

# Determination of the Characteristics of Tenturiye üzüm and Alicante Bouschet in Tekirdağ

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## Abstract

Grapes with dark red color on both the skin and the flesh are known as Teinturier grapes. Tenturiye üzüm, which is a new grape genotype grown in Tekirdağ province, is similar to Alicante Bouschet grape variety in terms of many characteristics. Total phenolic content, total anthocyanin content and total tannin content obtained from Alicante Bouschet cultivar were 6332.5 mg gallic acid equivalent kg<sup>-1</sup>, 1324.52 mg malvidin 3-glucoside kg<sup>-1</sup>, 9.6 mg tannic acid equivalent g<sup>-1</sup> and Tenturiye üzüm's results obtained were 5012.05 mg gallic acid equivalent kg<sup>-1</sup>, 1022.25 mg malvidin 3-glucoside kg<sup>-1</sup>, 5.55 mg tannic acid equivalent g<sup>-1</sup>, respectively. The determined values are higher than many table and wine grapes. Since teinturier grapes are important for the winemaking industry in terms of giving color to the wine, the Tenturiye üzüm genotype found in Tekirdağ is an important discovery for the region and country viticulture.

## Introduction

Teinturier is a term for grapes in which anthocyanins accumulate not only in the skin but also in the pulp of the berry (Chen et al., 2018). Anthocyanin is a class of red pigments responsible for the colour of grapes and wines (Jackson and Lombard 1993). The current trend of winemaking is the production of premium wines with good appearance. Since red wines obtained from a single cultivar sometimes do not exhibit good appearance, teinturier grapes may be mixed to enhance colour without dramatically impacting taste.

Generally, teinturier grape cultivars have greater anthocyanin per fresh mass than nonteinturier cultivars (Luan et al. 2014).

Phenolic compounds known as polyphenols play a key role in the quality criteria of grape berries such as antioxidant, taste and color (Shahidi and Ambigaipalan 2015). Many studies have reported that grapes are good sources of phenolic matters (Fernandez de

Simon et al., 1993; Kammerer et al., 2004; Hollecker et al., 2009).

However, studies on properties of teinturier grape varieties, especially local genotypes, are limited in Turkey. Climate and soil conditions, cultural processes, grape ripening status, crop load, organic or traditional cultivation method, green fertilization and rootstocks affect the status of phenolic substances, and also these substances may also vary by years (Cheng et al., 2017). Additionally, local differences affect the development of the vine, the ripening of the grape, the composition and sensory properties of the grape and wine (Bonino et al., 2003). Therefore, new studies are needed to determine the regional characteristics of local genotypes and reveal the differences between varieties.

Alicante Bouschet is the most famous wine grape variety of teinturier grapes. Tenturiye üzüm is a genotype with flesh colored fruit in Tekirdağ Viticulture Research Institute's Grapevine Field Gene Bank (TGFGFB). In this study, it was aimed to compare the properties of Tenturiye üzüm with

Alicante Bouschet, that was found during the survey study in Tekirdağ.

### Materials and Methods

In this study, two different grapes from TGFGFB were used in the coordinates of 40°58' N.- 27°28' E, in Tekirdağ, Turkey. Grapes harvested in September 2021 were compared in terms of some morphological and bioactive properties. Both grapes had the characteristics of tenturier grapes.

#### Alicante Bouschet

It was obtained by Henri Bouschet in France in 1865 as a Grenache x Petit Bouschet hybrid. Its clusters are of medium size, dense and winged conical. It matures in mid-season. It has medium-sized, round, dense blue-gray hazy grains and 1-2 seed. Its must is dark red in color and does not have a special aroma (Çelik 2002). It is a grape variety with medium-thick berry skin, black pulp, pulpy-juicy inside. The juice obtained from the variety has been identified as one of the black grape varieties with the highest compatibility with grape juice with its high anthocyanin content and high scores in the scaled rating. It may be mixed with other varieties to strengthen the black color in the production of grape juice (Gülcü et al. 2010). Alicante Bouschet was taken in TGFGFB during the 2021 harvest period (Figure 1).

