

TEOR TOTAL DE FENÓLICOS E ANTOCIANINA DE DOIS CULTIVOS DE UVA VERMELHA (*VITIS VINIFERA* L.) CVS SAHEBI E HALAGHO CONFORME AFETADOS PELA SEVERIDADE DA PODA E COMPRIMENTO DA CANATOTAL PHENOLICS AND ANTHOCYANIN CONTENT OF TWO RED GRAPE (*VITIS VINIFERA* L.) CVS SAHEBI AND HALAGHO CULTIVARS AS AFFECTED BY PRUNING SEVERITY AND CANE LENGTH

فنل كل و محتوای آنتوسیانین دو کلتیوار انگور قرمز صاحبی و حلقو و تاثیر پذیری آنها از شدت هرس و طول شاخه

MAHTAB, Zonouri¹; DAVOOD, Bakhshi^{2*}; ESMAEIL, Fallahi³; ISA, Arji⁴

¹University of Guilan, Faculty of Agricultural Sciences, University Campus 2, Department of Horticultural Science

²University of Guilan, Faculty of Agricultural Sciences, Department of Horticultural Science

³University of Idaho, Parma Research and Extension Center, Parma

⁴ Agricultural and Natural Resources Research and Education Center, Department of Crop and Horticultural Science Research

* Corresponding Author

e-mail: bakhshi-d@guilan.ac.ir

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RESUMO

Uma uva popular e importante a 'Sahebi' e uma uva local menos conhecida a 'Halagho' do Irã foram estudadas para desmascarar a influência da severidade da poda e do comprimento da cana nos potenciais bioativos da fruta; teor total de fenólicos e antocianinas nas quais eles afetaram significativamente. Nesta investigação, foram estudados os efeitos de 4, 6 e 8 gemas por cana com intensidade de poda leve, moderada e severamente podada e sua interação no curso de dois anos consecutivos, 2017 e 2018 nas variedades 'Sahebi' e 'Halagho'. Os resultados revelaram diferenças marcantes que, em qualquer cultivar, o conteúdo fenólico da polpa foi 2 a 5 vezes maior em 2017 em comparação com 2018 com a 'Halagho' severamente podada no mais alto (5,14 mg/g DW). Considerando que 'Halagho' podada levemente com 6 gemas por cana teve o maior conteúdo de antocianina da casca (1,09 mg/g DW), na mesma condição, mas com a poda severa 'Halagho' apresentou o maior conteúdo de antocianina na polpa (0,86 mg/g DW) enquanto no segundo ano, em 2018, podada severa ou levemente esse tipo com 6 gemas por cana teve um aumento significativo no acúmulo de compostos fenólicos (10 mg/g DW). A partir dos resultados deste experimento, pode-se concluir que a poda das videiras é um estressor e gera fitoquímicos aprimorados que podem possivelmente mitigar as consequências negativas. No entanto, a variação nas propriedades bioquímicas manifestou uma dependência notável da cultivar de uva, além do fator tempo, que foi mais pronunciado na variedade 'Halagho' do que na 'Sahebi'. Até certo ponto, o efeito das cultivares foi predominante nas diferenças de duas cultivares.

Palavras-chave: polpa de bagas; qualidade da baga; casca das bagas; fecundidade de brotos; fitoquímicos.

ABSTRACT

A popular and important grape 'Sahebi' and a less known local grape 'Halagho' in Iran, were studied to unmask the influence of pruning severity and cane length on fruit bioactive potentials; total phenolic and anthocyanin content in which they affected significantly. In this investigation, effects of 4, 6 and 8 buds per cane with pruning intensity as light, moderate and severely pruned and their interaction in the course two consecutive years, 2017 and 2018 in 'Sahebi' and 'Halagho' were studied. Results revealed striking differences which in either cultivar, pulp phenolic content was 2 to 5 fold higher in 2017 compared to 2018 with the severely pruned 'Halagho' at the highest (5.14 mg/g DW). Whereas, lightly pruned 'Halagho' with 6 buds per canes had the highest peel anthocyanin content (1.09 mg/g DW), in the same condition but with severely pruning 'Halagho' showed the highest anthocyanin content in pulp (0.86 mg/g DW) while in the second year, 2018, severely or lightly pruned this type with 6 buds per cane had a significant increase in phenolic compound accumulation (10 mg/g DW).

