

INFLUENCE OF SOME CULTURAL PRACTICES ON YIELD, FRUIT QUALITY AND INDIVIDUAL ANTHOCYANINS OF TABLE GRAPE CV. 'HOROZ KARASI'

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ABSTRACT

This study was conducted to determine the effects of different cultural practices on vine yield, bunch and berry characteristics, juice quality, total anthocyanins and individual anthocyanins for 'Horoz Karası' table grape which have been demanded in the recent years. In addition to the control vines, seven cultural practices were compared: cluster thinning, boron, topping, cluster thinning + boron, cluster thinning + topping, boron + topping, cluster thinning + boron + topping. The highest and the lowest yields were obtained from boron+topping (14.94 kg vine⁻¹) and cluster thinning (8.58 kg vine⁻¹) practices respectively. For cluster weight and number of berries/cluster; cluster thinning+boron+topping practice yielded the highest value, while cluster thinning practice yielded the lowest value. Total soluble solid (TSS), pH, TSS/acidity characteristics were the highest in cluster thinning practice. It was also determined that such practice increased amounts of total anthocyanin and individual anthocyanins significantly more than other practices. Proportional malvidin-3-glucoside percentage was significantly higher than other individual anthocyanins in this variety. Grape quality and composition were affected by the amount of products in vine. Negative correlations were identified between vine yield and TSS, pH, TSS/acidity, total anthocyanins, Cy-3-G, Dp-3-G, Mv-3-G, Pn-3-G, Pt-3-G.

Keywords: *Vitis vinifera* L., table grape, treatment, fruit quality, individual anthocyanins.

INTRODUCTION

Grape (*Vitis vinifera* L.) is considered as one of the most important commercial fruit crops of temperate to tropical regions (Gowda *et al.*, 2008). The grape is gaining popularity for its high nutritive value, excellent in taste, multipurpose use and better returns (Ghosh *et al.*, 2008). A constant and steady improvement is observed in worldwide table grape consumption (Celik *et al.*, 2005). Production of grapes for fresh consumption increased 18% over the past two years and reached 2,256,845 tons in Turkey in 2009 (TUIK, 2009). Besides; seeded black varieties with large berries (such as 'Horoz Karası', 'Michele Palieri', 'Bonnoir', 'Bilecik İrikarası', 'Kömüşmemesi') have been demanded in Turkey in the recent years (Celik *et al.*, 2005). 'Horoz Karası' is an important table grape variety with economic value.

There are many factors in grape growing enter into the production of quality such as pruning, crop load, thinning, girdling, topping and pinching, the use of plant growth regulators (Winkler *et al.*, 1974). American vine rootstocks used in vineyards also influence fruit quality (Celik, 1996; Howell, 1987). 'Rupestris du Lot' rootstock is one of the rootstocks used commonly in vineyard regions of Turkey (Celik *et al.*, 2005). Cultivation of this rootstock, with 'Horoz Karası' variety grafted on it, is commonly seen in Eastern Mediterranean Region. 'Rupestris du Lot' may cause indirect coulure as it grows grafted varieties vegetatively strong (Bahar *et al.*, 2009; Celik, 1996). However, coulure in vines may be caused

by many other factors besides rootstock (Bahar *et al.*, 2009). Rootstock choice is the main factor in modern viticulture and it takes a long time. However, increasing grape quality of regional vineyards is dependent on carrying out different practices. Desired quality in table grapes represents a combination of medium-sized clusters of uniformly large, perfect berries with the characteristic color, pleasing flavor and texture of the variety (Winkler *et al.*, 1974). Physical properties of products such as color, form and shape are more important for consumers (Francis, 1978). Therefore, intense studies are carried out on berry color development in grapes (Gao and Cahoon, 1998). Consumer attraction of many colored table grape types is closely related to the anthocyanin amount on berry skin (Kliever, 1970). Anthocyanins are responsible for many red, violet and blue colors in fruit and flowers. Anthocyanins are strong antioxidants (Romero *et al.*, 2008). The detailed study of individual anthocyanins is essential to the understanding of anthocyanin metabolism and fruit-color improvement (Gao and Cahoon, 1998). However, no studies have been conducted in Turkey on the contents of individual anthocyanins in table grapes. Influence of different cultural practices regarding 'Horoz Karası' grape variety on characteristics such as cluster characteristics, berry characteristics, juice quality and vine yield have been explored in this study. Also, individual anthocyanins and percentage of individual anthocyanins in the berry skin have been identified in order to have a better understanding of berry color development of this variety and to see a simple anthocyanin profile.