#### Tenturiye üzüm

Tenturiye üzüm found in TGFGFB was



**Figure 1.** Alicante Bouschet  
Photos by Tamer Uysal

determined as a result of the survey conducted in Tekirdağ province in 2018. Morphologically, it has a conical and dense cluster, rounded grain structure and 1-2 seed. In the ripening period of the grains, when the softening of the Tenturiye üzüm has not started yet, the whole bunch takes on the color of African violet. This is a distinctive feature of the variety. The color of the skin darkens with softening during the veraison period and turns blue-black during the harvest period. It is a seed variety with a unique aroma. Tenturiye üzüm was taken in TGFGFB during the 2021 harvest period (Figure 2). There are similarities between Teinturier ad acino rotondo grape variety found in Italy vitis database and Tenturiye üzüm. Considering its ampleographic features, it is very likely to be synonymous.

#### Determination of titration acidity

A few drops of phenolphthalein (1% in ethanol) were added to the must obtained by crushing and filtering fresh grape berries and then titrated with 0.1 N NaOH solution. The results were calculated in terms of "tartaric acid" (g 100 g<sup>-1</sup>) (Cemeroğlu 2007).

$$\text{Titration acidity} = \frac{V \cdot f \cdot E \cdot 1000}{A}$$

*V*: Amount of 0.1 N NaOH spent, ml

*f*: Factor of 0.1 N NaOH, 1

*E*: The amount of tartaric acid equivalent to 1 mL of 0.1 N NaOH (0.007505)

*A*: Amount of sample taken for titration, mL or g



**Figure 2.** Tenturiye üzüm

### Amount of Total Soluble Solids analysis

The brix degree % of grape berries must was determined by measuring with a hand-held refractometer (ATAGO Co Ltd.).

### Determination of pH

It was determined by measuring the must obtained by crushing and filtering fresh grape berries directly with a pH meter (Mettler Toledo FE20).

### Maturation index

It was calculated by dividing the total soluble solids value determined in the samples by the titration acidity (%) and multiplying by 10 (Blouin and Guimberteau 2000).

### Extraction of phenolic compounds

After the grape samples were harvested, they were quickly brought to the laboratory and stored at -20 °C until extracted. Before the extraction process, the seeds were removed from the berries and the remaining skin and pulp were extracted. Grape berries without seeds were grounded in a blender and then samples were placed in an eppendorf tube, were weighed, then were added an extraction solvent (80% aqueous methanol acidified with 0.1% HCl). With using a rotary shaker, the mixture was shaken (Rotator, Dragon Laboratory Instruments) at 50 rpm and room temperature for 1 h. Then, the extracts were centrifuged for 10 min at 4,500 rpm and to remove suspended materials. All extractions were performed in three replicates.

### Determination of total phenolic content (TPC)

The TPC was specified by use of the Folin–Ciocalteu method with micro scale protocol (Waterhouse, 2002). Briefly, 40 µl of grape extract or gallic acid standards (50–500 mg L<sup>-1</sup>), 200 µl of Folin–Ciocalteu reagent and 3.16 ml of water were pipetted into a 4 ml spectrophotometer cuvette. After 3–4 min, 600 µl solution of Na<sub>2</sub>CO<sub>3</sub> (20%) were added. The content was held at room temperature for 2 h, with using a spectrophotometer (Shimadzu UV–Vis Mini

1240, Tokyo, Japan), at 765nm against a blank, the absorbance of the sample was determined. TPC was specified as mg gallic acid equivalent per kilogram fresh weight (mg GAE kg<sup>-1</sup>).

### Total anthocyanin content (TAC)

Total monomeric anthocyanin content was determined by the pH differential method (Giusti and Wrolstad, 2001). Determinations were performed on a UV-Vis spectrophotometer (Shimadzu UV–Vis Mini 1240, Tokyo, Japan), measurements at 520 and 700 nm. Total monomeric anthocyanin concentration was expressed as mg malvidin 3-glucoside kg<sup>-1</sup> and calculated by the following formula.