From the results of this experiment, it could be concluded that the pruning of the vines is a stressor and generates enhanced phytochemical constituents to possibly mitigate the negative consequences. However, variation in the biochemical properties manifested a notable grape cultivar dependency in addition to the time factor, which was more pronounced in 'Halagho' than 'Sahebi'. To some extent the effect of cultivars on the differences of two cultivars was predominant.

Keywords: *berry pulp; berry quality; berry peel; bud fruitfulness; phytochemicals*

چکیده

یک رقم انگور محبوب و مهم 'صاحبی' و انگور محلی کمتر شناخته شده 'حلقو' در ایران، برای آشکار کردن تأثیر شدت هرس و طول شاخه بر پتانسیل‌های بیوشیمیایی میوه مورد مطالعه قرار گرفتند. میزان فنل کل و آنتوسیانین کل در آنها به طور قابل توجهی تحت تأثیر قرار گرفت. در این تحقیق، اثرات 4، 6 و 8 جوانه در هر شاخه با شدت هرس به عنوان سبک، متوسط و شدید و برهم کنش آنها در طی دو سال متوالی، 2017 و 2018 در 'صاحبی' و 'حلقو' بررسی شد. نتایج نشان داد که در هر دو رقم اختلاف قابل توجهی وجود دارد، میزان فنل پالپ در سال 2017 در مقایسه با سال 2018 با هرس شدید 'حلقو' 2 تا 5 برابر بیشتر بوده و در بالاترین سطح (5/14 میلی گرم در گرم در وزن خشک) قرار داشت. در حالی که، هرس متوسط صورت گرفته در 'صاحبی' با 6 جوانه در هر شاخه دارای بالاترین میزان آنتوسیانین پوست (1.09 میلی گرم در گرم وزن خشک) را داشت، در همان شرایط اما با هرس شدید 'حلقو' دارای بالاترین میزان آنتوسیانین در پالپ بود (0.86 میلی گرم در گرم وزن خشک). در حالی که در سال دوم 2018، 'حلقو' هرس شدید یا سبک با 6 جوانه در هر شاخه افزایش قابل توجهی در تجمع ترکیب فنلی (10 میلی گرم در گرم خشک) را داشت. از نتایج این آزمایش می توان نتیجه گرفت که هرس انگور یک عامل تنش زا است و باعث افزایش ترکیبات فیتوشیمیایی می‌گردد تا با پیامدهای منفی تنش مقابله نماید. با این حال، تغییر در خواص بیوشیمیایی علاوه بر عامل زمان، وابستگی قابل توجهی به رقم انگور را نشان می‌دهد، که این میزان در 'حلقو' از 'صاحبی' برجسته تر بود. تا حدودی اثر ارقام بر اختلاف دو رقم غالب بود.

کلمات کلیدی: پالپ حبه، کیفیت حبه، پوست حبه، باروری جوانه، فیتوشیمیایی

1. INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most valuable agricultural crops worldwide. Archeological and historical discoveries suggest the Near-East region between the Black and Caspian seas where its early domestication could have been taken place (Myles *et al.*, 2011).

The very first traces of grape cultivation detected in the north of Zagros mountains-Iran (McGovern, 2017). Considering the criticality of grape fruit, knowledge on almost every aspect in particular secondary metabolites in grapevine is expanding which helps vine growers to develop new cultivation techniques to modify and improve grape quality (Azuma, 2018). From which plant management can significantly contribute to improving viticultural practices in order to control plant physiological response, reflecting on enhancement table grape yield and quality. As a key task, pruning remains a manual operation that can largely influence a spectrum of quality and quantity properties (Billikopf and Norton, 1992; Keller, 2015).