MATERIALS AND METHODS

This study has been carried out in Hatay province in Eastern Mediterranean basin. 'Horoz Karası' variety; grafted on 'Rupestris du Lot' rootstock established at 4 × 4 m intervals and trained using goblet system, was used in the experiment. Besides control vines, seven cultural practices were experimented in the study. These practices are cluster thinning, boron, topping, cluster thinning + boron, cluster thinning + topping, boron + topping, cluster thinning + boron + topping. Topping practice was made by leaving five leaves above the last cluster on summer shoots. Cluster thinning was made by pruning 1/3 of clusters on the vine. Boron at the rate of 100 mg l⁻¹ as boric acid was applied as foliar. All cultural practices were carried out on May 16, 2009, approximately one week before blooming. The clusters were harvested on August 03, 2009. Temperature and precipitation values for the year of study have been depicted in Figure 1. The experiment was laid out in a Completely Randomized Design (CRD) with three replications. There were two vine per replication.

Measurements and analyses, made for the purpose of identifying quality characteristics in grape samples taken for each practice, are as follows:

1) Vine yield: It was found by weighing grapes obtained from each vine on 1g precision balance.

2) Cluster characteristics: Cluster weight, cluster width, cluster length and number of berries on cluster were identified using 10 clusters during each repeat.

3) Berry characteristics: 50 berries were used during each repeat for berry weight, berry width and berry length.

4) Must properties: 50 berries were squeezed for each repeat and grape juice was used for characteristics such as TSS, pH, acidity, TSS/acid.

Width and length values were measured during the study using a ruler on cluster and a caliper compass on berry. Weight measurements were made using a 0.01 g precise balance. Berries on the cluster were counted and identified by hand. TSS content (%) in the juice was determined using hand refractometer (Atago Model ATC-1E) and juice pH was determined by pH meter. Acidity measured using potentiometric method, while prepared juice was titrated with 0.1 N NaOH solution until 8.1 value was read on the pH meter and results were calculated in percentage of tartaric acid.

5) Anthocyanin contents: Anthocyanin extraction was carried out after Carreno *et al.* (1997) with some modifications. Briefly, skins of grape berries were homogenized by blender, ground in porcelain mortar with liquid nitrogen, and approximately 1 g of tissue sample was taken. 10 ml 0.01% HCL-methanol was added to it. It was mixed by vortex for 1 minute and then kept in shaker (Heidolph Unimax 2010) at 350 rpm speed for 6 hours. It was then centrifuged (Rotina 38 R Hettich,

Zentrifugen, Germany) at 4°C for 10 minutes at 9,000 rpm (13,000 g) speed. Clear liquid that remained on top as a result of centrifuge was taken and kept in an amber colored sample bottle at -20°C until the time of analysis. This process was repeated four times until the tissue was colorless. Extracts obtained from each extraction were combined and total volume was completed to 50 ml using 0.01% HCL-methanol. Total anthocyanins were identified using total anthocyanin pH difference method (Giusti and Wrolstad, 2001) on obtained skin extracts. Skin extracts were filtrated by using 0.45 µm syringe filter and then injected into 20 µl volume HPLC for individual anthocyanins (Dp-3-G, Cy-3-G, Pt-3-G, Pn-3-G, Mv-3-G). Used HPLC device (Shimadzu, Japan) model is LC-10A and consists of parts such as LC-10AD pump, in-line degasser, CTO-10A column oven, SCL-10A system controller and LC solution software. Individual anthocyanins (Dp-3-G, Cy-3-G, Pt-3-G, Pn-3-G, Mv-3-G) were separated by Nucleosil 100 C18 5 µm 250 mm × 4.6 mm i.d. column (Macherey-Nagel, Düren, Germany) at 1.5 ml min⁻¹ speed at 32°C and gradient mobile phase consisting of 10% formic acid (A) and 100% acetonitrile (B) (0-10 min 10% B, 10-20 min 15% B, 20-37.5 min 20% B, 37.5-70 min 30% B) and identified by photodiode array detector (PDA) at 520 nm using external standard method. Total and individual anthocyanin contents were specified as mg 100 g⁻¹ fresh berry weight. Furthermore, proportional amounts of individual anthocyanins were calculated using individual anthocyanin and total anthocyanin values.

