



THE EFFECTS OF CLUSTER THINNING YIELD AND QUALITY ON 'HORUZ KARASI' CULTIVAR OF GRAPEVINE

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Abstract: In this study, it was aimed to determine the effects of different levels of bunch thinning treatments (S1/Control: 20 clusters/vine; S2: 16 clusters/vine; S3: 12 clusters/vine; S4: 8 clusters/vine; S5: 4 clusters/vine) on yield and quality parameters in Horoz Karası (*Vitis vinifera* L.) grape variety. Within the scope of the study, the effects of different cluster thinning treatments on cluster weight, berry weight, berry weight, berry width, berry length, pH, TSS (water soluble dry matter), total acidity, total phenolic content and total antioxidant capacity of grapes were analyzed and evaluated. Considering the results; S2 cluster thinning level stood out with a cluster length of 21.26 cm, a cluster weight of 752.13 grams, and a berry width of 21.50 mm. S3 treatment gave the highest values in terms of berry length and hundred-berry weight (29.15 mm; 791.43 g). S1 cluster thinning level stood out with a cluster width of 15.75 cm and a vine head yield of 14.15 kg. When the effects of bunch thinning treatments on chemical and physiological characteristics were examined, it was determined that although there was no significant difference between the values obtained in terms of pH, total acidity (TA), TSS and maturity index (MI), the bunch thinning treatment at S3 level had the highest values in terms of both total phenolic matter content and total antioxidant capacity on phytochemical parameters. Considering the results, it was concluded that cluster thinning, currently applied to other grape varieties, also had a positive effect on the physical, chemical, and phytochemical properties of the 'Horoz Karası' variety. Implementing this type of treatment on this variety, which is used for various purposes (table and drying) across a wide geography, is anticipated to shed light on future studies by testing different levels of cluster thinning and incorporating it into different cultural practices.


Keywords: Total phenolic, Cluster width, pH, Yield

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1. Introduction

Viticulture, which is practiced almost everywhere in our country, is an important source of income for our producers. Increasing population, changing consumer demands lead producers towards a higher quality and efficient production. Ensuring the quality/product relationship is the main objective of almost all farming techniques. Some cultural practices are carried out to ensure this balance in grapevine plants. Panicle thinning, which is defined as the suppression of panicles and flowers before maturity, is one of them (Pallioti and Cartechini, 1998).

Pruning in viticulture is done in two periods as summer pruning and winter pruning. Winter pruning is mostly done to manage yield and summer pruning is done to improve quality. Summer pruning, which is done when the vines are in leaf, is also called green pruning. Summer pruning, which is applied to increase product quality, has benefits such as limiting the longitudinal growth of the vines, ensuring a good air intake and outflow in the vines, and providing optimum sunbathing in the area where the

clusters are formed. Cultural techniques applied within summer pruning include shoot removal, seat removal, cluster thinning and leaf removal (Özer et al., 2005; Sabir et al., 2010). The effect of the applications on yield and quality varies according to soil structure, ecology, climate characteristics and variety (Uzun, 1996).

Cluster thinning is defined as the removal of the clusters on the vine before maturity (Pallioti and Cartechini, 1998). Cluster thinning aims to reduce the fruit load on the vine and to obtain a more balanced and quality product (Çelik, 1998; Smart and Robinson, 2006). Cluster thinning improves the quality of clusters and berries by allowing more air and sunlight to penetrate between the clusters and into the crown, and by improving the conditions inside the crown of the vine. The period of thinning and the rate of thinning may vary depending on the purpose (Dumartin et al., 1990; Smithyman et al., 1998; Climaco et al., 2005; Martins, 2007; Mawdsley et al., 2019).

Cluster thinning can be done before flowering and during the small berry period. During the flowering period, one



cannot be sure how many berries the clusters will produce, especially if it is a shriveling variety. Clusters can be noticed and removed during panicle thinning after berry set. Cluster thinning practices are generally applied to table varieties. These practices have a direct impact on the product load. For this reason, many researchers emphasize that thinning should be carried out by taking into account the yield load of the vine, paying close attention to the time and rate of thinning (Jackson and Lombard, 1993; Reynolds et al., 1994; Martins, 2007).

