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CHANGES IN PHYSICAL AND CHEMICAL CHARACTERISTICS OF SYRAH GRAPES IN RESPONSE TO TIMING OF CLUSTER THINNING

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ABSTRACT

Cluster thinning was performed on vines of the Syrah variety eight days before the véraison (CTBV) and after the completion of the véraison (CTAV) in the 2019 and 2021 vegetation periods. The differences in physiological and chemical properties between the grapes were investigated to which cluster thinning was applied at two different times and those without cluster thinning. According to the research results, CTBV was more effective in the berry fresh weight, dry weight, berry volume, skin weight, skin surface, cluster width, cluster length, cluster weight, and cluster volume parameters than CTAV. Likewise, the levels of total phenolic compounds, total anthocyanins, and antioxidant capacity increased by 9%, 27%, and 30%, respectively, compared to the control group without cluster thinning. Among the phenolic compounds, *trans*-resveratrol showed the highest increase of 38%, and petunidin-3-glucoside increased the most (12%) among anthocyanins.

Key words: wine grape, phenolics, anthocyanins, antioxidant capacity

INTRODUCTION

Many factors affect the quality of wine grape growth. Cluster thinning is one of them, and it is a viticulture tool used to correct excessive crops, improve fruit composition, and balance shoot growth and fruit development. It is a known fact that quality wines are usually obtained from vineyards with low or medium yields depending on cultural practices [Condurso et al. 2016, Reeve et al. 2018]. Cluster thinning suppresses flowers or grape clusters before they fully mature [Poni et al. 2009]. It improves the kinetics of ripening by making physiological adjustments to the plant [Smithyman et al. 1998]. In recent years, there has been no consensus among researchers on the effect of cluster thinning on grape maturity. Several studies have reported that cluster thinning, mainly when applied in the early stages, improves berry ripening and results in higher sugar and lower acidity compared to fruit from un-thinned grapevines [Petrie and Clingeleffer 2006, Xi et al. 2016].

The timing of cluster thinning also affects yield and quality components such as berry per cluster, berry weight, and size [Fanzone et al. 2011]. The timing and effects of cluster thinning are debated among researchers today. There are studies [Bogicevic et al. 2015] that suggest that cluster thinning should be done for wine quality before the véraison [Fertel 2011] or during the véraison, which corresponds to the beginning of the final growth phase of grapes, when the green color begins to fade, and the berry begins to soften. After the clusters are removed, especially in the early stages

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(at or just after flowering), excessive photo-assimilate accumulation can inhibit leaf photosynthesis [Naor et al. 1997]. If cluster thinning is applied while shoot growth is still rapid, those assimilates can be directed to fruit [Keller et al. 2005]. In the Sangiovese variety, brix, total phenol levels, and total anthocyanin levels increased in grapes regardless of the cluster thinning time [Gatti et al. 2012]. In a study conducted on the Syrah variety, acidity decreased with the cluster thinning effect, while pH, total phenolic, and total anthocyanin levels increased [Condurso et al. 2016]. On the other hand, total phenol and antioxidant compounds remained unchanged, while total flavonoids decreased in another study on Syrah [Çelik and Ilgaz 2020]. In Cabernet Sauvignon and Ugni Blanc varieties, cluster thinning did not significantly affect the berries' chemical properties [Song et al. 2018]. Thus, the effects of cluster thinning vary depending on the variety and application. Many researchers have examined the effects of cluster thinning on wine quality in Syrah [Condurso et al. 2016, Liu et al. 2018, Wang et al. 2022]; however, there is a limited number of studies examining the effects of cluster thinning at two different phases on the physical and chemical properties of grapes in Syrah to the best of our knowledge. Furthermore, the effect of cluster thinning time should be examined in each wine grape growing region, as conflicting results have been reported regarding cluster thinning, which leads to better grape quality in some cases. Therefore, the aim of the present research is to examine the effect of cluster thinning applications carried out before and after the véraison period on Syrah grape quality, one of the most processed varieties in the world and in Turkey. In order to improve scientific understanding of vineyard practices, this study aims to guide proper management for red wine grapes growing in Güney-Denizli province, where most of Turkey's wine grapes are grown and the largest Syrah cultivation area is located.

