileri tespit etmek için *t*-testi ve CART analizi SPSS 23.0 kullanılarak yapıldı. CART analizinde ana düğümler, alt düğümler ve çapraz doğrulama sırasıyla 6, 3 ve 5 olarak alındı. CART algoritmasının değerlendirme kriterleri R yazılımı kullanılarak gerçekleştirildi.

**Bulgular ve tartışma:** Kapsamlı bir inceleme sonrasında bal Brix'inin %81,9 ile %84,5 arasında değiştiği ve ortalama %83,2±0,79 olduğu ortaya çıkmıştır. Ayrıca prolin içeriği 322.64 mg/kg ile 1114.03 mg/kg arasında değişmekte olup ortalama 678.83±192.16 mg/kg olmuştur. *T*-testi, *ML* ve *Mb\** değerlerinin her iki değişkende de istatistiksel olarak anlamlı ( $p < 0.05$ ) olduğunu göstermiştir. Ayrıca Brix için HB grubunun *L* (69,6±2,9) ve *b\** (29,26±1,4) değerlerinin LB grubuna (*L*: 56,9±4,8 ve *b\**: 21,75±3,1) göre istatistiksel olarak yüksek olduğu görülmüştür. CART analizi ile yüksek prolinli balların *L* değerlerinin 48.143'ün üzerinde ve *b\** değerlerinin ise 35.416'nın altında olduğu tespit edildi. Ancak, Brix değeri düşük olan balların ise sırasıyla 55.860 ve 53.660'ın altında *L* ve *a\** değerlerine sahip olduğu ve yeni hasat edildiği bulunmuştur. CART algoritması ile prolin ve Brix seviyeleri sırasıyla %95 ve %100 doğrulukla sınıflandırdı ( $p < 0.001$ ). Ayrıca, Brix ile *L* ve *b\** (spektrofotometre) ve *a\** (ImageJ) değerleri arasında sırasıyla %48,5, %50,5 ve %46,9 oranında anlamlı pozitif korelasyon belirlenmiştir ( $p < 0.05$ ). Ancak, prolin ile balın renk özellikleri arasında kayda değer bir korelasyon bulunmamıştır. Bu araştırma, baldaki Brix ve prolin içeriğinin hem spektrofotometreler hem de termal görüntüleme

kameraları tarafından ölçüldüğü gibi, fark edilebilir renk özelliklerine bağlı olarak dalgalanabileceğini ortaya çıkarmıştır.

**Sonuç:** Bu çalışmada elde edilen en önemli sonuç, beyazımsı, mavimsi, siyahımsı ve yeşilimsi balların yüksek prolin ve düşük Brix içeriği nedeniyle daha kaliteli olmasıdır. Ancak bal kalitesinin renk özelliklerine dayalı belirlenebilmesi için farklı bal kökenlerine ilişkin daha detaylı çalışmalara ihtiyaç vardır. Bu şekilde gelecekteki uygulamalar, bal kavanozlarına bir renk skalasının uygulanmasını ve bal kalitesinin renge dayalı olarak analiz edilmesine olanak sağlayacaktır.

### INTRODUCTION

Honey bees are known as the most efficiently managed pollinators worldwide (Gaines-Day and Gratton 2016). Honey and beeswax, the main products of beekeeping, play a significant role in both global agriculture and human nutrition. In 2022, Türkiye is an intensive beekeeping country holding 8.984.676 colonies, and 95.386 beekeepers, and has a honey yield of 118.297 tons/year (TSI 2023). Quality is just as important as yield in honey production and is influenced by numerous factors. Known for their nutritional and medicinal benefits, honey and pollen are widely harvested and used. However, the low quality of these products can lead to health risks (Bayır 2019). Due to the variety of flower species, Türkiye produces high-quality honey worldwide (Sancak et al. 2013).

In Türkiye, proline content in honey can vary greatly and is often used as a measure of ripeness or sugar adulteration. According to the Honey Communiqué in the Turkish Food Codex, flower honey must contain at least 300 mg/kg of proline (Anonymous 2020). In Germany, it is assumed that non-ripe or adulterated honey contains less than 180 mg/kg of proline (Bogdanov 2002). It was reported that the amino acids proline, lysine and glutamic acid are most abundant in honey (Hışıl and Börekçioğlu 1986).

In addition, a possible connection between the proline content in honey and its antioxidant properties is pointed out (Meda et al. 2005). Additionally, studies have found that there is a negatively strong and consistent relationship between Brix and moisture content in honey (Anupama et al. 2003, Kanbur et al. 2021). It has been reported that the unusual correlation between honey moisture and sugar concentration may reveal signs of adulteration (Conti 2000). It is reported that

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there is a noticeable difference between the honey jam (including 0.625 kilograms of honey in lieu of sugar) and the sucrose jam in terms of Brix, which suggests that the former has a lower sugar (Kapira et al. 2023). When analyzing the studies, it becomes clear that the Brix content of honey can provide information about its sugar content. However, the Turkish Food Codex Honey Communiqué does not provide details about the Brix value of honey. In addition to the Brix value and proline, some spectrophotometrically determined color parameters were also examined as quality criteria for honey (Polat 2007, Bayır 2019, Boistean et al. 2021).

It is important to know that honey can have a spectrum of colors ranging from yellow and amber tones to transparent white or deep red. This variance is influenced by elements such as the honey's botanical origin, nectar composition, harvesting techniques, and temperature and storage duration (Shafiee et al. 2013, Becerril-Sánchez et al. 2021). Interestingly, the effect of color properties obtained from thermal images (analyzed in the ImageJ program) on proline and Brix content in honey has not been investigated in the current literature. However, such research is crucial for evaluating the quality of honey.

In Türkiye it is possible to quickly determine the proline and Brix content of honey, but these are currently not required to be declared on the label. With comprehensive studies similar to the current study, it is expected that a smartphone application could allow consumers to analyze honey images and assess the quality of honey during the purchasing process. Therefore, the aim of this study is to find out whether the color properties of honey determined by ImageJ and spectrophotometry can estimate proline and Brix concentrations in honey. This contemporary research is the first to analyze the relationship between the color properties of honey (using thermal imaging) and its proline and Brix content on a global scale.