Variance analysis was carried out through COSTAT statistical software and means were compared by Duncan Multiple Range Test ($\alpha=0.05$). Correlation analysis of parameters in the study were conducted using COSTAT software.

RESULTS AND DISCUSSION

Boron + topping practice provided the highest value in terms of yield per vine and topping, control, cluster thinning + boron and boron practices were statistically at par. While cluster thinning practice provided the lowest yield per vine, it was followed by cluster thinning + topping and cluster thinning + boron + topping practices (Table 1). Palliotti and Cartechini (2000), Dami *et al.* (2006) and Diago *et al.* (2010) also identified cluster thinning reduced yield per vine in their studies.

Effect of practices on cluster width was not statistically important in terms of cluster characteristics. However, the highest values in terms of cluster weight and cluster length were obtained from cluster thinning + boron + topping practice. The same practice also increased number of berries per cluster and provided the highest value. Number of berries per cluster were the lowest in control and cluster thinning practices (Table 1).

Visual factors are very important in determining the quality of table grapes. These factors include berry size and color (Mullins *et al.*, 1992). The highest values in terms of berry weight and berry width were obtained from control practice while the lowest values were obtained from topping and boron + topping practices. No difference was identified among practices in terms of berry length (Table 2).

TSS and acidity play an important role in fruit quality improvement (Chanana and Gill, 2008). The highest value in terms of TSS was obtained from cluster thinning practice. This practice also provided the highest value in terms of pH and TSS/acidity ratio. Effect of practices on acidity content were not considered statistically important (Table 2). Our results are in agreement with the findings of Palliotti and Cartechini (2000) and Dami *et al.* (2006) and Diago *et al.* (2010). Dami *et al.* (2006) reported in their studies that cluster thinning improved juice composition by increasing TSS and pH but not acidity.

Fruit color is an important quality attribute in table grapes. 'Horoz Karası' berries contain anthocyanins only in their berry skins. Anthocyanins are the most prominent pigments in grape skin (Romero *et al.*, 2008). Total anthocyanin content varied between 24.46 and 56.50 mg for this variety. No studies were found in the literature on anthocyanin amount and contents of this variety. However, our findings were within total anthocyanin values (4-99 mg 100 g⁻¹) identified by Orak (2007) in 16 grape varieties. Cluster thinning practice provided the highest total anthocyanin content in the study (Table 3). These findings are in agreement with previous studies that cluster thinning was shown to enhance total anthocyanin concentration of grapes (Gao and Cahoon, 1998; Guidoni *et al.*, 2002; Prajitna *et al.*, 2007). Cluster thinning practice provided the highest values in terms of Cy-3-G, Dp-3-G, Mv-3-G, Pn-3-G, Pt-3-G amounts in our study (Table 3). Similarly, effect of this practice on individual anthocyanin percentages (except for Cy-3-G) was proportionally higher than other

practices (Table 4). Cluster thinning + boron + topping is practice provided the lowest value (71.78%) in terms of proportional total of individual anthocyanins, while cluster thinning provided the highest value (98.17%). Main anthocyanin is malvidin-3-monoglucoside in many *Vitis vinifera* varieties (Winkler *et al.*, 1974). Proportional malvidin-3-glucoside percentage was significantly higher than other individual anthocyanins also in 'Horoz Karası'. Cortell *et al.* (2007) reported in their study that a higher proportion of malvidin-3-O-glucoside and lower proportions of the other four anthocyanins (delphinidin-, cyanidin-, petunidin-, and peonidin-3-O-glucosides) were found in *Vitis vinifera* 'Pinot Noir'.