'Horoz Karası' variety is also a valuable and productive variety for Kilis province where the study was conducted. The 'Horoz Karası' variety has a very strong growing structure. With too frequent cluster formation, disease and pest effects can be seen in the variety and quality losses may occur. The aim of this study was to investigate the effects of the level of cluster thinning on the yield and quality of the 'Horoz Karası' variety, which is an important variety for the region.

2. Materials and Methods

The study was carried out in the producer vineyard located in Çayıraltı village of Musabeyli district of Kilis province, Türkiye (36.93° N, 36.99° E; characterized by, Mediterranean transitional climate and terra rossa soils). The vineyard where the study was conducted is 10 years old, has a goblet system and the distance between rows and above rows is 4×4 meters. In the vineyard where the vines were established, an average of 60 vines were planted per decare. Phenological observation was followed and harvested at 21°Brix level. Winter pruning was carried out in mid-February, leaving an average of 7 spurs shoots per vine, leaving an average of 3 buds on each shoot, leaving an average of 21 buds in total.

2.1. Plant Material

The berries of the 'Horoz Karası' cultivar are blue-black in color, elongated elliptical, very large and have 2-3 seeds. The clusters are very large, densely packed and have a winged conical structure. It is grown intensively in Gaziantep and Kilis and ripening takes place in the middle season. It is suitable for mixed - short pruning in terms of pruning characteristics (Çelik, 2006).

2.2. Methods

Cluster thinning was performed at the 31st developmental stage according to the Eichhorn-Lorenz (E-L) scale, which is the phenological stage during berry development when the berries reach pea size. In this study, cluster thinning, one of the widely practiced cultural treatments to improve quality parameters in viticulture, was applied. Before the application, the average number of bunches on each vine was fixed at 20 and these vines were considered as the control group. Cluster fixation was carried out by removing excess inflorescences during the flowering period. At this stage, five different dilution levels were established on each vine by removing 0 (control), 20, 40, 60, 60 and 80 % of the clusters, respectively. These levels were defined as S1 (20 cluster/vine), S2 (16 cluster/vine), S3 (12

cluster/vine), S4 (8 cluster/vine) and S5 (4 cluster/vine) (Cluster thinning was done at the EL-31 stage according to Lorenz et al. (1995)).

Grape samples were harvested on August 30, 2023, in accordance with the sampling plan. Analyses were conducted in the field at the Gaziantep University Ulubey Research Institute in Gaziantep province, under accredited laboratory conditions in accordance with the ISO/IEC 17025 standard. Samples were appropriately labeled and collected in sterile, leak-proof polypropylene sample containers. They were transported to the laboratory in insulated transport containers containing cooling gel packs at a temperature between +4±2 °C. The transport time did not exceed six hours, and temperature monitoring was conducted at regular intervals throughout this process. Depending on the analysis parameters, samples delivered to the laboratory were stored under appropriate conditions in a refrigerator at +4 °C or in a deep freezer at -20 °C. The parameters examined in the study are as follows.

2.2.1. Physical analyses (morphological and agronomic analyses)

Berry width and length (mm)

The width and length of the grape berries were measured with a digital caliper (0.01 cm precision) during the harvest period and the values obtained were recorded in mm (Tangolar et al., 2005).

100 berry weight (g)

For each treatment, 100 grapes randomly selected from the lower, middle and upper parts of four different bunches were weighed on an analytical balance (0.001 g precision) and the values were recorded in g (Tangolar et al., 2005).

Cluster weight and length (cm)

During the harvest period, the width of the bunch was measured using a ruler at its widest point and recorded in centimeters. The length of the bunch was determined by measuring the distance from the upper point where the bunch began to branch to the lower point of the outermost berry (Tangolar et al., 2005).

Cluster weight (g)

The average weight per cluster was calculated by dividing the total yield per vine by the number of bunches on the vine and the values were recorded in g. (Tangolar et al., 2005).

Yield (kg/vine)

The total product harvested from each vine was weighed separately and the yield per vine was determined in kg.

2.2.2. Chemical analyses

Total acidity (TA, g/L): The acidity of the fruit juice was analyzed using the titration acidity method, and the results were expressed as tartaric acid in g/L (Cemeroğlu, 1992).

pH: The pH values of the fruit samples pureed in the homogenizer were measured directly with a glass electrode pH meter.

Soluble solids (TSS, °Brix): The soluble solids content of fruit juice was determined in °Brix using a handheld

digital refractometer

Maturity index: Calculated by dividing the TSS value by the titratable acidity.