### MATERIALS AND METHODS

#### The collection and analysis of honey samples

Twenty honey samples (each weighing one kilogram) collected in 2020 and 2021 from 13 beekeepers in Bozkır District of Konya at latitude: 37.171860 and longitude: 32.216846 were used in this study. These honey samples were not differentiated according to regional differences, but rather according to the different care and feeding practices of the beekeepers. Although practices may

vary among beekeepers, bee colonies generally fed sugar syrup and cake during the summer and winter months. Samples were stored between 10 and 30 °C to replicate typical storage conditions and analyzed for proline and Brix content. The proline concentration was determined using the IHC method, which was carried out by specialists from various food analysis and research institutions (Bogdanov 2002). The digital refractometer (Atago PAL-1) was used to measure the Brix content, with three measurements being averaged (James et al. 2009). Before changing samples in the refractometer analysis, cleaning and calibration with distilled water were carried out for precise measurements.

#### Thermal image processing

Thermographic cameras have proven to be a reliable option for food analysis due to their ability to examine samples non-destructively and easily (Izquierdo 2020). In this research, honey images were captured utilizing a thermal imaging camera (Flir One Pro, Android version, P/N 435-0007-01) under controlled environmental conditions; temperature (24-26 °C), humidity (60-70%), the distance between samples and the camera (25 cm) and sample cup (150 mm gamma sterile). The thermal camera functions within a range of 0 to 35 °C and can identify different temperatures (-20 to 400 °C). Image values were obtained by creating uniform-width circles on the honey samples using the ImageJ. Subsequently; L\*, a\*, and b\* values were analyzed to ascertain the black/white, green/red, and blue/yellow color changes utilizing ImageJ. In addition, hue (H), saturation (S), brightness (B), red (R), green (G) and blue (B) were also defined. Finally, the color properties (L, a\* and b\*) were analyzed spectrophotometrically (Konica Minolta CM-700d). Four measurements were taken for each sample, and the mean values for mean L (ML), mean a\* (Ma\*) and mean b\* (Mb\*) were calculated from these measurements. The mean values of color properties of ImageJ and spectrophotometer were used in the analysis.

#### Statistical test

CART analysis was performed to detect the associations between the traits (proline and Brix) and the factors (color properties and year of harvest). The CART algorithm is a prevalent decision tree method in the academic literature that splits clusters into two distinct subsets (Breiman et al. 1984, Kayri and Boysan 2008, Coşkun et al. 2023). The CART algorithm obviates data normality

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and homogeneity that is needed for assumptions intrinsic to multiple regression analysis (Kayri and Boysan 2008). For this investigation, a threshold of 650 mg/kg demarcated a decision tree, classifying honey as either high or low in proline content. Specifically, these are honeys of classes 1 (proline > 650 mg/kg) and 0 (proline < 650 mg/kg). Similarly, an 83% Brix threshold was set to devise a decision tree, differentiating honey into high Brix (marked as 0) or low Brix (marked as 1) classifies. When allocating Brix metrics, the nexus between Brix and honey quality was acknowledged, with honey registering low Brix values (attributed to reduced sugar content) being designated as 1 (Kapira et al. 2023).

The Gini index (L) served as the basis for classification. It was calculated based on the

equation  $L = 1 - \sum_{i=1}^j p_i^2$ . Where;  $j$  is the number of classes in the data and  $p_i$  is the relative frequency of each class in the total dataset. When the dataset is divided into two subsets ( $L_1$  and  $L_2$ ) based on feature A, the Gini index ( $L_A$ ) is computed using the respective dimensions  $n_1$  and  $n_2$  (Tangirala 2020).  $L_A$  was calculated with subsequent formula;  $L_A = (\frac{n_1}{n} * L_1) + (\frac{n_2}{n} * L_2)$ . The depletion of impurity ( $\Delta A$ ) was calculated using the following equation:  $\Delta A = L - L_A$  (Tangirala 2020). Accuracy, sensitivity and specificity were calculated using the equations (Mikail and Keskin 2015, Aytekin et al. 2018). The confusion matrix of the CART algorithm classifier is presented in Table 1.

**Table 1.** CART algorithm classifier's confusion matrix

Studied Traits Variable	Observed High Low	Estimated	
		High k m	Low l n
$Accuracy = (k + n)/(k + l + m + n)$		1	
$Sensitivity = k/(k + l)$		2	
$Specificity = n/(m + n)$			3
$Error\ proportion = 1 - [(k + n)/(k + l + m + n)]$		3	
$se_{AUC} = \sqrt{\frac{AUC(1-AUC)+(n_A-1)(q1-AUC^2)+(n_B-1)(q2-AUC^2)}{n_A n_B}}$		4	
$n_a = k + m$ and $n_b = b + d$		5	
$q1 = \frac{AUC}{2-AUC}$ and $q2 = \frac{2AUC^2}{1+AUC}$		6	

In the equation; k and m indicate true and false positives, while l and n indicate true and false negatives. The area under the ROC (AUC) was determined with the formula provided by Hanley and McNeil (1982). Analysis of CART, including parent nodes (6), child nodes (3), and cross-validation (5), as well as t-test, was applied utilizing SPSS 23.0 (IBM Corp. 2015). The evaluation criteria for the CART algorithm were performed using R software (R Core Team 2019, Eydurán 2020).

### RESULTS

The minimum, maximum and mean values were 81.9, 84.5 and 83.2±0.79% for Brix and 322.64,

1114.03 and 678.83±192.16 mg/kg for proline. Furthermore, L, a\*, and b\* values were determined as 63.87, 1.38, and 25.88 using the spectrophotometer, respectively. Additionally, the following values were obtained by image processing: L = 52.405, a\* = 52.619, b\* = 20.309, hue = 177.450, saturation = 203.616, brightness = 192.199, red = 183.903, green = 48.967, and blue = 121.078.