MATERIAL AND METHODS

Syrah vineyard. The research was undertaken in 2019 and 2021 vegetation periods in guyot training, the nonirrigated vineyard of Syrah (*Vitis vinifera* L.) grafted to 41 B stock at Güney (38°09'45.29" N and 29°07'14.46" E, 791 m above sea level), Denizli, Turkey. Vine spacing was 2 × 3 m. The vines were pruned in winter by leaving two renewals (two buds for each) and two crops (six buds for each) in March. The study was conducted in a randomized block design with 20 vines for each cluster thinning application (cluster thinning before véraison and cluster thinning after véraison) and 60 vines. Meteorological data were obtained from the climatic station of the Turkish State Meteorological Service located in the research area. The main climatic conditions in the 2019 and 2021 vegetation periods are given in Table 1.

Cluster thinning treatments. After cluster thinnings (CT), only one cluster per shoot was left (ap-

Table 1. The climatic conditions of the experiment vineyard (Güney, Denizli) during 2019 and 2021 vegetation period

Months	Min temp. (°C)		Max temp. (°C)		Mean temp. (°C)		Rainfall (mm)		Day and night temp. difference (°C)	
	year									
	2019	2021	2019	2021	2019	2021	2019	2021	2019	2021
March	4.2	2.0	14.8	11.3	9.0	6.1	0.5	2.1	10.1	9.3
April	7.0	7.8	17.2	18.4	11.5	12.5	1.0	0.4	10.1	10.1
May	13.1	13.8	24.1	27.5	18.2	20.2	0.7	0.0	11.0	13.7
June	16.7	14.9	28.9	27.3	22.0	20.1	2.1	0.6	12.1	12.4
July	18.1	20.1	31.3	33.4	24.3	26.5	0.2	0.3	13.2	13.3
August	19.4	20.0	33.2	34.2	25.8	26.8	0.7	0	13.8	14.2
September	15.4	15.0	28.5	27.4	21.0	26.8	2.0	0.2	13.1	12.5

proximately 50% of clusters were removed). Phenology was determined using the BBCH scale [Lorenz et al. 1995]. The treatments were as follows:

– UNT (untreated): only winter pruning without cluster thinning,
– CTBV (cluster thinning before véraison): cluster thinning was performed on 2 July 2019 and 25 June 2021, 8 days before the start of véraison (BBCH 81),
– CTAV (cluster thinning after véraison): cluster thinning was performed on 23 July 2019 and 20 July 2021, one day after all the grape clusters became colored (BBCH between 83–85).

Harvest was carried out manually on 22–29 August 2019 and 2021 for each application when the grapes reached approximately 24 °Bx (BBCH 89). The harvested grapes were transported in iced boxes to the Horticulture Department, Faculty of Agriculture, Ankara University, the same day.

Grape juice analyses at harvest. Total acidity (in terms of tartaric acid, mg g^{-1}), pH, and °Bx of grape juices were measured on the harvest day according to OIV [2009]. The yield values of the vines belonging to the treatments were also recorded.

Analyses of the physical characteristics of berries and clusters. From the harvested grapes, 100 grape berries were randomly selected for each treatment, and berry width (mm), berry length (mm), berry fresh weight (g), berry dry weight (g), berry volume (cm^3), and skin weight (g) were measured according to OIV [2009], and skin surface (cm^2/berry) was measured according to Palma et al. [2007]. Additionally, cluster width (cm), cluster length (cm), cluster weight (g), cluster volume (cm^3), and cluster density were measured for 6 clusters taken randomly on the same day for each treatment according to OIV [2009].

Extraction of grape samples for chemical analysis. The grapes were extracted according to Colombo et al. [2019]. Accordingly, the samples frozen with liquid nitrogen and weighed 2 g were homogenized for 3 minutes by adding 3 mL of methanol/distilled water (1:1, v/v). After homogenization, the samples were placed in an ultrasonic bath for 15 minutes and then centrifuged at 14,000 rpm for 15 minutes. The samples whose supernatants were taken into a separate tube were again treated with the same extraction procedures on the solid parts. The supernatants were combined by being filtered through 0.45 µm PVDF filters, and the final volume was adjusted up to 10 mL. The obtained extracts were preserved in the dark at +4°C for spectrophotometer analysis.