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}$$

$$TAC (mg\ kg^{-1}) = ((A)(MW)(Df)(1000)) / ((\epsilon) l)$$

*A*: Absorbance difference

*MW*: Molecular weight of malvidin-3-glucoside: 493,5

*Df*: Dilution factor

*ε*: Molar absorption coefficient for malvidin-3-glucoside: 28,000

*l*: Cuvette layer thickness:1

### Total tannin content

According to Association of Official Analytical Chemists (AOAC, 1998), the total tannin content was determined by a colorimetric assay. Briefly, extraction of grape samples (40 µl), 3.36 ml water and 200 µl of Folin-Denis reagent were added to a 4 ml spectrophotometer cuvette. After 3–5 min, 400 µl saturated solution of Na<sub>2</sub>CO<sub>3</sub> were added to cuvette. The contents were mixed and kept at room temperature for 30 minutes. The absorbance of the sample was measured at 760 nm against a blank (water substitute for the sample) using a UV-Vis spectrophotometer (Shimadzu UV–Vis Mini 1240, Tokyo, Japan). The amount of tannic acid in the sample was detected according to the r<sup>2</sup> curve drawn using tannic acid standards (100–1000 mg L<sup>-1</sup>). Total tannin content was calculated as mg tannic acid equivalent per gram fresh weight (mg TAE g<sup>-1</sup>).

**Table 1.** Characteristic properties of the two grape varieties (OIV 2009)

Characteristics	Alicante bouschet	Tenturiye üzüm
Young shoot (OIV 003)*	3 weak	7 strong
Young Leaf (OIV 051)**	2 green with bronz spots	3 bronze color
Mature leaf (OIV 070)***	3/5 between weak and medium	5 medium
Berry: color of skin (OIV 225)	6 blue-black	6 blue-black
Berry: color of flesh (OIV 230)	2 colored	2 colored
Intensity of the color of the flesh (OIV 231)	9 very strongly colored	9 very strongly colored
Seedy (OIV 241)	3 present	3 present
Cluster weight (OIV 502)	5/7 between medium to high	3 low (174.46 g)
Grain weight (OIV 503).	5 medium	3 low (1.48 g)

\*shoot Intense anthocyanin coloration on oblique hairs on the tip

\*\*leaf upper surface color (4th leaf)

\*\*\*Anthocyanin coloration of the main veins on the upper surface of the leaf was detected as red after the second branching

### Determination of phenolic compounds

Phenolic compounds of samples were determined by a SPD-M20A PDA detector (HPLC Shimadzu LC-20A) and an Inertsil ODS-3 (5 µm; 4.6 × 250 mm) column. 2% acetic acid in water (A) and acetonitrile (B) mixture were used as mobile phases. The flow rate of the mobile phase and the injection volume were 1.5 ml min<sup>-1</sup> at 30°C and 20 µl, respectively. The analysis was performed by filtering the methanolic extracts with 0.45 µm membrane filters. The gradient program was as noted: 0–10 min 5% B; 10–25 min 15% B; 25–30 min 15% B; 30–45 min 40% B; 45–50 min 80% B; and 50 to 60 min 5% B. The gradient program's time for each sample was 60 min. The peak records were carried out at 280, 320, and 360 nm (Halişçelik & Turmuş, 2017).

### Data analysis

JMP© 7.0 (SAS Institute, Inc.) statistical program was used to perform statistical data analysis. The significance of the differences between treatments was determined using oneway analysis of variance (ANOVA) and significant differences were grouped with the Tukey test at 5% significance level.

### Results and discussion

The International Organisation of Vine and Wine (OIV) is an intergovernmental organisation of a scientific and technical nature of recognised competence for its works concerning vines, wine, wine-based beverages, table grapes, raisins and other vine-based products. Morphological

characteristics of both two grapes are compared in Table 1 according to the international criteria determined by the OIV.

When the characteristic features determined by the OIV between the two grapes are examined, they had similar qualities. Based on OIV 003 criteria, Alicante Bouschet was 3 notes in young shoot, whereas it was 7 notes in Tenturiye üzüm. According to the OIV 502 and OIV 503 criteria, Alicante Bouschet has a slightly larger structure compared to the Tenturiye üzüm, both in cluster structure and berry size. In addition, Alicante Bouschet, like many other grapes, did not become colored during the unripened period, while the Tenturiye üzüm became colored. This feature is the most important feature that distinguishes the Tenturiye üzüm from Alicante Bouschet.

The ripening values of harvested grapes are shown in Table 2. The values of Alicante Bouschet and Tenturiye üzüm are close to each other.