### Some descriptive statistics

Some descriptive statistics and the t-test results related to the color properties for Brix and proline are presented in Table 2.

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**Table 2.** T-test results of effective factors on proline and Brix

Effective factors	Traits	Variable	Code	N	Minimum	Maximum	$\bar{X} \pm S_x$	$S_x$	
Year	Brix	High Brix	0	11	2020	2021	2020.55±0.16	0.522	
		Low Brix	1	9	2020	2021	2020.56±0.18	0.527	
	Proline	High Proline	1	9	2020	2021	2020.56±0.18	0.527	
		Low Proline	0	11	2020	2021	2020.55±0.16	0.522	
Spectrophotometer	ML	Brix	High Brix	0	11	56.21	80.37	69.6±2.9 <sup>a</sup>	9.55
			Low Brix	1	9	30.72	79.44	56.9±4.8 <sup>b</sup>	14.50
		Proline	High Proline	1	9	30.72	80.37	63.4±5.5	16.4
			Low Proline	0	11	47.27	80.27	64.3±3.3	11.1
	Ma*	Brix	High Brix	0	11	-2.243	4.862	0.64±0.77	2.54
			Low Brix	1	9	-1.407	4.748	2.29±0.62	1.87
		Proline	High Proline	1	9	-1.407	4.748	1.61±0.86	2.57
			Low Proline	0	11	-2.243	4.862	1.20±0.69	2.29
	Mb*	Brix	High Brix	0	11	18.21	36.58	29.26±1.4 <sup>a</sup>	4.57
			Low Brix	1	9	12.35	35.14	21.75±3.1 <sup>b</sup>	9.39
		Proline	High Proline	1	9	14.71	34.60	26.80±2.5	7.37
			Low Proline	0	11	12.35	36.58	25.13±2.6	8.62
L	Brix	High Brix	0	11	46.545	55.613	51.456±0.988	3.275	
		Low Brix	1	9	48.294	57.807	53.564±1.041	3.124	
	Proline	High Proline	1	9	48.294	55.891	52.837±0.941	2.822	
		Low Proline	0	11	46.545	57.807	52.051±1.131	3.750	
a*	Brix	High Brix	0	11	50.324	55.636	53.390±0.565	1.873	
		Low Brix	1	9	48.343	53.988	51.675±0.789	2.366	
	Proline	High Proline	1	9	48.526	54.653	52.322±0.751	2.253	
		Low Proline	0	11	48.343	55.636	52.861±0.690	2.288	
b*	Brix	High Brix	0	11	-2.241	36.304	16.392±4.511	14.962	
		Low Brix	1	9	2.632	47.356	25.096±4.741	14.222	
	Proline	High Proline	1	9	2.632	34.782	21.473±4.125	12.376	
		Low Proline	0	11	-2.241	47.356	19.356±5.208	17.274	
Hue	Brix	High Brix	0	11	23.630	235.066	177.436±21.139	70.111	
		Low Brix	1	9	12.025	235.875	177.468±24.486	73.458	
	Proline	High Proline	1	9	93.924	235.066	196.918±16.049	48.146	
		Low Proline	0	11	12.025	235.875	161.522±24.786	82.206	
Saturation	Brix	High Brix	0	11	175.911	233.114	205.221±6.234	20.676	
		Low Brix	1	9	178.585	229.359	201.655±6.355	19.064	
	Proline	High Proline	1	9	175.911	229.359	200.788±6.783	20.350	
		Low Proline	0	11	179.890	233.114	205.930±5.879	19.498	
Brightness	Brix	High Brix	0	11	162.680	230.975	190.320±7.948	26.362	
		Low Brix	1	9	163.177	233.442	194.496±8.725	26.176	
	Proline	High Proline	1	9	163.177	222.432	189.903±7.694	23.083	
		Low Proline	0	11	162.680	233.442	194.077±8.617	28.580	
Red	Brix	High Brix	0	11	120.130	231.151	180.352±11.785	39.086	
		Low Brix	1	9	134.421	233.473	188.244±11.876	35.628	
	Proline	High Proline	1	9	134.421	222.564	183.687±10.827	32.480	
		Low Proline	0	11	120.130	233.473	184.080±12.541	41.593	
Green	Brix	High Brix	0	11	14.334	105.102	47.036±9.385	31.128	
		Low Brix	1	9	17.145	104.362	51.326±9.938	29.814	
	Proline	High Proline	1	9	17.145	82.100	45.285±7.955	23.866	
		Low Proline	0	11	14.334	105.102	51.979±10.494	34.804	
Blue	Brix	High Brix	0	11	49.944	163.196	123.754±12.271	40.700	
		Low Brix	1	9	48.723	162.953	117.807±13.148	39.445	
	Proline	High Proline	1	9	75.846	162.953	125.848±10.735	32.205	
		Low Proline	0	11	48.723	163.196	117.175±13.657	45.296	

a, b: p < 0.05,  $\bar{X}$ : Mean,  $S_x$ : Standard error,  $S_x$ : Standard deviation

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T-test analysis revealed that some color properties, derived from thermal image processing and spectrophotometric techniques, affected proline and Brix (Table 2). The *t*-test outcomes indicated that the ML and Mb\* values were statistically significant ( $p < 0.05$ ) across both variables. Additionally, for Brix, the L ( $69.6 \pm 2.9$ ) and b\* ( $29.26 \pm 1.4$ ) values of the HB group were found to be statistically higher than the

LB group (L:  $56.9 \pm 4.8$  and b\*:  $21.75 \pm 3.1$ ). All other independent variables were found to be insignificant (Table 2).