Total phenolic compound, total anthocyanin, and antioxidant capacity (ABTS, DPPH, and FRAP). Total phenolic compounds, total anthocyanin, and antioxidant capacity analyses were performed with Shimadzu UV 1208 model UV VIS spectrophotometer (Japan). Total phenolic compounds were determined according to Singleton and Rossi [1965], and the results are expressed as mg gallic acid equivalent (GAE) kg^{-1}. According to Giusti and Wrolstad [2001], total amounts of anthocyanins were determined, and the results are expressed as mg kg^{-1}. Antioxidant capacity levels were determined based on three different methods: ABTS [2,2′-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)], DPPH (2,2-diphenyl-1--picrylhydrazyl), and FRAP (ferric reducing antioxidant power). ABTS method was performed according to Re et al. [1999]; DPPH was performed according to Katalinić et al. [2004]; and the FRAP method was performed according to Benzie and Strain [1996]. The results are expressed in Trolox equivalents (µmol Trolox g^{-1}) to facilitate comparability.

Phenolics and anthocyanins. *Trans*-resveratrol, *cis*-resveratrol, (+)-catechin, (-)-epicatechin, rutin, and quercetin amounts of grape berries and delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3--glucoside, peonidin-3-glucoside, and malvidin-3--glucoside from anthocyanins levels in grape skins were determined by HPLC-DAD (Shimadzu, Japan) [Downey and Rochfort 2008]. The phenolic compounds were identified based on the retention times and spectra of the standard substances used. Since there are no commercial standards to determine *cis*-resveratrol, the prepared *trans*-resveratrol standards were exposed to UV-C light with a wavelength of 254 nm (Philips TUV PL-L) to transform the *trans* into *cis* form. The standards were prepared at 1–50 ppm concentrations, and standard curves were generated and used to determine the phenolic compound amounts of the samples. Gemini Phenomenex C18 (Calif., USA): 4.6 × 260 mm column was used. Two different solvents were used in the mobile phase: 10% formic acid in water (solvent A) and 10% formic acid in methanol (solvent B). The flow rate of the solvents was

1.0 mL min^{-1}, and the gradient conditions were set as 0 min 18% B, 14 min 29% B, 16 min 32% B, 18 min 41% B, 18.1 min 30% B, 29 min 41% B, 32 min 50% B, 34.5 min 100% B, and 35–38 min 18% B. HPLC-DAD readings were performed in the range of 210–600 nm. The results are expressed as mg kg^{-1}.

Statistical analyses. IBM SPSS 20 software was used to perform the statistical data analysis. One-way ANOVA was performed on the data to investigate the effects of cluster thinning time on each parameter. The analyses were performed in 3 replications. The means of both years were presented because no statistical difference was detected between the 2019 and 2021 data. Statistical significance level was considered 5% (*) and 1% (**) SPSS statistical program was used for all statistical analyses.

RESULTS AND DISCUSSION

Parameters of grape juice. The differences in pH, total acidity, and °Bx levels of cluster thinning before véraison and cluster thinning after véraison compared to untreated group grape juice are given in Table 2. While the lowest pH is 3.11, the total acidity is measured inversely with 7.53 g L^{-1} in the UNT group, which had the highest value. °Bx value, which was 23.90 in UNT, increased by 10.7% with CTBV application and 0.7% with CTAV application. Kök [2011] applied cluster thinning in the Sauvignon Blanc variety at different times and acquired the highest °Brix value after cluster thinning application eight weeks before véraison. Although the application time in this study was not the same, pre-véraison (CTBV) application caused higher °Bx accumulation compared to the application performed after (CTAV). Condurso et al. [2016] stated in their research that there was an increase in Brix and pH values and a decrease in acidity value in the Syrah cultivar by thinning the clusters in the early stages of véraison compared to the control group. Therefore, the amount of sugar increased, and the acidity decreased; therefore, the sugar/acid ratio increased in the present study in line with previous studies [Gil et al. 2009, Yağcı and Bozkurt 2020]. In general, high-quality red wine production is recommended that the total acidity value should be less than 135 meq L^{-1} and that pH should be less than 3.5 [Jackson 2014]. It may be seen that the recommended values were not exceeded by either cluster thinning in this research.

