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Relationships between anthocyanin content and some pomological and colour characteristics of black mulberry (*Morus nigra*) fruit

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A B S T R A C T

The analytical method used to determine the total monomeric anthocyanin content of fruits is costly and labour intensive. Researchers are endeavouring to develop prediction models to determine anthocyanin content in a simpler and more accurate way. The aim of this study was to investigate whether there is a relationship between anthocyanin and some fruit characteristics (width, length, weight, L*, a*, b*, chroma, hue) in black mulberry (*Morus nigra*) fruit. With the outputs of the study, it is aimed to provide preliminary information for the models to be developed for anthocyanin estimation in future studies. The study material, black mulberry fruits, was collected from a single black mulberry tree in Kemalpaşa village of Tokat province in July 2022. Harvesting of the fruits continued for two weeks as raw, semi-ripe and ripe. A total of 586 fruits were individually evaluated and the weight, width, length, colour parameters (L*, a*, b*, chroma, and hue) and total monomeric anthocyanin contents of each fruit were determined. Then, Pearson correlation coefficients between the variables were determined. Stepwise regression analysis was used to find the appropriate model to explain the change in the dependent variable anthocyanin with independent variables (length, width, weight, L*, a*, b*, chroma, hue). After the multiple regression model was established, residual analysis was performed to see the outliers in the full model and to check the accuracy of the model. As a result of the study, it was observed that anthocyanin content could be predicted by colour parameters up to a certain maturity stage. This relationship was found to weaken at the ripeness stage when the fruit colour turns black.

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1. Introduction

Scientific studies have reported the positive effects of phytochemicals found in fruits and vegetables on human health through their antioxidant capacities (Koca and Karadeniz, 2009; Veberic et al., 2009; Arozarena et al., 2012; Aman and Masood, 2020; Naja and Hamadeh, 2020; de Faria Coelho-Ravagnani et al., 2021). The obtained findings emphasize the importance of fruit consumption in the daily diet. Many researchers have concluded that the amount of fruits consumed should be considered as well as the ratio of beneficial compounds in them. Researchers believe that dark red and purple coloured fruits such as black mulberry, raspberry, blackberry, pomegranate, strawberry, sour cherry, cherry, plum and grape, which are especially rich in anthocyanins, are very effective in preventing the emergence of some diseases that cause premature death such as some types of cancer, diabetes vascular and heart diseases (Zafra Stone et al., 2007; Castaneda-Ovando et al., 2009; Castro-Acosta et al., 2016; Feng et al., 2016). Colour is therefore one of the first perceived property of fruit and is a characteristic that greatly influences the choice of consumers, as it leads to an idea of the taste, smell and composition of the fruit.

Plants with different colours often reflect the presence and distribution of essential compounds such as flavonoids, carotenoids and chlorophyll that contribute to plant biochemistry and physiology (Manetas, 2006; Li et al., 2024). For example, the pigment responsible for the blue, red or purple hues in plants is the water-soluble anthocyanins. They are part of flavonoids of plant origin and are transported to the vacuole as glutathione conjugates via glutathione transporters and stored there. In the plant kingdom, with the exception of betalain producers, anthocyanins are present as glycosidic compounds in various of plant tissues, mainly flowers and fruits, but also leaves, stems, tubers and roots. Anthocyanins are aglycone (anthocyanidin) glycosides containing one or more hexose sugar groups (glucose, galactose, rhamnose, arabinose, rutinose, sambubiose and soforose), usually linked to the -OH group of the pyrylium ring. Aglycone is a polyphenolic ring structure based on diphenylpropane. The most common anthocyanin aglycones found in plants are pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (Kong et al., 2003; Hou et al., 2004; Deroles, 2009; Heldt and Piechulla, 2015; Yang et al., 2023; Chu et al., 2024).

The number and localisation of hydroxyl and methoxyl groups in the flavium cation and the presence of glycosyl and acyl groups attached to the aglycone affect the stability or reactivity of anthocyanins (Guidi et al., 2015). As a result of these events, anthocyanidins and their related structures make fruits and vegetables appear in many different colours and hues. The stability of anthocyanins is very sensitive and depends on the pH level of the environment, the type of anthocyanin pigment, copigments, enzymes, antioxidants, temperature, light, metal ions and oxygen (Khoo et al., 2017).