2.2.3. Phytochemical analyses:

Total phenolic content ($\mu\text{g GAE/g}$): Total phenolic content was determined using the Folin-Ciocalteu reagent according to the method reported by Singleton and Rossi (1965). The results were calculated as gallic acid equivalent (GAE), $\mu\text{g GAE/g}$ per fresh fruit.

Total Antioxidant Capacity (TAC, $\mu\text{mol TE/g}$): Antioxidant capacity was determined according to the TEAC (Trolox equivalent antioxidant capacity) method reported by Özgen et al. (2006). The absorbance values

obtained were evaluated according to the Trolox standard curve prepared in the range of 10–100 $\mu\text{mol/L}$, and the results were presented as $\mu\text{mol Trolox equivalent (TE)/g}$ fresh fruit weight.

2.3. Statistical Analysis

The experiment was conducted using a randomized complete block design with three replicates, each replicate containing five vines, for a total of 75 vines. The collected data were subjected to a variance analysis using SAS statistical analysis software at 0.05 level of significance. Duncan's multiple comparison test was employed to compare the significance of the means (Genç and Soysal, 2018).

Table 1. Effect of cluster thinning treatments on berry characteristics of 'Horoz Karası' grape variety

Cluster Thinning Levels	Berry Width (mm)	Berry Length (mm)	Hundred Berry Weight (g)
S1 (control)	21.16 \pm 0.1963 ^A	27.77 \pm 0.1419 ^A	721.0 \pm 18.4228 ^A
S2	21.50 \pm 0.1963 ^A	28.69 \pm 0.1419 ^A	746.18 \pm 18.4228 ^A
S3	20.96 \pm 0.1963 ^{BA}	29.15 \pm 0.1419 ^A	791.43 \pm 18.4228 ^A
S4	19.44 \pm 0.1963 ^B	26.29 \pm 0.1419 ^B	610.04 \pm 18.4228 ^B
S5	20.30 \pm 0.1963 ^{AB}	28.60 \pm 0.1419 ^A	774.69 \pm 18.4228 ^A

Mean values with different superscripts within the same column indicate a significant difference (Duncan, $P < 0.05$), Standard error of the mean.

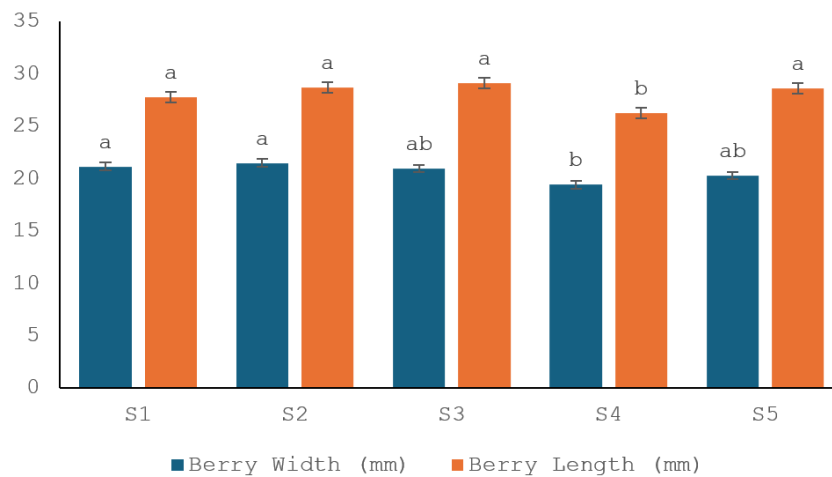


Figure 1. Effect of cluster thinning treatments on berry width and length. (One-way ANOVA with Duncan, $P < 0.05$; S1-20 cluster/vine, S2-16 cluster/vine, S3-12 cluster/vine, S4-8 cluster/vine and S5 -4 cluster/vine).