### The CART analysis

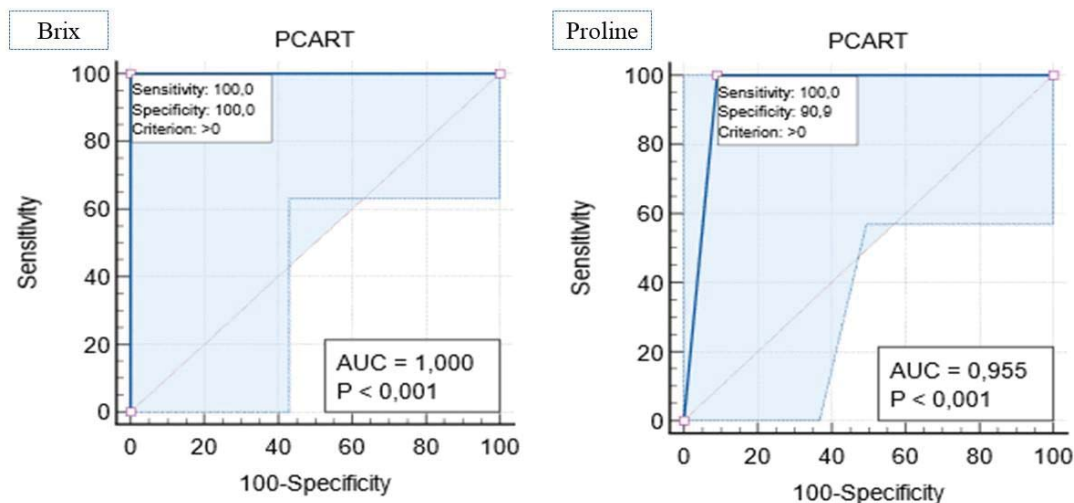
The precision of the CART algorithm in classification is shown in Table 3.

**Table 3.** CART algorithm's Confusion Matrix for Traits

Traits	Observed	Estimated		
		High Proline	Low Proline	Accuracy (%)
Proline	High Proline	10	1	90.9
	Low Proline	0	9	100.0
	Total (%)	50.0	50.0	95.0
Brix		High Brix	Low Brix	Accuracy (%)
	High Brix	11	0	100.0
	Low Brix	0	9	100.0
	Total (%)	55.0	45.0	100.0

The algorithm exhibited a high accuracy rate, classifying high proline honey samples with 90.9% accuracy and achieving perfect accuracy for low proline honey samples. Similarly, for Brix values, the

algorithm maintained a consistent 100% accuracy for both high and low classes. Figure 1 displays the ROC curve, further illustrating the classification effectiveness of the CART algorithm for traits.



**Figure 1.** ROC Curves for Traits via CART Algorithm

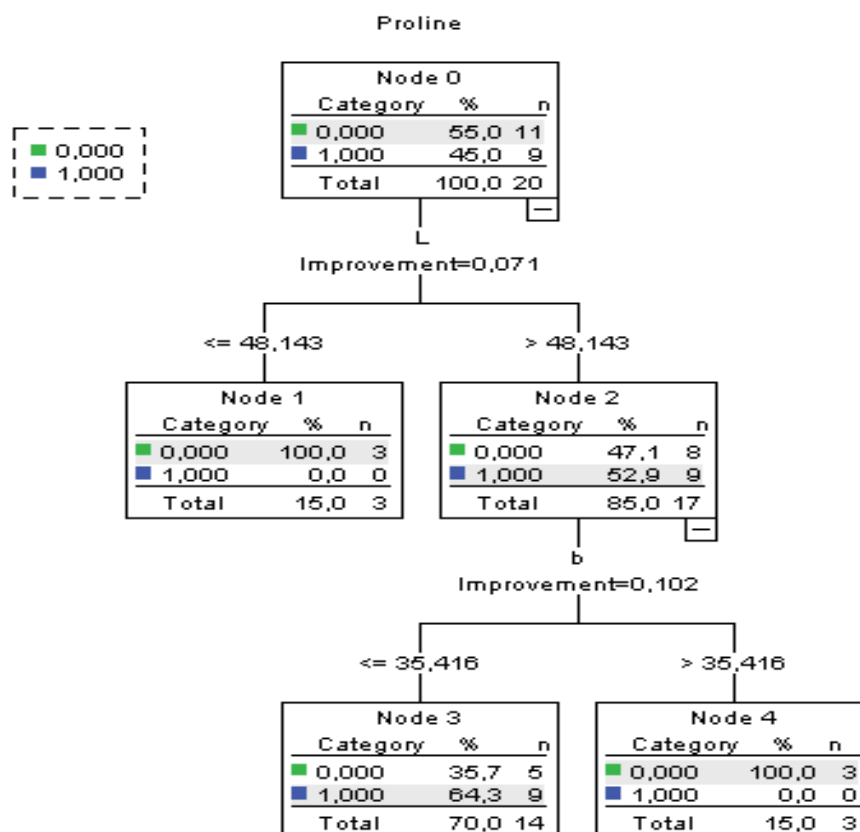
According to the area under the ROC curve (AUC), the classification performance of the CART algorithm in determining traits was significant ( $p < 0.01$ ). In addition, the performance of the CART algorithm for feature classification was presented in Table 4.

The accuracy, sensitivity, and specificity rates of the CART algorithm were 95.00%, 100.00%, and 90.91% for proline and 100.00% of all rates for Brix. Furthermore, the classification tree diagram of the CART algorithm for proline was shown in Figure 2.

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**Table 4.** The CART Algorithm's Classification Performance for Traits

Traits	Sensitivity	Specificity	AUC	Accuracy	P	Pairwise comparison of ROC curves
Proline	100.00	0.9091	0.955±0.0455	0.9500	<0.0001	P=0.0014
Brix	100.00	100.00	1.000±0.000	100.00	<0.0001	



**Figure 2.** The CART Algorithm's Classification Tree Diagram for proline

Node 0 included HP (9) and LP (11) honeys with 45% and 55%. Within Node 0, two distinct sub-branches emerged, predominantly based on the L value, the pivotal independent variable for delineating proline levels in honey samples (Nodes 1 and 2). Specifically, in Node 1, all specimens possessing L values  $\leq 48.143$  were designated as LP. Conversely, within Node 2, 52.9% of samples with L values  $> 48.143$  were identified as HP, while the remaining 47.1% were classified as LP. Node 2

is further separated into Node 3 (64.3% of honey samples with  $b \leq 35.416$  values were labeled as HP and the remaining 35.7% as LP) and Node 4 (exhibiting b values  $> 35.416$ , all samples were classified as LP. Notably, proline values across Nodes 1, 3, and 4 demonstrated homogeneity, denoting a comprehensive differentiation culminating in the terminal node. The classification tree diagram of the CART algorithm for Brix was presented in Figure 3.