When the phenolic content was examined, it was detected that all values of Alicante Bouschet were higher than Tenturiye üzüm (Table 3). However, it has been reported that climate and soil conditions, cultural processes, grape ripening status, crop load, organic or traditional cultivation method, green fertilization and rootstocks affect the status of phenolic substances, and also these substances may also vary by years (Cheng et al., 2017). The grapes we examined were processed under the same conditions and harvested from the same place. It is, therefore, thought that the difference between the values is owing to the difference between the two grape varieties. The total amount

**Table 2.** The ripening values of harvested the two grape varieties

Varieties	Titrateable acidity (g 100 g <sup>-1</sup> )	Amount of total soluble solids (%)	pH	Maturation index
Alicante Bouschet	6.3	21.8	3.62	34.6
Tentüriye üzüm	6.3	21.4	3.78	33.9

**Table 3.** Phenolic matter contents of the two grape varieties

Varieties	Total phenolic matter (mg GAE kg <sup>-1</sup> )	Total anthocyanin matter (mg kg <sup>-1</sup> )	Total tannin matter (mg TA g <sup>-1</sup> )
Alicante Bouschet	6332.5 ± 285.23 A	1324.52 ± 126.02 A	9.6 ± 0.4 A
Tentüriye üzüm	5012.05 ± 7.96 B	1022.25 ± 59.93 B	5.55 ± 0.45 B

Each value is expressed as mean ± standard deviation; values in each column with different letters are significantly different ( $p < 0.05$ ).

**Table 4.** Phenolic substances of grapes

Phenolic substances of grapes (mg kg <sup>-1</sup> fw)	Alicante bouschet	Tenturiye üzüm
Gallic acid	0.35 ± 0.003 A	0.21 ± 0.02 B
3,4-Dihydroxybenzoic acid	1.38 ± 0.03 A	0.99 ± 0.02 B
Catechin	30.19 ± 1.88 A	6.33 ± 2.02 B
Vanillic acid	2.82 ± 0.07	2.04 ± 0.14
Syringic acid	0 B	105.95 ± 12.71 A
Epicatechin	50.93 ± 4.76 A	32.2 ± 1.26 B
Caftaric acid	133.39 ± 6.44 A	16.27 ± 0.18 B
Clorogenic acid	49.61 ± 4.92 A	6.12 ± 0.3 B
Cafeic acid	22.93 ± 0.95 A	14.35 ± 0.36 B
Cumaric acid	0.53 ± 0.27 A	0.61 ± 0.07 B
Ferulic acid	5.18 ± 0.01 A	2.36 ± 0.01 B
trans-Resveratrol	9.94 ± 1.29 <sup>ns</sup>	11.45 ± 0.11 <sup>ns</sup>
Rutin trihydrate	7.19 ± 2.19 <sup>ns</sup>	7.36 ± 0.03 <sup>ns</sup>
Kaempferol 3-glucoside	2.04 ± 0.03 A	0.9 ± 0.45 B
Quercetin	1.29 ± 0.04 B	1.67 ± 0.02 A

Each value is expressed as mean ± standard deviation; values in each row with different letters are significantly different ( $p < 0.05$ ). ns: not significant

of phenolic matter (TPM) varies greatly according to grape varieties. Lorenzo et al. (2019) found TPM ranged between  $440 \pm 0.02$  and  $7940 \pm 0.19$  mg gallic acid equivalents kg<sup>-1</sup> of fresh weight in their study on 16 grape varieties. They reported that TPM of red wine varieties was higher than that of red table grapes. Özcan et al. (2017) revealed that the TPM amounts in three different grapes at the time of harvest varied between 5,000 and 8,937 mg gallic acid equivalents kg<sup>-1</sup>. Although the values of Tenturiye üzüm were low, it was determined

as better than many other wine grapes. Candar et al. (2019) found TPM, TAM and TTM values on Merlot grapes in their study in 3 harvest years between 1866.38 - 2974.58 mg GAE kg<sup>-1</sup>, 535.97 - 639.88 mg kg<sup>-1</sup>, 2.59 - 4.32 mg TA g<sup>-1</sup>, respectively. Particularly, the total anthocyanin matter content was very high due to their teinturier variety. Chen et al. (2018) determined the total anthocyanin amounts in the pulp as 1,280 mg kg<sup>-1</sup> and 1,490 mg kg<sup>-1</sup> in two different grapes. The total anthocyanin values of the two grapes we studied were also high. Anthocyanins