Physical characterization of berries and clusters. The changes due to the cluster thinning application in the physical parameters of grapes concerning the UNT group are given in Table 3. Yield decreased in both cluster thinning treatments as expected and as reported in previous studies [Gatti et al. 2012]. There was a 49% decrease in yield with CTBV and a 40% decrease in yield with CTAV. It confirmed that thinning the grapes before véraison reduced yield more. While berry width and berry length reached the highest levels with CTAV, berry fresh weight, berry dry weight, berry volume, skin weight, skin surface, cluster width, cluster length, cluster weight, and cluster volume levels were affected more by CTBV application. Cluster density decreased with cluster thinning applications. The highest increase among the parameters representing the physical characteristics of grapes (Tab. 3) was observed in the berry dry weight parameter in CTBV and CTAV, at 146% and 121%, respectively. Marcon et al. [2018] reported that cluster thinning during and after véraison reduced cluster weight compared to cluster thinning before véraison in Montepulciano and Caber-

Table 2. Parameters of grape juice (pH, total acidity, °Bx) samples from Syrah grapevines subjected to different cultural practices

Parameter	UNT	CTBV	CTAV	ANOVA
pH	3.11 c	3.22 b	3.41 a	*
Total acidity (g L^{-1})	7.53 a	7.23 b	7.10 c	*
°Brix	23.90 c	26.37 a	24.07 b	**

UNT – untreated, CTBV – cluster thinning before véraison, CTAV – cluster thinning after véraison. A different letter indicates significant differences for each parameter within a line; * $P < 0.05$, for **: $P < 0.01$

Table 3. Physical characterization of berry and cluster samples from Syrah grapevines subjected to different cultural practices

Parameter	UNT	CTBV	CTAV	ANOVA
Yield (kg/vine)	3.21 a	1.63 c	1.91 b	*
Berry width (mm)	12.08 c	13.20 b	13.99 a	*
Berry length (mm)	12.13 c	13.72 b	14.08 a	*
Berry fresh weight(g)	1.66 b	2.01 a	2.00 a	*
Berry dry weight (g)	0.24 b	0.59 a	0.53 a	**
Berry volume (cm^3)	3.25 c	4.19 a	3.89 b	*
Skin weight (g)	0.26 c	0.32 a	0.29 b	*
Skin surface (cm^2/berry)	7.46 c	9.05 a	8.01 b	*
Cluster width (cm)	10.77 b	11.21 a	11.20 a	*
Cluster length (cm)	17.33 c	21.23 a	19.27 b	*
Cluster weight (g)	250.00 b	290.00 a	255.00 b	**
Cluster volume (cm^3)	202.33 c	261.00 a	258.33 b	**
Cluster density	0.76 a	0.62 c	0.70 b	*

UNT – untreated, CTBV – cluster thinning before véraison, CTAV – cluster thinning after véraison. A different letter indicates significant differences for each parameter within a line; * $P < 0.05$, for **: $P < 0.01$

net Franc cultivars. In the present study, while cluster thinning caused higher cluster weights, the application that increased the cluster weight the most was CTBV. It is considered the plant's reaction to compensate for the reduced yield. Contrary to the present study, the researchers mentioned above did not observe any effect of cluster thinning on berry sizes.

Total phenolic compound, total anthocyanin, and antioxidant capacity of grapes. The changes in total phenolic compound, total anthocyanin capacity, and total antioxidant capacity as a result of cluster thinning compared to UNT grapes are given in Table 4. As the antioxidant capacity of grapes results from phenolic compounds, antioxidant capacity values, measured with three different methods, are higher in CTBV and higher total phenolic compound content. CTAV application also gave higher results compared to the UNT group. CTBV application resulted in 9%, 30%, 25%, and 86% increases in TPC, ABTS, DPPH, and FRAP levels, respectively, compared to UNT. CTAV application increased the same levels by 7%, 18%, 17%, and 47%, respectively. As the phenolic content of the grape and, therefore, the wine rises, the quality of the wine improves. In addition to the organoleptic effect, phenolic compounds are essential with their antioxidant effects. Modern society is trying to consume foods that can treat and prevent diseases and increase longevity. In this context, functional foods with proven health benefits, rich in antioxidant compounds, and widely used beverages that provide health benefits are highlighted, and wine is one of them [Wurz 2019]. The way to obtain wine with these characteristics is by cultivating quality wine grapes. Therefore, attention is diverted to viticulture practices that increase the antioxidant effect.