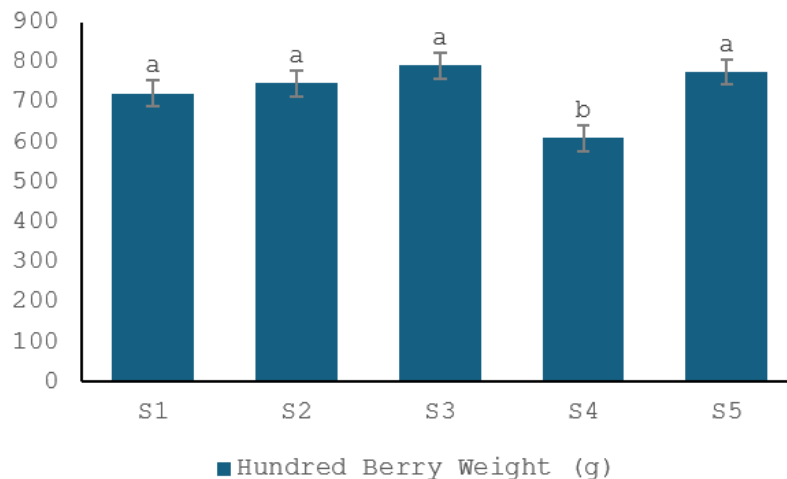


Figure 2. Effect of cluster thinning treatments on hundred berry weight. (One-way ANOVA with Duncan, $P < 0.05$, S1-20 cluster/vine; S2-16 cluster/vine; S3-12 cluster/vine, S4-8 cluster/vine and S5 -4 cluster/vine).

3. Results and Discussion

3.1. Effect of Cluster Thinning Treatments on Berry Characteristics

For a quality grape cultivation, intervention on the vine organs (stems, clusters, shoots, berries, etc.) is often the preferred method. In the study, 20, 16, 12, 8 and 4 clusters were left on the vine (S1, S2, S3, S4 and S5, respectively); S2 with 16 clusters left on the vine in terms of berry width, S3 with 12 clusters left on the vine in terms of berry length and hundred berry weight (Table 1, Figure 1, and Figure 2).

In a study in which 10, 15 and 20 clusters/vine were left on the vine in Red Globe and Sultani seedless grape varieties, it was reported that berry width, length and weight increased with the thinning rate (Tosun, 2019). In another study using Cabernet Sauvignon and Vranac cultivars, the separate and combined effects of cluster thinning and defoliation treatments were investigated and it was reported that only cluster thinning treatment did not affect berry weight, while both defoliation and cluster thinning treatments decreased cluster weight, grain weight and number of berries per cluster (Bogicevic et al., 2015). In a study in which 8, 16, 24 and 32 clusters were left on the vine, it was concluded that

the highest berry weight among physical characteristics was obtained from 16 clusters/vine treatment when yield and quality characteristics were evaluated (Pehlivan and Uzun, 2015). In another study in which cluster thinning was carried out at a rate of ¼ before flowering and at berry set by topping, the table grape variety Alphonse Lavallée was used. Treatments had no effect on cluster width, length and density and berry width and length, while berry weight increased with thinning (Akural, 2016). In the present study, the effect of cluster thinning treatments on berry traits was similar to the previous studies. It is thought that the differences between this study and other studies are due to reasons such as variety selection, cluster thinning rates, cluster thinning times and whether it is applied in combination with other cultural practices or not.

3.2. Effect of Cluster Thinning Treatments on Cluster Characteristics and Yield Value

The increase in total leaf area per unit area of crop with cluster thinning practices has been reported by many researchers as a reason for improved fruit quality (Fisher et al., 1977; Prajitna et al., 2007; Intrigliolo and Castel, 2011).

Table 2. Effect of cluster thinning treatments on cluster characteristics and yield value in Horoz Karası grape variety

Cluster Thinning Levels	Cluster Width (cm)	Cluster Length (cm)	Cluster Weight (g)	Yield per Vine (kg)
S1 (Control)	14.75 ± 0.3382 ^A	20.50 ± 0.3214 ^{AB}	707.80 ± 23.8976 ^A	14.15 ± 0.0 ^A
S2	13.43 ± 0.3382 ^{AB}	21.26 ± 0.3214 ^A	752.13 ± 23.8976 ^A	12.03 ± 0.0 ^B
S3	12.53 ± 0.3382 ^{BC}	20.80 ± 0.3214 ^A	723.47 ± 23.8976 ^A	8.68 ± 0.0 ^C
S4	11.16 ± 0.3382 ^C	19.36 ± 0.3214 ^B	517.87 ± 23.8976 ^B	4.14 ± 0.0 ^D
S5	11.56 ± 0.3382 ^C	20.33 ± 0.3214 ^{AB}	733.13 ± 23.8976 ^A	2.93 ± 0.0 ^E

Mean values with different superscripts within the same column indicate a significant difference (Duncan, P<0.05).