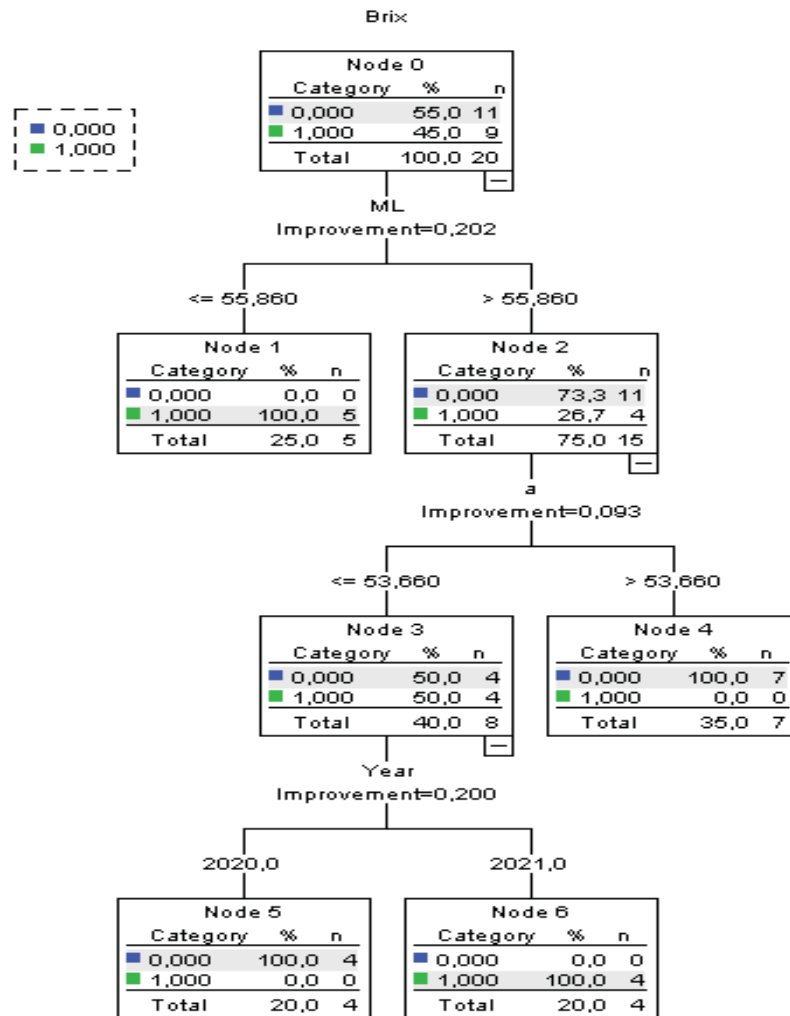


Figure 3. The CART Algorithm's Classification tree diagram for Brix

Node 0 contained HB (11) and LB (9) honey samples with 55% and 45%. The ML in Node 0 is divided into two distinct subnodes (honeys with  $ML \leq 55.860$  were designated as LB in Node 1, on the other hand, when the  $ML > 55.860$ , 26.7% of the samples are labeled as LB, while 73.3% are identified as HB in Node 2). In the structure where node 2 branches based on the  $a^*$  value, the samples were equally classified as HB and LB in node 3 ( $a^* \leq 53.660$ ), while all samples were classified as HB in node 4 ( $a^* > 53.660$ ). More detailed, Node 3 branches into Nodes 5 and 6 based on the year the honey was harvested, and these nodes identify all samples as LB (2021)

and HB (2020), respectively. Concerning the Brix values, uniformity is achieved across Nodes 1, 4, 5, and 6, culminating in a finalized differentiation at the terminal node.

**Correlation analysis**

In this section, the associations between dependent and independent variables are given in Table 5.



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**Table 5.** Correlation matrix for effective factors and traits

Traits/ Factors	Brix	Proline	Year	ML	Ma*	Mb*	L	a*	b*	Hue	Sat.	Bri.	Red	Green	Blue
Brix	1														
Proline	.04	1													
Year	-.06	-.16	1												
ML	.49 <sup>a</sup>	-.16	.11	1											
Ma*	-.23	.35	-.19	-.78 <sup>A</sup>	1										
Mb*	.51 <sup>a</sup>	.12	.22	.58 <sup>A</sup>	-.15	1									
L	-.28	.12	-.63 <sup>A</sup>	-.28	.14	-.36	1								
a*	.47 <sup>a</sup>	.04	.30	.17	.04	.26	-.80 <sup>A</sup>	1							
b*	-.26	.10	-.71 <sup>A</sup>	-.24	.12	-.37	.99 <sup>A</sup>	-.78 <sup>A</sup>	1						
Hue	.02	.09	.59 <sup>A</sup>	.04	-.08	.05	-.55 <sup>a</sup>	.57 <sup>A</sup>	-.63 <sup>A</sup>	1					
Sat.	-.01	-.23	.79 <sup>A</sup>	.05	-.09	.28	-.78 <sup>A</sup>	.34	-.80 <sup>A</sup>	.33	1				
Bri.	-.03	.10	-.88 <sup>A</sup>	-.03	.12	-.19	.82 <sup>A</sup>	-.51 <sup>a</sup>	.88 <sup>A</sup>	-.74 <sup>A</sup>	-.85 <sup>A</sup>	1			
Red	-.03	.14	-.82 <sup>A</sup>	-.12	.15	-.24	.89 <sup>A</sup>	-.51 <sup>a</sup>	.92 <sup>A</sup>	-.60 <sup>A</sup>	-.91 <sup>A</sup>	.97 <sup>A</sup>	1		
Green	-.03	.07	-.84 <sup>A</sup>	-.07	.14	-.17	.83 <sup>A</sup>	-.57 <sup>A</sup>	.89 <sup>A</sup>	-.82 <sup>A</sup>	-.78 <sup>A</sup>	.98 <sup>A</sup>	.95 <sup>A</sup>	1	
Blue	0.04	-.07	.85 <sup>A</sup>	.06	-.13	.18	-.82 <sup>A</sup>	.57 <sup>A</sup>	-.89 <sup>A</sup>	.82 <sup>A</sup>	.78 <sup>A</sup>	-.99 <sup>A</sup>	-.94 <sup>A</sup>	-1.0 <sup>A</sup>	1