As mentioned earlier, pruning is a cultural task with multidimensional effects which

improving bud fruitfulness by selecting buds in appropriate positions as well as control of shoot numbers and their placement of vine and enhancement of fruit quality in general and lessening the fluctuation in average annual yield in long-term (Groumpos *et al.*, 2015). Moreover, grapes enriched of an array of phenolic constituents in the peel, seeds, and pulp which possess notable biological properties specifically antioxidant activity (Casazza *et al.*, 2010). Being known for high nutritional and health values, fresh grape consumption is experiencing a significant and constant increase. In fresh fruits, texture and color attributes for the assessment of freshness and enjoyment of eating (Peneau *et al.*, 2006).

Also, the peel of the red grape compose of a large number of phytochemicals, because polyphenolics specifically anthocyanins in berry distributed unevenly with main concentration in peels (Adams, 2006; Godevac *et al.*, 2010) which confers numerous health benefits (Zhang *et al.*, 2011), and since all phenolics contain at least one aromatic ring, they can consequently absorb UV light effectively (Cote *et al.*, 2010). The grape polyphenol compounds are chiefly classified as

flavonoids (C6C3C6 skeleton) with a high level of anthocyanins (Garrido and Borges, 2013). Owing to their capability in quenching oxidants, anthocyanins are found to be accumulated in response to myriad number of environmental stresses (Braidot *et al.*, 2008; Song *et al.*, 2015). Additionally, they are potent bioactive constituents with anti-inflammatory and anticancer activities (Xia *et al.*, 2010).

The quantification of the quality traits of the grapevine can indeed provide viticulturists with invaluable practical knowledge, enable them to operate field tasks in a more efficient manner which ultimately leads to higher yield and filling the gap between supplies vs. demand (Barbagallo *et al.*, 2011). The concentration and composition of phenolic compounds in red grapes differ with grape cultivars (Costa *et al.*, 2014). In mountain vineyards, vines are trained by the head training systems, which due to its cost-effectiveness as well as low maintenance requirement is economically feasible. The lack of incident radiation in the clusters and excessive crop loads decrease quality.

Iran is one of the renown producers of table grapes and processed products such as juice and raisin in the world (FAO, 2014). Having an enormous variability of grapes in Iran, now various local grape cultivars being bred, albeit so far no significant attention on behalf of scholars has been paid to them. As a case in point, there is no published report concerning the berry phenolic content of 'Halagho' and 'Sahebi' grapes, native red grape cultivars which the former cultivar mostly cultivated in the west of Iran while the later cultivar is popular and cultivated widely. Hence, the purpose of current research was to evaluate the phenolic content and total anthocyanins of berries of those two abovementioned cultivars in the full ripening stage (e.g., in various parts: peel, pulp, and whole berry) to explore the effects of combined pruning severity and different bud numbers per cane in common vineyard conditions.

2. MATERIALS AND METHODS

2.1. Site description

The experiment was carried out in two consecutive growing seasons, 2017 and 2018, in a commercial vineyard located in Sarbarzeh region of Kermanshah province, in the west of Iran (34°45'46"N; 47°11'34" E, 1923 m asl). Two seeded red table grape varieties, 'Sahebi' and 'Halagho' from 20 year-old vines with head

training at a spacing of 1.5 by 2.5 m in an east-west row orientation in a relatively equal status were used. Both cultivars were hardwood cutting-originated, irrigated with drip irrigation. During each phenological period the irrigation applied in addition to possible precipitation.

2.2. Pruning programs

The 20 years old vines that annually moderately pruned by the owner in this study defined as 'control'. Our concentration in this experiment was on exploring the possible impacts of the length of canes and the severity of pruning. Pruning plan was applied in late - Apr in 2017 and late- Mar in 2018 (Because of local weather conditions. Pruning severity treatments have consisted of two types of pruning; lightly pruned with the formula 40 + 20 and severely pruned with 20 + 20 formula (Ahmedullah and Himelrick, 1989). Afterward, in the number of buds per cane, substitute cane (two-node spur) were pruned with 2 bud lengths and by randomly with respective treatments: pruning, 4, 6 and 8 buds on the cane and different pruning intensity of light and intensity). To repeat this experiment in 2018, the substitute canes (two-node spur) pruned and each on cane, two buds remained exchanged.