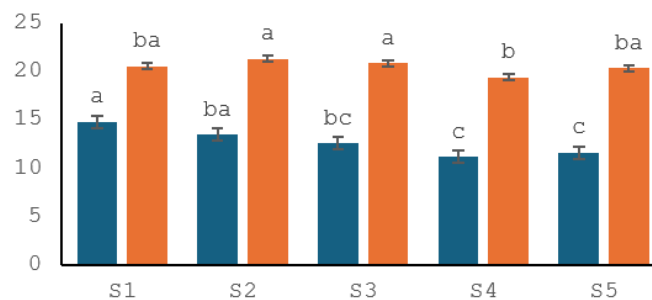


Figure 3. Effect of cluster thinning treatments on cluster width and length. (One-way ANOVA with Duncan, P<0.05, S1-20 cluster/vine; S2-16 cluster/vine; S3-12 cluster/vine, S4-8 cluster/vine and S5 -4 cluster/vine).

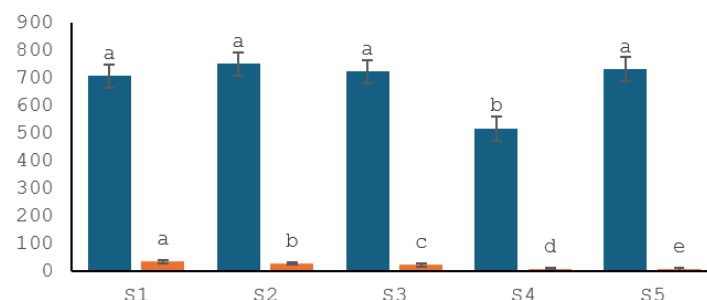


Figure 4. Effect of cluster thinning treatments on cluster weight and yield per cluster. (One-way ANOVA with Duncan, P < 0.05, S1-20 cluster/vine; S2-16 cluster/vine; S3-12 cluster/vine, S4-8 cluster/vine and S5 -4 cluster/vine).

In this study, when cluster characteristics were analyzed with cluster thinning, S1 (20 clusters/vine) in terms of cluster width and yield per cluster, and S2 (16 clusters/vine) in terms of cluster weight and length, cluster thinning came to the fore (Table 2, Figure 3, and Figure 4).

In a study conducted on Crimson Seedless cultivar, it was reported that cluster thinning and leaf removal treatments positively affected cluster weight (Abd El-Razek et al., 2010). Shiraz grape variety was used in the thinning study applied after berry set in the vines and cluster thinning was performed at different rates of 8, 16, 24 and 32 clusters/vine. In parallel with our study, the highest yield was obtained from the thinning treatment in which the most clusters were left; unlike the previous study, the differences between cluster width, length and weight values were not statistically significant in this study (Kalinkara, 2012). In another study conducted by Ilgaz and Çelik (2020), leaf removal and cluster thinning were carried out in Syrah grape variety, and while yield decreased with cluster thinning, cluster weight and cluster length values increased. In a study of Tempranillo grape variety under semi-arid conditions with water restriction and early cluster thinning practices, yield loss occurred with cluster thinning practices, while must characteristics were partially positively affected (Mancha et al., 2020).

3.3. Effect of Cluster Thinning Treatments on Chemical Parameters

Although the effects of cluster thinning treatments on the chemical parameters; water soluble dry matter (WDSM), pH, total acidity (TA) and ripening index (RI) differed according to the levels in terms of the values obtained, these differences were not statistically significant. The values obtained for TSS with cluster thinning treatment varied between 20.00 brix and 21.36 brix. The mean values for pH value were between 4.43 and 4.54. The mean pH values were between 4.43 and 4.54. TA values were in the range of 3.74-3.81 with the treatments. Maturity index values varied between 53.09-56.90 (Table 3).