The method used to determine the total anthocyanin content in fruits is both laborious and costly because it consists of many steps. Researchers are in search of models to predict the amount of anthocyanin instead of the current method. For this purpose, it is investigated whether there is a relationship between anthocyanin and other fruit characteristics. Some researchers have reported a high correlation between colour parameters (L, a, b, chroma, hue) and colour pigments (Itle and Kabelka, 2009; Shibghatallah et al., 2013; Yan et al., 2023). Based on this relationship, it is thought that anthocyanin amounts can be estimated by determining the colour values for species in the berry fruit group, which do not have large differences between skin colour and flesh colour. The extraordinary colour and rich anthocyanin content of "Black Mulberry" fruit known as Morus nigra (Özgen et al., 2009) indicates that there will be a high correlation between colour values and anthocyanin values.

The aim of this study was to investigate whether there is a relationship between anthocyanin and some fruit characteristics (width, length, weight, L*, a*, b*, chroma, hue) in black mulberry fruit. The outputs obtained from the study will provide preliminary information for the models to be developed for predicting anthocyanin in further studies.

2. Materials and methods

2.1. Plant materials

The fruits were picked from a single black mulberry tree in Kemalpaşa village of Tokat province in July 2022.

The harvesting and analysis process of raw, semi-ripe and ripe black mulberry fruits continued for one month. A total of 586 fruits were individually evaluated and the weight, width, length, colour parameters and total monomeric anthocyanin contents of each fruit were examined.

2.2. Measurement and analyses

Each fruit was weighed using a balance with a precision of 0.01 and the length and width were measured using a compass. Fruit colour parameters (L*, a*, b*) were measured with a colorimeter (Minolta, model CR-400, Tokyo, Japan) from the outside of the fruit (4 measurements for each fruit). L* represents values from black (0) to white (100), a* from green (-) to red (+) and b* from blue (-) to yellow (+). The hue angle is defined as a colour circle, with red-purple at 0° and 360°, yellow at 90°, bluish green at 180° and blue at 270°. Chroma values indicate the saturation of the colour. Chroma values decrease in dull colours and increase in vivid colours. Hue angle (h^o) and chroma values were calculated according to Equations 1 and 2 (McGuire, 1992). Total monomeric anthocyanins were analysed using the pH differential method and the results were given as µg cyanidin-3-glucoside equivalent (µg cy-3-glu g^{-1} fw) per g fresh weight (Giusti and Wrolstad 2001; Özgen et al., 2009).

$$h^{\circ} = \tan^{-1}\left(\frac{b}{a}\right) \quad \text{.................(1)}$$

$$C = (a^2 + b^2)^{1/2} \quad \text{..................... (2)}$$

2.3. Statistical analysis

First, Pearson correlation coefficients between variables were determined. Stepwise regression analysis was used to find the appropriate model to explain the change in the dependent variable, anthocyanin, by independent variables (length, width, weight, L, a, b, chroma, hue). After the multiple regression model was created, residual analysis was performed to see the outliers in the full model and to check the accuracy of the model. Finally, the simple regression line of the independent variable hue value, which has the highest R value in the model, and the anthocyanin content of the dependent variable, and the distribution of errors on this line are given. All statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Pearson correlation analysis

The correlations between the variables and anthocyanin are shown in Table 1. As seen in the table, high, negative and significant correlations were found between anthocyanin and colour parameters (L*, a*, b*, hue and chroma) ($p<0.0001$). It has also been reported in some previous studies that L* value decreases with the advancement of maturity in mulberry fruit, indicating darkening of fruit colour (Lin and Lay, 2013; do Lago et al., 2020). The negative correlation between L* and anthocyanin content indicates that anthocyanin content increases as fruit colour darkens. In agreement with the findings of this study, it was also reported by do Lago et al. (2020) and Smrke et al. (2023) that the intensity of colour pigments increased as the fruit ripened, while the brightness (L*) and vividness (C*) of the colour decreased and the fruit gained a dull appearance.