Correlation analysis results have been presented in Table 5. Negative correlation was found between vine yield and TSS ($r = -0.49$), pH ($r = -0.52$), TSS/Acidity ($r = -0.58$), total anthocyanin ($r = -0.47$) and individual anthocyanins ($r = -0.44 - -0.56$) according to practices. Diago *et al.* (2010) also determined in their study that

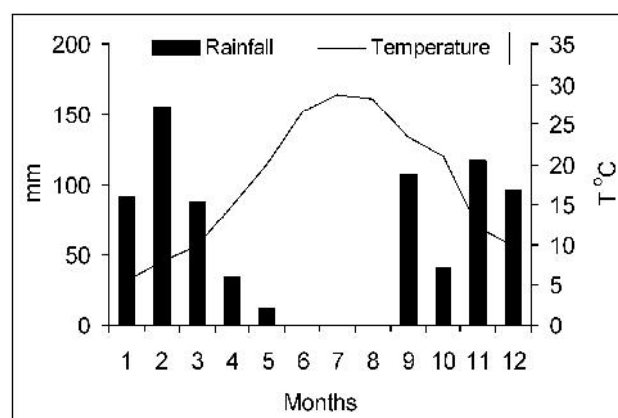


Figure 1. Monthly air temperature and rainfall of the experimental site in 2009.

Table 1. Effect of some cultural practices on yield and some of the cluster properties of 'Horoz Karası' table grape

Treatments	Yield (kg vine ⁻¹)	Cluster weight (g)	Cluster width (cm)	Cluster length (cm)	Total berries/cluster
Control	13.74 a ⁽¹⁾	429.44 cde	11.83	17.48 ab	111.27 b
Cluster thinning	8.58 b	384.58 e	11.50	17.46 ab	108.01 b
Boron	12.57 a	392.87 de	11.60	16.86 b	120.83 ab
Topping	14.03 a	438.37 bcde	11.94	16.98 b	130.47 ab
Cluster thinning +Boron	13.23 a	506.55 abc	11.97	18.59 ab	141.34 ab
Cluster thinning +Topping	11.17 ab	511.82 ab	12.15	17.54 ab	144.33 ab
Boron+Topping	14.94 a	466.84 bcd	12.26	17.52 ab	134.23 ab
Cluster thinning +Boron+ Topping	11.71 ab	549.01 a	12.23	20.08 a	158.64 a
Significant ⁽²⁾	3.61	73.19	ns	2.44	34.25

⁽¹⁾ : The differences between the means marked with different letters.

⁽²⁾ : *= $p \leq 0.05$, ns: not significant.

Table 2. Effect of some cultural practices on berry properties and grape juice composition of ‘Horoz Karası’ table grape

Treatments	Berry weight (g)	Berry width (mm)	Berry length (mm)	TSS (%)	pH	Acidity (%)	TSS/acidity
Control	4.92 a ⁽¹⁾	18.28 a	23.51	14.80 b	3.32 b	0.55	26.83 ab
Cluster thinning	4.72 ab	17.83 ab	23.34	17.13 a	3.45 a	0.57	30.43 a
Boron	4.31 ab	17.51 ab	22.34	14.83 ab	3.36 ab	0.55	27.04 ab
Topping	4.25 b	17.27 b	22.44	15.53 ab	3.29 b	0.60	26.04 b
Cluster thinning +Boron	4.71 ab	17.93 ab	23.26	14.63 b	3.28 b	0.62	23.72 b
Cluster thinning +Topping	4.39 ab	17.45 ab	22.76	15.53 ab	3.31 b	0.62	25.29 b
Boron+Topping	4.21 b	17.46 ab	22.21	14.73 b	3.26 b	0.62	23.88 b
Cluster thinning +Boron + Topping	4.28 ab	17.43 ab	22.79	15.33 ab	3.32 b	0.64	23.93 b
Significant ⁽²⁾	0.59	0.83	ns	1.69	0.11	ns	4.00

⁽¹⁾ : The differences between the means marked with different letters.⁽²⁾ : *p≤0.05, ns: not significant.**Table 3. Effect of some cultural practices on individual anthocyanin and total anthocyanin content of ‘Horoz Karası’ table grape**