Phenolics and anthocyanins of grapes. The effect of cluster thinning application at different times on selected phenolics and anthocyanins in grapes can be followed from Table 5. Phenolics are a large and highly complex group of compounds of primary importance to the properties and quality of red wine. They affect the appearance, taste, mouthfeel, odor, and antimicrobial properties [Jackson 2008]. Since they are mainly of grape origin, their amounts in wine vary with the effect of viticulture techniques. The amounts of phenolic compounds that are beneficial to human health were determined in the present study. CTBV samples are impressively richer in phenolic compounds than UNT samples. For example, while trans-resveratrol is 4.24 mg kg^{-1} in UNT, it reached 5.84 mg kg^{-1} with

Table 4. Total phenolic compound and antioxidant capacities of grape samples from Syrah grapevines subjected to different cultural practices

Parameter	UNT	CTBV	CTAV	ANOVA
TPC (mg GAE kg^{-1})	6433 d	7036 a	6892 b	*
antioxidant capacities (µmol Trolox mL^{-1})				
ABTS	35.20 c	45.89 a	41.54 b	*
DPPH	28.01 e	35.09 a	32.83 b	**
FRAP	10.16 d	18.85 a	14.96 b	*

UNT – untreated, CTBV – cluster thinning before véraison, CTAV – cluster thinning after véraison. TPC – total phenolic compound, ABTS – 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid), DPPH – 2,2-diphenyl-1-picrylhydrazyl, FRAP – ferric reducing antioxidant power. A different letter indicates significant differences for each parameter within a line; * $P < 0.05$, for **: $P < 0.01$

an increase of 38% with CTBV. As a critical phenolic compound in wine, catechin is 31.1 mg kg^{-1} in UNT, while it increased by 28% with CTBV application. *Cis*-resveratrol decreased due to both applications, suggesting that as the number of clusters decreases, UV-C exposure in the clusters also decreases, and *cis* formation does not occur due to the decline in exposure to the sun. Epicatechin, rutin, and quercetin levels increased by 7%, 9%, and 10% in CTBV samples, and 5%, 5%, and 10% in CTAV samples, respectively. They accumulate in the cellular vacuoles of the epidermis and outer hypodermis in the skin, and together with anthocyanins, they absorb ultraviolet radiation, protecting the inner tissues from the harmful effects of UV radiation [Jackson 2008]. Although the biosynthesis of phenolic compounds is complex and depends heavily on genetic expression in vines, the amount of phenolic compounds in wine is affected by the grape variety, the level of ripening of the grape at harvest, grape growing technology, soil, and climatic conditions, the winemaking process, and the evolution of phenolic compounds during aging in wine [Cravero et al. 2012, González-Neves et al. 2012, Giuffrè 2013, Tahmaz and Söylemezoğlu 2017].

The highest skin surface parameter of grape berries belongs to CTBV samples, and this expansion on the skin surface is evidenced mostly by anthocyanin accumulation. The highest total anthocyanin accumulation is measured in the samples of this application. In addition, the levels of all the examined anthocyanins, especially malvidin-3-glucoside, responsible for the color of grapes and wine, are also the highest with CTBV. The application that increased the anthocyanin compounds in Syrah grapes the most is CTBV, and CTAV application contains more intense anthocyanin compounds than the UNT group. As shown in Table 5, anthocyanin compounds increased more due to CTAV. Compared to UNT, delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside levels are 7%, 12%, 5%, 7%, 7% higher in CTBV, respectively. In CTAV, these compounds increased by 5%, 3%, 4%, 5%, and 2%, respectively. On the other hand, the total anthocyanin level increased by approximately 27% due to both treatments.