are responsible for the color of red wine. The total anthocyanin content in red wine grapes ranges from 300 to 7,500 mg kg<sup>-1</sup> fresh weight (Mazza & Miniati, 2018), which is consistent with our results. The amounts of phenolic substances detected in grapes are shown in Table 4. Caftaric acid was found in high amounts in Alicante Bouschet, as syringic acid was found in high amounts in Tenturiye üzüm. Uysal Seçkin (2019) found the highest values of gallic acid (3.757±2.022 mg kg<sup>-1</sup> dw) in white grapes, while caftaric acid (634±26 mg kg<sup>-1</sup> dw) was found higher in red varieties in the study on 10 grapes. These values were found to be similar for the two grape varieties examined.

Gutiérrez-Gamboa and Moreno-Simunovic (2018) found the amount of (+)-catechins varying between 25.80 mg kg<sup>-1</sup> and 50.58 mg kg<sup>-1</sup>, the amount of (-) epicatechin varying between 10.40 mg kg<sup>-1</sup> to 26.02 mg kg<sup>-1</sup>, the amount of kaempferol 3-glucoside varying between 8.20 g kg<sup>-1</sup> to 16.06 mg kg<sup>-1</sup>, the amount of caftaric acid varying between 15.32 mg kg<sup>-1</sup> to 22.38 mg kg<sup>-1</sup>, the amount of caffeic acid varying between 0.84 mg kg<sup>-1</sup> to 0.92 mg kg<sup>-1</sup> in 4 different grapes grafted on the same rootstock in Chile. Di Lorenzo et al. (2019) found the amount of (-) epicatechin varying between trace amount to 72.31 mg kg<sup>-1</sup>, the amount of kaempferol 3-glucoside varying between not detected to 21.94 mg kg<sup>-1</sup>, the amount of caftaric acid varying between not detected to 17.90 mg kg<sup>-1</sup>, the amount of rutin varying between not detected to 2.29 mg kg<sup>-1</sup> in 16 different grapes. While some of the phenolic content values of the investigated grapes were similar, some of them were different. This might be affected by the grape variety, regional differences, climatic conditions, growing conditions, etc.

Resveratrol, which is the most important of the phenolic compounds in terms of health benefits, is known to have healing properties against allergies, inflammation, hypertension and carcinogens. In addition, it has been proven as a result of studies that it has beneficial effects against disorders in the liver, skin, heart, circulation and lipid metabolism in humans (La Torre et al. 2004). Otağ (2015) determined the amount of t-resveratrol in the samples taken at different maturity periods in 4 different grape varieties grown in Denizli, at rates varying between 0.06 mg kg<sup>-1</sup> and 7.25 mg kg<sup>-1</sup>, and

higher in black varieties. Kim et al. (2003) found t-resveratrol values of 32 grape varieties, 9 wines, and 9 grape juices were ranged from trace amount to 2.07 mg kg<sup>-1</sup>, from 5.4 to 275.7 µg L<sup>-1</sup>, and from 63.3 to 751.6 µg L<sup>-1</sup>, respectively. In general, t-resveratrol values detected in both grape varieties were high, but the amount found in Tenturiye üzüm was much higher. This increases the value of this grape.

When evaluated in general, the phenolic content values of Alicante Bouschet variety were higher, but the high amount of t-resveratrol in Tenturiye üzüm shows that it may be an important variety for human health. In addition, while syringic acid could not be detected in Alicante Bouschet variety, Tenturiye üzüm were also determined at a high rate.

Although the amounts of phenolic substances detected in the study are compatible with the literature, there are also some differences. This may be thought to be due to both cultivar characteristics and growing conditions.

## Conclusion

A genotype similar to Alicante Bouschet, which is the most well-known tenturier cultivar all over the world, has been detected in Tekirdağ province. Tenturiye üzüm was discovered during the surveys of Tekirdağ Viticulture Research Institute's technical personnel and was taken under protection in TGFGB. The differences and similarities between the two varieties have been revealed with the examinations and analyzes made. The teinturier grapes, which are rich in phenolic substances, are important for the wine industry as they are also used to give color to the wine. Finding and promoting such a grape in Tekirdağ will be beneficial for the region and country viticulture.

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## Conflicts of Interest

The authors declare that there is no conflict of interest.

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