Treatments	Total anthocyanins (mg 100 g ⁻¹)	Cy-3-G (mg 100 g ⁻¹)	Dp-3-G (mg 100 g ⁻¹)	Mv-3-G (mg 100 g ⁻¹)	Pn-3-G (mg 100 g ⁻¹)	Pt-3-G (mg 100 g ⁻¹)
Control	29.73 b ⁽¹⁾	0.09 b	0.38 b	21.78 b	0.54 b	1.66 b
Cluster thinning	56.50 a	0.19 a	1.14 a	47.22 a	1.38 a	5.55 a
Boron	39.41 ab	0.12 ab	0.68 b	29.14 ab	0.66 b	3.03 b
Topping	40.83 ab	0.14 ab	0.77 ab	32.09 ab	0.87 ab	3.41 b
Cluster thinning + Boron	24.46 b	0.09 b	0.35 b	16.31 b	0.39 b	1.44 b
Cluster thinning + Topping	34.63 b	0.12 ab	0.66 b	25.94 b	0.56 b	2.84 b
Boron + Topping	26.03 b	0.10 b	0.40 b	20.24 b	0.53 b	1.62 b
Cluster thinning + Boron + Topping	31.87 b	0.11 b	0.54 b	22.79 b	0.66 b	2.22 b
Significant ⁽²⁾	17.08	0.06	0.40	17.91	0.55	2.12

⁽¹⁾ : The differences between the means marked with different letters.⁽²⁾ : *p≤0.05**Table 4. Effect of some cultural practices on the percentages of individual anthocyanin of ‘Horoz Karası’ table grape**

Treatments	Cy-3-G (%)	Dp-3-G (%)	Mv-3-G (%)	Pn-3-G (%)	Pt-3-G (%)	Total (%)
Control	0.30	1.31 b ⁽¹⁾	73.26 ab	1.79 ab	5.61 b	82.27 ab
Cluster thinning	0.33	2.01 a	83.59 a	2.45 a	9.79 a	98.17 a
Boron	0.31	1.68 ab	73.15 ab	1.59 b	7.22 ab	83.94 ab
Topping	0.34	1.89 ab	78.55 ab	2.13 ab	8.37 ab	91.29 ab
Cluster thinning + Boron	0.35	1.41 ab	66.78 ab	1.57 b	5.87 b	75.98 ab
Cluster thinning + Topping	0.36	1.92 ab	75.00 ab	1.65 b	8.25 ab	87.18 ab
Boron + Topping	0.39	1.54 ab	77.91 ab	2.02 ab	6.27 b	88.13 ab
Cluster thinning + Boron + Topping	0.35	1.61 ab	62.11 b	1.77 ab	5.93 b	71.78 b
Significant ⁽²⁾	ns	0.58	17.99	0.71	3.09	20.44

⁽¹⁾ : The differences between the means marked with different letters.⁽²⁾ : *p≤0.05, ns: not significant.

there was an increase in the anthocyanin content, soluble solids and pH in response to the decrease in yield reduction. Yield reduction should lead to higher leaf/yield ratios as well as changes in the source-sink balance, with

these two phenomena leading to enhanced translocation of assimilates towards the cluster (Diago *et al.*, 2010). Positive correlation was found between number of berries in cluster and cluster weight ($r = 0.86$), cluster width ($r =$

0.61), cluster length ($r = 0.64$) and acidity ($r = 0.75$) while there was a negative correlation with TSS/acidity ($r = -0.74$). It was determined that there were positive correlations between TSS and pH ($r = 0.72$), TSS/Acidity ($r = 0.54$), total anthocyanins ($r = 0.72$) and individual anthocyanins ($r = 0.68-0.76$). Correlations of TSS/acidity

between total anthocyanins ($r = 0.61$) and individual anthocyanins ($r = 0.58-0.64$) were positive. Matsumoto *et al.* (2007) also reported in their study that there was a highly positive correlation between total anthocyanin content and TSS/acidity.

Table 5. Correlation coefficients between variables on 'Horoz Karası' table grape