The effect of cluster thinning was examined due to the literature search because it is difficult to find studies that examined similar parameters by comparing the cluster thinning applications performed before and after véraison. It has been stated in previous studies on the Syrah variety that cluster thinning increases the quality characteristics and color compounds of the grape [Condurso et al. 2016], phenolic compounds and anthocyanins increase along with a decrease of approximately 40% in yield [Gil et al. 2013], it may cause an increase, especially in oenological compounds related to wine color. It also increases the catechin content [Peña-Neira et al. 2007]. In addition to researchers who state that early cluster thinning (flowering and fruit set) has no effect on maturity and anthocyanins

Table 5. Phenolics and anthocyanins of grape samples from Syrah grapevines subjected to different cultural practices

Phenolics and anthocyanins	UNT	CTBV	CTAV	ANOVA
Trans-resveratrol (mg kg^{-1})	4.24 b	5.84 a	4.86 b	*
Cis-resveratrol (mg kg^{-1})	0.23 a	0.20 b	0.19 b	*
Catechin (mg kg^{-1})	31.10 c	39.75 a	35.95 b d	*
Epicatechin (mg kg^{-1})	37.13 c	39.81 a	39.11 b c	*
Rutin (mg kg^{-1})	12.38 c	13.44 a	13.04 b	*
Quercetin (mg kg-^{1})	6.83 b	7.49 a	7.50 a	*
Delphinidin-3-glucoside (mg kg^{-1}) (mg/kg)	69.30 c	74.06 a	70.61 bc	*
Cyanidin-3-glucoside (mg kg^{-1}) (mg/kg)	46.75 b	49.84 a	49.29 a	*
Petunidin-3-glucoside (mg kg^{-1})	9.16 c	10.23 a	9.43 b	*
Peonidin-3-glucoside (mg kg^{-1})	17.31 c	18.18 a	18.01 ab	*
Malvidin-3-glucoside (mg kg^{-1})	722.67 c	771.67 a	761.67 b	**
Delphinidin-3-glucoside (mg kg^{-1})	69.30 c	74.06 a	70.61 bc	*
Total anthocyanin (mg kg^{-1})	1289 b	1639 a	1618 a	**

UNT – untreated, CTBV – cluster thinning before véraison, CTAV – cluster thinning after véraison. A different letter indicates significant differences for each parameter within a line; * $P < 0.05$, for **: $P < 0.01$

[Fanzone et al. 2011, Wang et al. 2018], some studies claim that cluster thinning during véraison generally has a positive effect on the composition and quality of grapes [Pastore et al. 2011, Xi et al. 2016, Song et al. 2018, Mawdsley et al. 2019]. In the present study, both cluster thinnings performed before and after véraison were influential in the parameters examined compared to the control group.

CONCLUSIONS

The results show that cluster thinning applications can modify grapes' physical and chemical characteristics at different times. The "véraison", where the grapes lose their green color and which results in ripening, is a turning point that can significantly affect the results of cluster thinning applications. Therefore, the decision on the timing of cluster thinnings before and after this point affects the composition of the final grape. The main factor that affects the wine quality most is the viticulture techniques. As a prerequisite for wines rich in phenolics, optimum synthesis and accumulation of polyphenols must be ensured during the grape ripening phase. In the present study, the effects of cluster thinning applications, which were carried out for two years before and after véraison in the Syrah grape variety on the physical and chemical properties of grapes were examined, and it is concluded that the most effective application on the examined parameters is cluster thinning before véraison (CTBV). Although CTAV is effective on the same parameters compared to UNT, berry fresh weight, berry dry weight, berry volume, skin weight, skin surface, cluster width, cluster length, cluster weight, cluster volume parameters, which are physical properties of berries and clusters, were more affected by CTBV. As a result of CTBV, the contents of TPC, TA, and antioxidant capacity increased, as well as the amounts of individual phenolic compounds and anthocyanins. The present research may be functional to advise grape growers and oenologists to use pre-véraison cluster thinning (CTBV) compared to post-véraison cluster thinning (CTAV) in the Syrah variety. Since the responses of grape cultivars to cultivation practices can differ, examining the effects of the same practices in different cultivars in future research is crucial.

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