2.3. Sampling

Specimens were collected at the berry fully ripe stage, based on the red color appearance. Picking grape bunches was carefully randomized throughout the vines for each cultivar. Vines were well managed, uniform in size, and had no nutrient deficiency or pest damage. At least 10 cluster samples were taken from each vine.

2.4. Biochemical analyzes

2.4.1. Fruit material and extract preparation

Briefly, grape samples were manually separated into peel and seeds. Berries were cut longitudinally with a razor blade, and seeds were carefully removed from each berry. The pulp on the inner face of berry peel was removed using an end-flattened spatula. Peel of freshly harvested grapes was directly separated, weighed and then pulverized with liquid nitrogen to ensure full phenolic extraction. Of each specimen 500 mg was mixed with 1.5 ml solvent containing 85% methanol and 15% acetic acid. Mixtures then incubated at 4 °C in the dark for 24 h. The extracts were centrifuged at 10000 rpm for 10 min, using Whatman filter papers the solvents removed from the extracts. The mixtures stored at 4 °C before use.

2.4.2. Total phenolic content

Total phenolic content (thereafter; TPC) in the grape extracts were evaluated colorimetrically according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965) with slight modification. Briefly, 70 μ l the grape extracts were mixed with 1 ml Folin-Ciocalteu reagent 10%, then added to 130 μ l double distilled water. After 6 min, 7.5% sodium carbonate solution was added. The mixture was allowed to react at room temperature in the dark for 90 min, and then, the absorbance was measured at 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight. Gallic acid was employed as a calibration standard. The measurements were compared to a standard curve of prepared gallic acid solutions and expressed as mg gallic acid equivalents per gram dry weight (GAE/g DW) ($y=3.0912x+0.0502$, $y^2= 9962$) of peel and pulps. The standard curve was generated with 0.10, 0.20, 0.30 and 0.40 mg gallic acid/L by a UV-vis double-beam spectrophotometer (T80+UV/VIS PG Instrument Ltd) at the wavelength of 750 nm.

2.4.3. Total anthocyanin content

Using Wrolstad (1976) method with some change the total anthocyanin content (thereafter; TAC) was measured. The absorbance of the anthocyanin containing extract, described above, was then measured at 520 and 700 nm in two reference pH 1.0 and 4.5 buffers against a blank of reagents according to Longo and Vasapollo (2006). The calculation of cyanidin 3-glucoside (C3G) was done using Equations 1 and 2.

$$A = (A_{510} - A_{700})_{\text{pH } 1.0} - (A_{510} - A_{700})_{\text{pH } 4.5} \quad (\text{Eq. 1})$$

$$\text{TMA content} = (\Delta A \times \text{MW} \times \text{DF} \times \text{Ve} \times 1000) / (\xi \times 1 \times C) \quad (\text{Eq. 2})$$

Where A is absorbance, MW is the molecular weight of cyanidin-3-glucoside (449.2), DF is the dilution factor (1), Ve is the extract volume, ξ is the molar absorptivity coefficient of cyanidin-3-glucoside (29,600), and M is the mass of the berries extracted and C is the concentration of the grape extract in milligrams per milliliter. The final results expressed as mg/g DW of anthocyanin.

2.5. Statistical analysis

The experimental design used was a

split-split plot with three replicates. The main factors consisted of time (year) with two levels (2017 and 2018) and grape cultivars with two levels ('Sahebi' and 'Halghoo'). The severity of pruning (light, moderate and severe) was considered as sub-plot factor and number of buds per cane (4, 6, 8 buds per cane) as sub-sub (or split-split) factors. Analysis of variance was performed using the SAS statistical package (version 9.4; SAS Institute, Cary, NC, USA). Differences among treatments were assessed by Duncan's range test at the level of 1% and 5%.