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0.38 ^{ns}	0.643*	0.20 ^{ns}	0.43*	-0.04 ^{ns}	0.06 ^{ns}	-0.05 ^{ns}	-0.49*	-0.52**	0.31 ^{ns}	-0.58**	-0.47*	-0.52**	-0.53**	-0.45*	-0.44*	-0.56**
	0.60**	0.64**	0.86**	-0.15 ^{ns}	-0.12 ^{ns}	0.04 ^{ns}	-0.17 ^{ns}	-0.40 ^{ns}	0.67**	-0.67**	-0.50*	-0.49*	-0.48*	-0.48*	-0.39 ^{ns}	-0.51*
		0.05 ^{ns}	0.61**	0.12 ^{ns}	0.04 ^{ns}	0.32 ^{ns}	0.03 ^{ns}	-0.19 ^{ns}	0.52**	-0.41*	-0.14 ^{ns}	-0.08 ^{ns}	-0.16 ^{ns}	-0.10 ^{ns}	-0.01 ^{ns}	-0.18 ^{ns}
			0.64**	-0.18 ^{ns}	-0.13 ^{ns}	-0.06 ^{ns}	-0.25 ^{ns}	-0.19 ^{ns}	0.48*	-0.55**	-0.41*	-0.49*	-0.40 ^{ns}	-0.41*	-0.35 ^{ns}	-0.41*
				-0.37 ^{ns}	-0.38 ^{ns}	-0.16 ^{ns}	-0.17 ^{ns}	-0.32 ^{ns}	0.75**	-0.74**	-0.39 ^{ns}	-0.39 ^{ns}	-0.34 ^{ns}	-0.40 ^{ns}	-0.34 ^{ns}	-0.40 ^{ns}
					0.93**	0.92**	-0.02 ^{ns}	0.13 ^{ns}	-0.33 ^{ns}	0.26 ^{ns}	0.13 ^{ns}	0.14 ^{ns}	0.05 ^{ns}	0.15 ^{ns}	0.15 ^{ns}	0.10 ^{ns}
						0.78**	-0.17 ^{ns}	-0.10 ^{ns}	-0.31 ^{ns}	-0.14 ^{ns}	-0.05 ^{ns}	-0.05 ^{ns}	-0.13 ^{ns}	-0.01 ^{ns}	-0.01 ^{ns}	-0.07 ^{ns}
							0.09 ^{ns}	0.22 ^{ns}	-0.24 ^{ns}	0.27 ^{ns}	0.16 ^{ns}	0.19 ^{ns}	0.11 ^{ns}	0.18 ^{ns}	0.23 ^{ns}	0.14 ^{ns}
								0.60**	0.15 ^{ns}	0.54**	0.72**	0.68**	0.76**	0.73**	0.76**	0.76**
									-0.31 ^{ns}	0.69**	-0.64**	0.55**	0.62**	0.59**	0.60**	0.63**
										-0.74**	-0.14 ^{ns}	-0.22 ^{ns}	-0.15 ^{ns}	-0.10 ^{ns}	-0.07 ^{ns}	-0.16 ^{ns}
											0.61**	0.64**	0.63**	0.58**	0.58**	0.64**
												0.93**	0.94**	0.98**	0.95**	0.97**
													0.95**	0.92**	0.89**	0.96**
														0.91**	0.87**	0.99**
															0.97**	0.96**
																0.91**

** Significant correlation ($p \leq 0.01$); * Significant correlation ($p \leq 0.05$); ^{ns}: not significant; 1: Yield (kg vine⁻¹); 2: Cluster weight (g); 3: Cluster width (cm); 4: Cluster length (cm); 5: Total berries/cluster; 6: Berry weight (g); 7: Berry width (mm); 8: Berry length (mm); 9: TSS (%); 10: pH; 11: Acidity (%); 12: TSS/acidity; 13: Total anthocyanins (mg 100 g⁻¹ berry); 14: Cy-3-G (mg 100 g⁻¹ berry); 15: Dp-3-G (mg 100 g⁻¹ berry); 16: Mv-3-G (mg 100 g⁻¹ berry); 17: Pn-3-G (mg 100 g⁻¹ berry); 18: Pt-3-G (mg/100g berry).

Conclusion: This study yielded information about influence of some cultural practices on yield, fruit quality and individual anthocyanins of table grape 'Horoz Karası' in Mediterranean climatic conditions. Effect of cluster thinning practice on number of berries in the cluster was similar to the control. However, it was found out that other practices partially increased number of berries in the cluster. The highest value was obtained from cluster thinning + boron + topping practice. Vine yield was found to be the highest in boron + topping (14.94 kg vine⁻¹) practice. Cluster thinning (8.58 kg vine⁻¹) practice reduced vine yield. However, juice quality (TSS, pH, TSS/acidity) increased as compared to other practices. Total anthocyanin and individual anthocyanin amounts were significantly increased by this practice. Grape quality and composition were affected by amount of products in the vine. Negative correlations were identified between vine yield and TSS, pH, TSS/Acidity, total anthocyanins and individual anthocyanins.

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