<sup>a</sup>: p < 0.05, <sup>A</sup>: p < 0.01, Sat: Saturation, Bri: Brightness

**Table 6.** Literature on the honey proline and Brix content as well as the color properties

References	Honey origin	N	Proline (mg/kg)	Brix (%)	Spectrophotometer									
					L	a*	b*							
Meda et al. (2005)	Various (Burkina Faso)	27	989.5±407.4	-	-	-	-							
Anupama et al. (2003)	Various (Indian)	11	-	78.6±1.88	34.65±5.20	12.44±6.41	49.92±7.96							
Bayram (2019)	Various (Türkiye)	12	747.67±144.37	-	-	-	-							
Kanbur et al. (2021)	Highland	6	755.14±114.8 <sup>b</sup>	82.5±2.43	75.85±5.26 <sup>A</sup>	13.03±5.81 <sup>B</sup>	77.5±8.87							
	Chestnut							Türkiye	5	925.16±126.0 <sup>a</sup>	81.2±0.84	43.53±3.34 <sup>B</sup>	41.4±1.42 <sup>A</sup>	68.07±5.12
Conti (2000)	Various (Lazio)	84	-	82.03±1.07	-	-	-							
Polat (2007)	Various (Türkiye)	40	-	80.9	23.01	0.24	0.13							
Boistean et al. (2021)	Multiflora (Tunisia)	-	-	-	24.51±0.10	3.96±0.05	8.64±0.08							
	Multiflora (Romania)	-	-	-	37.64±0.06	1.40±0.01	20.39±0.05							
	Multiflora (Moldova <sub>1</sub> )	-	-	-	27.99±0.12	3.25±0.04	12.63±0.07							
	Multiflora (Moldova <sub>2</sub> )	-	-	-	33.03±0.09	2.27±0.01	18.96±0.11							
Haroun (2006)	Pine	7	853.86±163.48	82.45±0.83	26.36±0.83	1.69±0.81	9.63±1.25							
	Cotton							Türkiye	5	712.80±151.21	81.07±1.40	29.42±2.91	-0.046±0.52	6.96±1.54
	Chestnut								4	1292.75±176.05	81.41±2.59	25.09±1.71	3.81±1.11	9.13±2.44
	Sunflower								3	680.33±97.45	80.91±0.73	27.45±1.31	0.33±1.02	12.01±1.88
	Highland								3	609.33±147.89	82.55±1.75	29.12±0.84	0.34±0.27	6.90±2.70
	Multiflora								4	966.75±366.50	80.42±1.43	28.18±0.66	1.12±0.80	9.15±1.24
	Oak								1	544	84.47	23.84	3.77	7.35
	Citrus								1	574	82.48	27.90	0.60	8.62
	Various								14	888.86±263.16	81.79±1.12	27.38±1.01	0.70±0.67	7.95±1.72
Adulterated	2	0.00±0.00	82.54±0.52	28.57±0.64	0.66±0.84	12.89±0.35								
Eker et al. (2017)	Various (Centre)	-	-	83.6±1.4	60.47±2.1 <sup>ab</sup>	-0.81±0.12 <sup>c</sup>	6.17±0.84 <sup>b</sup>							
	Various (Kagızman)							Kars/Türkiye	-	-	84.9±1.0	59.99±4.7 <sup>ab</sup>	-0.81±0.36 <sup>c</sup>	4.76±0.5 <sup>bc</sup>
	Various (Digor)								-	-	81.8±0.8	56.05±7.6 <sup>ab</sup>	-0.27±0.3 <sup>abc</sup>	4.63±1.1 <sup>bc</sup>
	Various (Selim)								-	-	79.4±1.0	64.50±7.80 <sup>a</sup>	-0.44±0.28 <sup>bc</sup>	3.43±0.1 <sup>cd</sup>
	Various (Sarıkamış)								-	-	80.6±1.4	46.12±0.40 <sup>b</sup>	-0.51±0.07 <sup>bc</sup>	10.30±1.2 <sup>a</sup>
	Various (Susuz)								-	-	82.6±2.8	66.62±1.88 <sup>a</sup>	0.36±0.08 <sup>a</sup>	-0.58±0.1 <sup>c</sup>
	Various (Arpacay)								-	-	82.8±1.0	66.17±1.40 <sup>a</sup>	-0.30±0.0 <sup>ab</sup>	1.76±0.1 <sup>d</sup>
Geană et al. (2020)	Acacia	16	-	81.62±2.24	-	-	-							
	Rape							Romania	14	-	81.05±2.17	-	-	-
	Polyfloral								3	-	81.27±4.02	-	-	-
	Linden								2	-	81.12±0.99	-	-	-
	Honeydew								4	-	85.36±1.73	-	-	-
	Sunflower								4	-	83.62±2.73	-	-	-
	Mixed								5	-	82.02±1.02	-	-	-
Commercially	12	-	82.37±1.18	-	-	-								