3. RESULTS AND DISCUSSION

A notably valuable insight into the impacts of pruning practice on phytochemical contents of two important Iranian red grapes was acquired, in which in terms of time factor the TPC of the grape peel, as well as pulps in either cultivar, was significantly higher in 2017 than 2018, by comparison (Table 1 and 2). Interestingly enough, TPC of the whole berry in 2018 was significantly higher when compared with 2017 (Table 3). Whereas to some extent, similar to the TPC behavior pattern, in 2017, TAC of peel, pulp, and whole berry was higher in 2017 than in 2018 (Tables 4, 5 and 6). In 2017, as negative consequences of pruning, imposing stress, and significant decrement in yield and fruit size by comparison to control were witnessed.

By 2018, vines reached stable physiological conditions, which as a result, enhancement in fruit growth was observed. Increasing the amount of phenolic compounds in peel and pulp in 2017 compared to 2018 can also be justified by the fact that due to increased light penetration into the crown of vines, in all forms of pruning severity light, moderate and severe.

Archer and Strauss (1989) reported that the fruit composition is affected by the microclimate inside the crown, in shade conditions, the quality of fruit in grapes is greatly reduced. In fact, with the increase of light penetration into the vines, phenolic compounds increased in 2017, but in 2018, by creating a coating on grape fruit clusters, reduced light intensity and phenolic compounds decreased. The phenolic content was significantly lower in the grape pulp when compared with peel (Table 2). The content of phenolics seems to be a tissue specific trait. Phenolics were lower in pulp compared to peel which is in accordance with other scholars (Pantelic, 2016), unlike pulp, the grape peel contains a large array of phenolic compounds (Nile *et al.*, 2013). The accumulation of secondary metabolites is a strategy of plant

defense against stress.

Vine response to stress at the berry level resulted in the accumulation of secondary metabolites in pulp and peel as a defense against cell degradation (Ferrandino and Lovisolo, 2013).

On the other hand, considering the fact that parameters such as yield, TSS, TA and number of berries per bunch showed a statistically significant increase in 2018 (data not shown), hence, it could be pointed that increase in volume of yield may lead to lessen the concentration of phytochemicals of interests in this experiment. In 2017, peel phenols of 'Sahebi' were significantly lower than 'Halagho' in all pruning levels and number of buds per cane (Table 1).

Differences in the phenolic compounds of various grape cultivars have also been proven in others' studies (Rodriguez-Montealegre, 2006; Orak, 2007; Pantelic, 2016). The grape cultivar exerts a significant influence on the phenolic composition (Dani *et al.*, 2007; De Pascali *et al.*, 2014). The concentration and composition of phenolic compounds in red grapes differ with grape cultivars (Costa *et al.*, 2014).

In which in this study regardless of the factor of year, the two grapes type almost followed a consistent pattern since the compounds targeted in 'Sahebi' were lower in general when compared to 'Halagho'. The difference in phenolic contents in the peel of cultivated grapes could be partly attributed to the genotypes (Montealegre *et al.*, 2006). Peel morphological and structural characteristics, as dictated by the cultivar, seem to exert a direct effect on the extractability of phenolic compounds (Rolle *et al.*, 2009). Biochemically, flavonoids and anthocyanins are important determinants of antioxidant capacity and quality of fruit.

Acylated derivatives of anthocyanin greatly affect its stability and color intensity. In this study, there was a significant difference between the anthocyanin and phenol compounds in 'Halagho' and 'Sahebi', indicating the genetic difference and the enzymes involved in the bio production of the compounds in different cultivars reported by other researchers (Yonekura-Sakakibara, 2009).