In the study, although some differences were observed in the effect of cluster thinning on the chemical properties of the must, these differences were not found to be statistically significant. Akçay (2012) thinned 25% of the clusters in Mourvedre, Grenache and Syrah wine grape varieties after the mole fall period. In parallel with the study, it was found that the treatments did not have a

significant effect on the pH value. In the study on the effect of Cabernet Franc on grape yield and quality characteristics, 3 different cluster thinning treatments (0%, 25% and 50%) were applied. According to the data obtained, it was determined that the values of TSS increased with the dilution rates (Koskoşlu, 2021). In a study in which 30% and 60% somak thinning was applied on Cardinal and Amasya grape varieties one week before flowering, it was reported that bunch thinning applications decreased the fruit yield and the amount of acid in the fruit, while the SÇKM value increased (Dardeniz and Kismalı, 2002). Nail (2010) reported that °Brix value increased with cluster thinning in Cabernet Franc variety. In the study in which 40, 50, 60 and 80% clusters were left per vine in Tash-A-Ganesh table grape variety, the lowest Brix value was calculated in 80% clusters/vine and the highest in 60% clusters/vine. Regarding the acidity percentage, the lowest values were found in 40% clusters/vine and the highest values were found in 60% clusters/vine treatments (Somkuwar and Ramteke, 2006). There are similarities and incompatibilities between the chemical parameters obtained in this study and the data obtained in other studies. The reasons for this situation may be the difference in grape varieties used in the studies, differences in cultural practices, ecological differences, differences in thinning rates, thinning levels and thinning times.

Similar to this study, Fanzone et al (2011) reported an increase in phenolic content in the berries thanks to 50% cluster thinning application during the flowering and mole verasion. In Cabernet-Sauvignon and Carmenere grape varieties, topping and cluster thinning treatments were applied and as a result of the study, cluster thinning treatment caused an increase in phenolic compounds (Bordeu et al., 2014). Bekar and Cangi (2018) reported that the highest values in terms of the amount of phenolic-flavonoid substances were obtained from 30% and 60% cluster thinning applications in their study in Narince grape variety. In a study using red grape varieties (Syrah, Cabernet Sauvignon), cluster thinning was performed in 3 different periods from ripening to harvest. It was determined that the grapes treated with thinning were a good source of phenolic compounds. It was reported to show a higher antioxidant activity in the thinning performed during the intermediate ripeness period (Carmona-Jiménez et al., 2021).

Table 3. Effect of cluster thinning treatments on chemical parameters in Horoz Karası grape variety

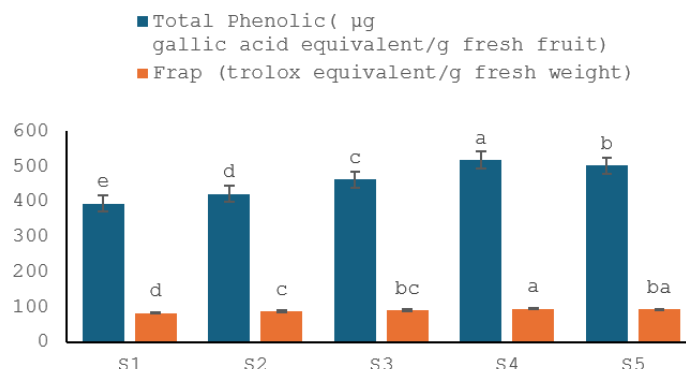
Cluster Thinning Levels	TSS (°Brix)	pH	TA(g/L)
S1 (Control)	21.36 ± 1.5176 ^{N.S}	4.54 ± 0.079 ^{N.S}	3.75 ± 0.0260 ^{N.S}
S2	20.53 ± 1.5176 ^{N.S}	4.52 ± 0.079 ^{N.S}	3.76 ± 0.0260 ^{N.S}
S3	20.00 ± 1.5176 ^{N.S}	4.49 ± 0.079 ^{N.S}	3.76 ± 0.0260 ^{N.S}
S4	21.20 ± 1.5176 ^{N.S}	4.43 ± 0.079 ^{N.S}	3.81 ± 0.0260 ^{N.S}
S5	20.40 ± 1.5176 ^{N.S}	4.49 ± 0.079 ^{N.S}	3.74 ± 0.0260 ^{N.S}

Mean values with different superscripts within the same column indicate a significant difference (Duncan, P<0.05).