It is known that while the red colour intensity (a*) is high until the semi-ripe stage of the fruits, this colour intensity and the shades of yellow colour (b*) decrease with ripening. The purplish-blackish colour intensity that occurs with the ripening of the black mulberry fruit is located close to the 0 and 360° angles on the colour angle. This indicates that the hue value decreases with the advancement of ripening (Ercisli and Orhan, 2007; Lin and Lay, 2013; do Lago et al., 2020). The negative correlations determined between colour parameters and anthocyanin content in this study are consistent with the above literature information. Significant correlations between colour values and anthocyanin content of the fruit were also reported for other fruits other than mulberry. For example; Karaat et al. (2019) reported that there was a high negative correlation between anthocyanin content and Chroma values ($r = -0.63$).

In a similar study with strawberry fruit, in parallel with our findings, Hernanz et al. (2008) reported negative correlations between colour values and colour pigments, with the best correlations found between pelargonidin-3-rutinoside and outer a* ($r = -0.87$), followed by pelargonidin-3-glucoside and inner L* ($r = -0.72$).

In another study, Yan et al. (2023) determined the total amount of 25 anthocyanins and 14 flavonols in 20 blueberry genotypes and examined their relationship with the external colour of the fruit. As a result of the study, it was reported that L*, C* and h° values showed weak correlations ($r < 0.4$) with anthocyanin concentrations measured at harvest and during post-harvest storage, but colour changes (Δ colour) after four weeks of storage showed a strong correlation ($r > 0.7$) with changes in anthocyanin concentrations. Vieira et al. (2018) reported that the correlation between L* value and anthocyanin content was higher for extracts produced with 70% ethanol and diluted 20 times ($r = -0.95$). Although our findings are consistent with the literature, they also contain inconsistencies. This inconsistency may be due to different solvent and dilution ratios as well as different coloured fruits and different molecular structures of the anthocyanins they contain (Han et al., 2008). Positive and significant correlations were found between length, width and weight parameters and anthocyanin ($p<0.0001$). This is due to the increase in the size and chemical content of the fruit as it passes from raw, semi-ripe and full-ripe levels.

Table 1. Pearson correlation analysis, N=586

	Anthocyanin	Length	Width	Weight	L*	a*	b*	Chroma
Length	**0.25**							
	0.0001							
Width	**0.56**	0.61						
	0.0001	*0.0001*						
Weight	**0.39**	0.84	0.72					
	0.0001	*0.0001*	*0.0001*					
L*	**-0.79**	-0.36	-0.59	-0.54				
	0.0001	*0.0001*	*0.0001*	*0.0001*				
a*	**-0.85**	-0.42	-0.63	-0.55	0.73			
	0.0001	*0.0001*	*0.0001*	*0.0001*	*0.0001*			
b*	**-0.82**	-0.39	-0.66	-0.55	0.86	0.894		
	0.0001	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*		
Chroma	**-0.86**	-0.42	-0.66	-0.56	0.79	0.98	0.96	
	0.0001	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	
Hue	**-0.86**	-0.38	-0.65	-0.55	0.83	0.90	0.96	0.93
	0.0001	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*

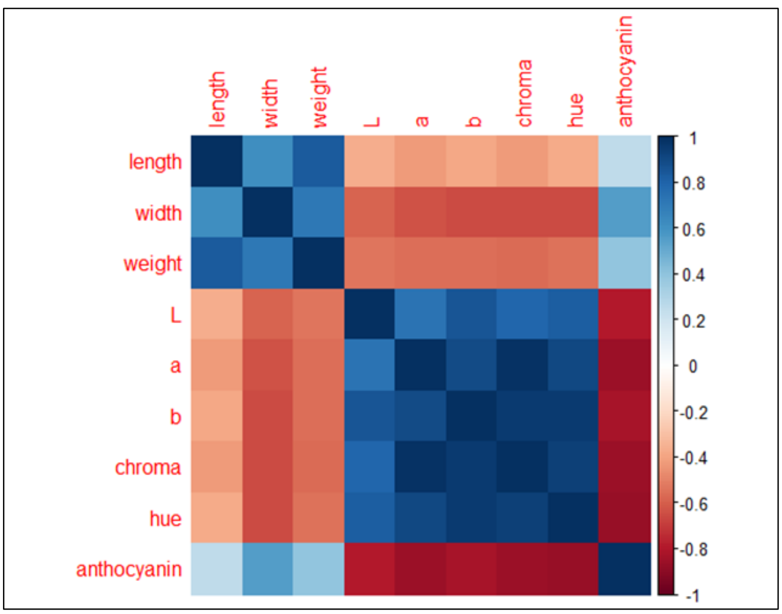

Figure 1. Colour representation of correlations between variables (Blue areas indicate positive correlation, red areas indicate negative correlation. As the darkness of the colours increases, the correlation between the variables increases)