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Continuation of Table 6

References	Honey origin	N	Proline (mg/kg)	Brix (%)	Spectrophotometer			
					L	a*	b*	
Khalafi et al. (2016)	Coriander (Tiran and Karvan)	Iran	1	-	-	18.98±0.03 <sup>i</sup>	23.01±0.02 <sup>a</sup>	31.64±0.04 <sup>f</sup>
	Dill (Tiran and Karvan)		1	-	-	29.94±0.02 <sup>i</sup>	18.55±0.03 <sup>b</sup>	47.68±0.02 <sup>c</sup>
	Ziziphus (Borazjan)		1	-	-	41.19±0.16 <sup>d</sup>	14.29±0.03 <sup>c</sup>	59.98±0.04 <sup>a</sup>
	Thyme (Damavand)		1	-	-	41.39±0.00 <sup>c</sup>	11.34±0.00 <sup>d</sup>	49.81±0.02 <sup>b</sup>
	Parsley (Tiran and Karvan)		1	-	-	45.63±0.00 <sup>a</sup>	5.14±0.00 <sup>f</sup>	36.50±0.02 <sup>e</sup>
	Qangal (Shahr-e-Kord)		1	-	-	45.05±0.03 <sup>b</sup>	5.41±0.01 <sup>e</sup>	37.67±0.03 <sup>d</sup>
	Astragal (Shahr-e-Kord)		1	-	-	30.12±0.02 <sup>h</sup>	2.63±0.01 <sup>i</sup>	23.91±0.01 <sup>h</sup>
	Alfalfa (Baharestan)		1	-	-	31.03±0.01 <sup>g</sup>	2.94±0.01 <sup>g</sup>	24.85±0.01 <sup>g</sup>
	Tamarisk (Shahr-e-Kord)		1	-	-	32.47±0.00 <sup>f</sup>	2.27±0.01 <sup>i</sup>	21.80±0.02 <sup>j</sup>
Orange blossom (Shiraz)	1	-	-	33.10±0.10 <sup>e</sup>	2.77±0.03 <sup>h</sup>	23.22±0.11 <sup>i</sup>		
Kek et al. (2017)	Tualang (Malaysia)	2	-	-	26.52±0.24 <sup>bc</sup>	1.42±0.04 <sup>a</sup>	2.96±0.11 <sup>bc</sup>	
	Gelam (Malaysia)	2	-	-	26.06±0.11 <sup>bcd</sup>	2.10±0.10 <sup>a</sup>	2.78±0.11 <sup>bc</sup>	
	Pineapple (Malaysia)	2	-	-	27.31±0.91 <sup>ab</sup>	2.05±0.70 <sup>a</sup>	3.59±0.26 <sup>b</sup>	
	Borneo (Malaysia)	2	-	-	25.86±0.26 <sup>cd</sup>	1.45±0.16 <sup>a</sup>	2.66±0.16 <sup>bc</sup>	
	Kelulut (Malaysia)	3	-	-	24.90±1.38 <sup>de</sup>	1.90±0.49 <sup>a</sup>	2.52±1.05 <sup>c</sup>	
	Manuka (Australia)	1	-	-	23.70±0.04 <sup>e</sup>	0.09±0.02 <sup>b</sup>	0.15±0.02 <sup>d</sup>	
	Commercial Y (Malaysia)	1	-	-	29.03±0.01 <sup>a</sup>	2.02±0.03 <sup>a</sup>	7.68±0.07 <sup>a</sup>	
	Commercial Z (Malaysia)	1	-	-	25.94±0.02 <sup>bcd</sup>	1.90±0.03 <sup>a</sup>	2.64±0.03 <sup>bc</sup>	
Al-Farsi et al. (2018)	Sidr	Oman	29	449	82.5	-	-	-
	Sumer		21	877	82	-	-	-
	Multiflora		8	487	82.2	-	-	-
Cengiz et al. (2018)	Pure floral (Türkiye)	19	546±80	82.8±1.1	-	-	-	
Nagai et al. (2018)	Acacia (Hungary)	1	-	80.9±0.1	11.97±1.03	5.19±0.32	24.18±2.11	
	Orange (Mexico)	1	-	79.7±0.1	3.58±0.19	4.24±0.25	3.09±0.14	
	Lavender (Spain)	1	-	82.1±0.1	4.83±0.37	5.96±0.49	4.91±0.33	
	Blueberry (Canada)	1	-	82.2±0.1	5.65±0.51	10.57±0.84	6.30±0.61	
	Litchi (China)	1	-	79.2±0.1	4.20±0.22	3.85±0.17	3.08±0.19	
	Coffee (Guatemala)	1	-	79.7±0.1	3.32±0.09	1.55±0.04	1.77±0.06	
Islam et al. (2022)	Jarrah honey	11	-	82.62±0.23	-	-	-	
Aykas (2023)	Various (Türkiye and USA)	147	-	78.7±3.2	-	-	-	
Mehdi et al. (2023)	Euphorbia (Morocco)	1	-	80.67	-	-	-	
	Euphorbia (Morocco)	1	-	81.13	-	-	-	
	Carob (Morocco)	1	-	80.60	-	-	-	
	Carob (Morocco)	1	-	78.53	-	-	-	
	Arbutus (France)	1	-	81.67	-	-	-	
	Arbutus (Morocco)	1	-	80.07	-	-	-	

a, b: p<0.05, A, B: p<0.01

There was a significant positive correlation between Brix and the values of L and b\* (spectrophotometer), and a\* (ImageJ) at rates of 48.5%, 50.5%, and 46.9%, respectively (p<0.05). Conversely, there was an absence of a notable correlation between proline and the color characteristics of honey. Nevertheless, a positive correlation of 58.8%, 79.0% and 85.3% was found between crop year and hue, saturation and blue values (p< 0.01). Furthermore, pronounced

inverse relationships (p< 0.01) were noted between the harvest year and values of L, b\*, brightness, red, and green, with percentages of 63.1%, 71.3%, 87.7%, 82.2%, and 84.4%, respectively. In the study, a positive correlation of 77.9% was found between L and a\*, while a negative correlation of 57.5% was observed between L and b\* (p< 0.01). Additionally, substantial relationships, both positive and negative, were evident across color properties extracted from

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thermal imaging. In the correlation matrix, the honey color tends to be white, red, and yellow as Brix content increases. Nonetheless, no linkage was identified between proline and Brix contents of honey samples. Furthermore, fluctuations were observed in the thermal image-derived color properties across years, implying alterations in honey color over storage durations (Table 5).