Table 4. Effect of cluster thinning treatments on phytochemical parameters in 'Horoz Karası' grape variety

Cluster Thinning Levels	Total Phenolics Total Phenolics (µg GAE/g FW)	FRAP (TE/g FW)
S1 (Control)	394.38 ± 0.4255 ^E	83.16 ± 0.6373 ^D
S2	421.54 ± 0.4255 ^D	87.37 ± 0.6373 ^C
S3	462.36 ± 0.4255 ^C	90.49 ± 0.6373 ^{BC}
S4	518.41 ± 0.4255 ^A	95.44 ± 0.6373 ^A
S5	502.37 ± 0.4255 ^B	92.45 ± 0.6373 ^{AB}

Mean values with different superscripts within the same column indicate a significant difference (Duncan, P<0.05).

**Figure 5.** Effect of cluster thinning treatments on total phenolic and total antioxidant activity. (One-way ANOVA with Duncan, P<0.05, S1-20 cluster/vine; S2-16 cluster/vine; S3-12 cluster/vine, S4-8 cluster/vine and S5 -4 cluster/vine).

In Cabernet Sauvignon and Probus (*Vitis vinifera* L.) wine grape varieties, both cluster thinning and leaf removal treatments caused changes in anthocyanin ratios in wines obtained from the grapes (Ivanišević et al., 2020). As a result of a study investigating the effects of cluster thinning on the amounts of phytochemical substances in Narince grape variety, it was reported that a positive effect occurred in all phytochemical substance amounts (Demirer, 2017). In the Blauer Portugieser grape variety Blauer Portugieser, along with cluster thinning treatments, thinning treatments significantly increased the content of total anthocyanins, flavonols and hydroxycinnamic acids in grapes and wine, but not total flavanols (Reščič et al., 2015).

3.4. Effect of Cluster Thinning Treatments on Phytochemical Parameters

When the effect of cluster thinning treatments on phytochemical properties was analyzed, the lowest values were obtained at cluster thinning level S1, where no thinning treatment was applied, while the highest values were obtained at cluster thinning level S4, where 8 clusters/vine treatment was applied (Table 4 and Figure 5).

Similar to this study, Fanzone et al (2012) reported an increase in phenolic content in the berries thanks to 50% cluster thinning application during the flowering and mole verasion. In Cabernet-Sauvignon and Carmenere grape varieties, topping and cluster thinning treatments were applied and as a result of the study, cluster thinning treatment caused an increase in phenolic compounds (Bordeu et al., 2014). Bekar and Cangi (2018) reported that the highest values in terms of the amount of phenolic-flavonoid substances were obtained from 30% and 60% cluster thinning applications in their study in

Narince grape variety. In a study using red grape varieties (Syrah, Cabernet Sauvignon), cluster thinning was performed in 3 different periods from ripening to harvest. It was determined that the grapes treated with thinning were a good source of phenolic compounds. It was reported to show a higher antioxidant activity in the thinning performed during the intermediate ripeness period (Carmona-Jiménez et al., 2021). In Cabernet Sauvignon and Probus (*Vitis vinifera* L.) wine grape varieties, both cluster thinning and leaf removal treatments caused changes in anthocyanin ratios in wines obtained from the grapes (Ivanišević et al., 2020). As a result of a study investigating the effects of cluster thinning on the amounts of phytochemical substances in Narince grape variety, it was reported that a positive effect occurred in all phytochemical substance amounts (Demirer, 2017). In the Blauer Portugieser grape variety Blauer Portugieser, along with cluster thinning treatments, thinning treatments significantly increased the content of total anthocyanins, flavonols and hydroxycinnamic acids in grapes and wine, but not total flavanols (Reščič et al., 2015).

4. Conclusion

In this study, five different levels of cluster thinning were applied on Horoz Karası grape variety and yield and quality characteristics were analyzed. Cluster thinning did not have a significant effect on chemical traits, but had an effect on physical and phytochemical traits of Horoz Karası grape variety. When the data obtained were analyzed, especially the cluster thinning treatments at S2 and S3 levels came to the forefront with their grape quality improving properties. The positive effect of S3 cluster thinning treatment on phytochemical parameters

can be considered as an alternative method for grape production, especially for functional food production. Although the data obtained with the results of the study are a source for further studies, there is always a need for further applications with other varieties.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	B.G.	S.S.D.	N.Ş.
C	20	60	20
D	20	60	20
S	20	60	20
DCP	20	60	20
DAI	20	60	20
L	20	60	20
W	20	60	20
CR	20	60	20
SR		100	
PM	20	60	20
FA	30	40	30

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

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