3.2. Stepwise analysis

Stepwise analysis was performed to determine the appropriate model and the results of the analysis are given in Table 2. In Stepwise analysis, variables up to a significance level of 0.15 are included in the model. Variables with a significance level above 0.15 are excluded from the model and cannot enter the model (Hosmer and Lemeshow, 1999). 'Hue' entered the model first and 'hue' alone explained 74% of the variation in anthocyanin. In the presence of 'hue', the parameter 'a' explained 03% of the change in anthocyanin. Both together explained 77%. In total, when all variables were evaluated together (8 variables), it was determined that 85% of the change in anthocyanin was explained. In a previous study, Vieira et al. (2018) developed prediction models for the anthocyanin content of 13 different fresh fruits and vegetables using the stepwise method. For this purpose, L*, a*, b*, C* and h* values of colorimetry were used. As a result of the study, it was reported that linear equations with R2 values ranging from 0.80 to 0.99 were obtained. In this respect, our findings were in agreement with the literature.

Table 2. Stepwise analysis

Independent variable	Number of variables in the model	Partial R^2	Cumulative R^2	F value	P (significant)
Hue	1	0.7415	0.7415	1675.61	0.0001
a*	2	0.0314	0.7729	80.59	0.0001
L*	3	0.0239	0.7969	68.53	0.0001
Weight	4	0.0246	0.8215	80.05	0.0001
b*	5	0.0170	0.8385	61.17	0.0001
Chroma	6	0.0101	0.8486	38.51	0.0001
Width	7	0.0029	0.8515	11.34	0.0008
Length	8	0.0006	0.8521	2.44	0.1192

3.3. Multiple regression analysis

The multiple regression equation obtained as a result of stepwise regression analysis was as follows. This equation expresses that: 1 unit increase in fruit length corresponds to 1.65 unit increase in anthocyanin content while other variables are constant. Similarly, a unit increase in hue value corresponds to a decrease of 7.95 units in anthocyanin content.

Anthocyanin = 841.93 – (1.65 x **Length**) + (6.52 x **Width**) – (20.87 x **Weight**) – (11.2 x **L***) + (30.29 x **a***) + (49.43 x **b***) – (57.07 x **chroma**) – (7.95 x **hue**)

In order to test the accuracy of this regression equation and to identify outliers, standardised residual analysis was performed. When the errors shown in Figure 2 are examined, it is seen that the model is compatible and there are few outliers in the range of 0 to 300 μg cy-3-glu g^{-1} anthocyanin, while the predictive power of the model decreases and the number of outliers increases when the anthocyanin amount exceeds 300 μg cy-3-glu g^{-1}. These deviations are seen especially in black mulberry fruits that have reached full ripeness. Black mulberry fruits gain a darker appearance when they reach full ripeness. At this stage, L*, a*, b*, C* and h° values of the fruit decrease and anthocyanin values increase. A similar situation was observed in blueberry fruit (Smrke et al., 2023). These colour parameters and anthocyanin content, which change with ripeness, weaken the predictive power of the model.

The weakening relationship between colour parameters and anthocyanin content at high anthocyanin values was also observed in the simple regression analysis between hue values and anthocyanin content (Figure 3). At hue values between 50 and 0, there is a linear relationship between anthocyanin content and hue value, while this relationship breaks down at lower hue values.

4. Conclusion

In this study, which was carried out to develop a model for easy estimation of anthocyanin content in fruits as an alternative to labour-intensive and costly analysis methods to determine this content, it was seen that anthocyanin content up to a certain maturity period can be predicted by colour parameters. This relationship was found to be weakened at the ripeness stage when the fruit colour turns black. Other criteria are needed for accurate estimation of anthocyanin content at this stage.