### DISCUSSION

The color of honey is an important sensory characteristic in the apiculture sector; Consumers pay particular attention to this quality feature. Studies suggest that the HSB color model is an alternative to the Pfund colorimeter, which is based on an optical comparison method characterized by labor intensity, time waste and subjectivity (Dominguez and Centurión 2015). Table 6 provides a comprehensive overview of various research findings on the proline and Brix content as well as the color properties of honey.

The mean Brix content of honey, determined in this study, was lower than that reported for oak, honeydew, sunflower honey and various types of honey (Haroun 2006; Eker et al. 2017; Geană et al. 2020), conversely higher than in other studies. The mean proline content of honey, detected in the current study, was higher than different studies of the amounts found in highland, oak, citrus, Sidr, multiflora and pure floral honey (Haroun 2006, Al-Farsi et al. 2018, Cengiz et al. 2018), however lower than in other studies. In summary, the proline and Brix content of honey determined in this study was different from the other studies. The L value of honey in this study was lower compared to specific varieties from Kars (Selim, Susuz, and Arpacay) and highland honey (Eker et al. 2017, Kanbur et al. 2021), but higher than others. Regarding  $a^*$ , the mean  $a^*$  value of the honey samples in this study was higher than of cotton, sunflower, highland, multiflora, citrus, diverse, adulterated, Manuka and various honey types (Haroun 2006, Polat 2007, Eker et al. 2017, Kek et al. 2017), but lower than other studies. The  $b^*$  value for the honey samples in this study was lower than the values reported for coriander, ziziphus, thyme, various types of honey, highland and chestnut honey (Anupama et al. 2003, Khalafi et al. 2016, Kanbur et al. 2021), but higher than in other studies. Overall, the mean L,  $a^*$  and  $b^*$  values in this study were comparatively high, low, and high, respectively, compared to other studies. It can be

concluded that differences between Brix and proline content in different honey samples are mainly due to their different origins.

The results of this study suggest that the color of honey changes from a darker shade reminiscent of black to a lighter shade as the Brix value of its sugar concentration increases. In addition, a shift in the color spectrum towards yellow and blue can be observed with an increase or decrease in the Brix value of honey. From the present results, it can be deduced that honey varieties with darker and bluish tones have a reduced Brix value and therefore have a lower sugar content than honey varieties with lighter and more yellowish tones. However, there is no compelling evidence of a direct relationship between Brix levels, proline content and other color properties of honey. In another study, highland and chestnut honey varieties were compared; Proline-rich honey samples were found to have decreased L values but increased  $a^*$  values (Kanbur et al. 2021). This contradicts the claims of the current study, which indicated that honeys with darker and redder hues contained a higher concentration of proline than honeys with lighter and greener hues. Using regression tree analysis, this research also found that honeys that appear whiter or bluer generally contain higher levels of proline than honeys that appear darker or yellowish.

Anupama et al. (2003) found that the L,  $a^*$ , and  $b^*$  values for honey (measured by spectrophotometer) ranged from 23.77 to 43.69, 3.4 to 27.83, and 39.11 to 68.54, respectively. Their study found that the L,  $a^*$  and  $b^*$  values had no significant impact on the Brix value of the honey. In contrast, Eker et al. (2017) claimed that although honey samples from different sources had comparable Brix values, they had differences in their L,  $a^*$  and  $b^*$  values. Kapira et al. (2023) highlighted a significant decrease in Brix value of honey jam compared to sucrose jam after 14 days of storage, suggesting possible fermentation or degradation of sucrose due to microbial activity. On the contrary, the results of the current study are different from reported studies in terms of L value (Anupama et al. 2003, Eker et al. 2017, Kapira et al. 2023). This study found that honey samples that had darker tones, such as blackish or greenish tones, and were freshly harvested generally had lower Brix levels than honey with lighter tones, such as whitish or reddish tones, that was previously harvested. Also, no significant correlation was observed between the color of honey and the content of proline and Brix as well as harvest

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time. The variation in color traits in different harvest years as well as the consistent proline and Brix values indicate that color is not the only indicator of honey quality. Polat (2007) highlighted that darker honeys have high mineral concentrations, while Haroun (2006) found a notable negative correlation of 60.6% and 46.3% between L value and total phenolic and antioxidant components of honey, respectively. Furthermore, Haroun (2006) observed a significant positive association between the total phenolic (79%) and antioxidant levels (58.9%) in honey with its  $a^*$  value ( $p < 0.05$ ). Similarly, Haroun (2006) indicated a positive correlation between the total phenolics (33.5%) and antioxidants (42.3%) content of honey and the  $b^*$  value ( $p < 0.05$ ). Meda et al. (2005) suggested a possible correlation between the proline content of honey and its inherent antioxidant abilities. Anupama et al. (2003) showed a significant negative correlation between honey moisture and Brix (99%) and viscosity (100%). The same researchers found that there was a positive correlation between viscosity and sweetness from sensory evaluation and viscosity (69%) and sweetness (76%) from instrumental measurements.

### Conclusion

This study revealed that the content of proline and Brix in honey can vary based on its color traits, determined by both spectrophotometers and thermal imaging. In particular, honey samples with whitish and bluish tones may have high proline concentrations, while honeys with darker tones like blackish and greenish tones may have higher Brix values. Honey of the highest quality is generally characterized by decreased Brix values and simultaneously increased proline content. Nonetheless, no significant relationship was found between the proline and Brix content of honey.

As a result, it can be said that the values obtained from spectrophotometers and thermal imaging devices should not be used interchangeably to assess color characteristics of honey. However, to accurately assess honey quality based on its color traits, comprehensive studies with more honey samples and origin, are required.

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**Ethics:** No animal or human was used in this research.

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