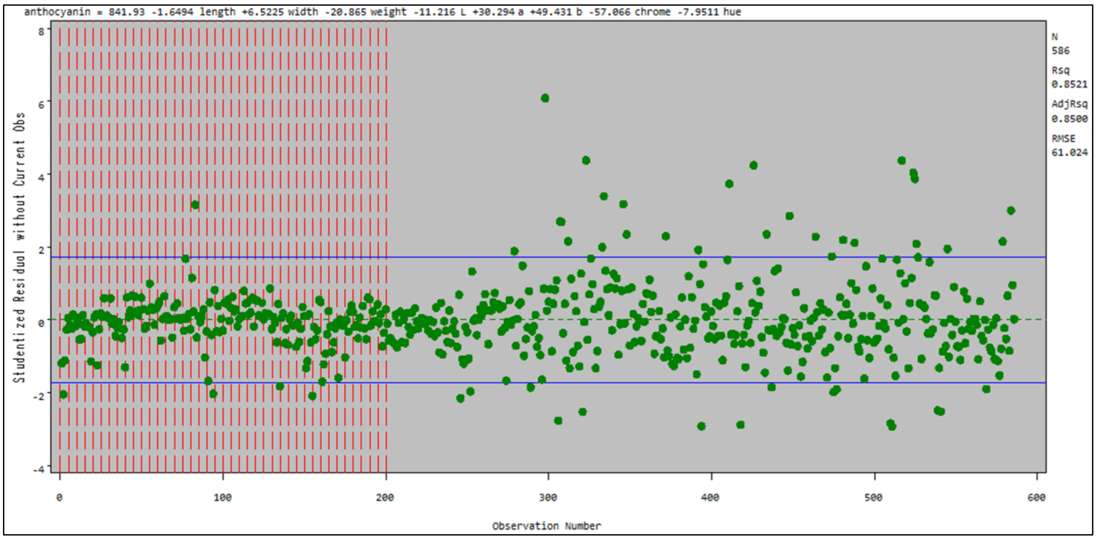

Figure 2. Scatterplot of errors in multiple models

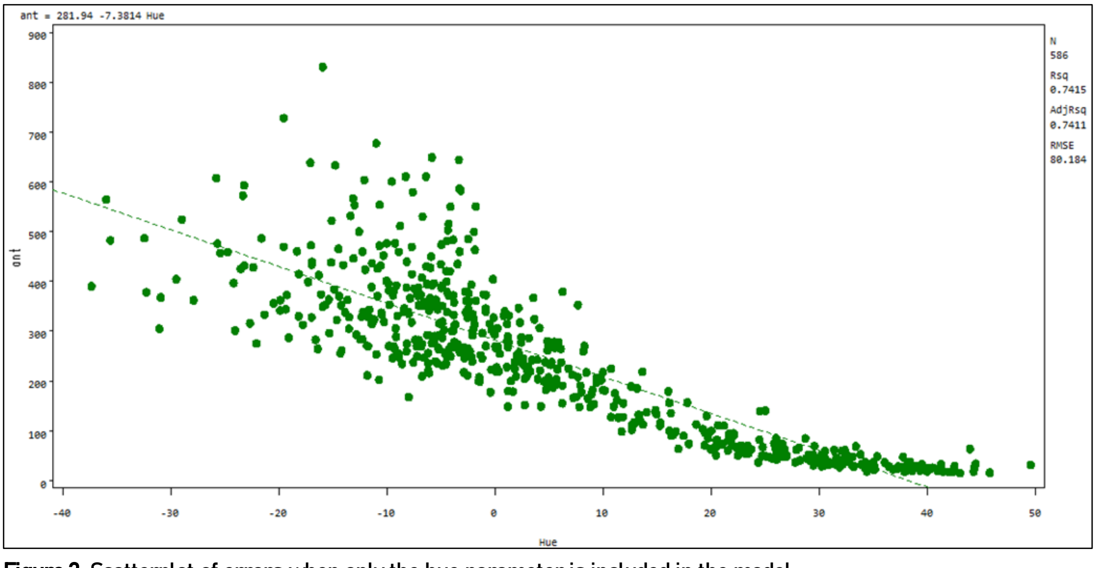

Figure 3. Scatterplot of errors when only the hue parameter is included in the model

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Osman Nuri ÖCALAN: Investigation, statistical analysis and writing. **Onur SARAÇOĞLU**: Writing, review and editing.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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