Severe and moderate pruning of 'Halagho' during 2018 lead to a significant increase in TPC in the whole berry. In 2017, severely and lightly pruned of 'Sahebi' compared with control

treatment, and maintenance of 4, 6 or 8 buds per cane showed a significant decrease in total phenol content in the whole berry which showed no significant difference at all (Table 3).

In the case of the interaction between years, grape cultivars, pruning severity and the number of buds per cane showed that 'Halagho', when pruned in 2017 with 6 or 4 buds per cane, showed peel anthocyanin content which was not significantly affected by treatments. In 2018, peel anthocyanin content in both cultivars decreased without any significant difference (Table 4). In severely pruned 'Halagho' in 2017 and the maintenance of 6 buds per cane increased pulp anthocyanin significantly compared to other treatments.

The pulp anthocyanin in both cultivars decreased significantly in 2018 (Table 5). Severe pruning of 'Sahebi' in 2017 with 4 buds per cane increased TAC in the whole berry which was not significantly different from the other treatments. In 2018, TAC was significantly decreased in both cultivars compared with 2017. However this difference was not significantly different (Table 6). The results of this experiment indicated that the pruning of two cultivars, 'Halagho' and 'Sahebi' (in particular the 'Halagho'), in 2017, increased anthocyanin content in the peel, pulp, and whole berry as compared in 2018. In all pruning levels, a self-regulating branch growth was found leading to an increase in compounds such as anthocyanin and total phenols. In red grapes, such as 'Halagho' and 'Sahebi', anthocyanins are an important group of phenolic compounds (Yang *et al.*, 2009) because of their valuable nutritional and medicinal benefits and visual quality of their color. The peel color plays an important role in determining fruit quality of table grapes, mainly due to the composition and the content of anthocyanins (Paredes-Lopez *et al.*, 2010). It is shown that the quantity and quality of anthocyanins in grape berries greatly influence the quality of red grapes (Boss *et al.*, 1996). Phenolics in the plant kingdom are important because of their function in the defense system. Pruning has a significant effect on the accumulation of these compounds. In order to synthesize anthocyanin in grapevine berries and to balance juice compounds, clusters should receive direct sunlight (Spayd *et al.*, 2002). Due to the high temperature for producing high quality fruits and berries with maximum color, it should be prevented from exposing clusters to long-term exposure to sunlight (Bergqvist *et al.*, 2001). In confirmation of this point, the results of this research showed that the pruning of the

'Halagho' in light form in 2017 and keeping 6 buds per cane or the severe pruning of this cultivar in 2017 and keeping 4 buds per cane increased anthocyanin content in the peel. These results are consistent with reports from Shinomiya *et al.* (2015). Vine or cluster exposure to low temperature and high light intensity enhances anthocyanin accumulation which was not seen in the current work. This might be due to the training system used in this study. High temperature and/or shading treatments reduce berry peel coloration. Reports of increasing anthocyanin accumulation due to sunlight exposure have helped growers to avoid poor peel coloration by incorporating light quality control methods, such as shoot positioning and light reflectors around the fruiting zone. In a vineyard, the training system determines the light interception for a certain amount of leaf area and consequent bud and fruit exposure to sunlight (Reynolds and Heuvel, 2009). Bunch sunlight and UV exposure significantly increase anthocyanins content in the grape (Song *et al.*, 2015). In particular, temperature and irradiance are critical because of their direct effect on the synthesis and accumulation of polyphenols in the berries (Keller, 2010). The effects of light on grapevine physiology have been exploited to address grape berry quality: different training systems, influencing the vine light use efficiency exert pivotal consequences on berry quality (Petrie *et al.*, 2009).

The reduction of the anthocyanin level in 2018 can also be due to branch growth. The anthocyanin levels in the whole berry, pulp and peel are significantly lower than in 2017. It has been proved that phenolic biosynthesis may be induced in response to different abiotic elicitors (Song *et al.*, 2015). In 2017, the number of canes was reduced due to differences in pruning intensity compared to 2018. The number of cane per plant in the year 2016 was significantly higher than in 2017 and 2018, and there was a significant difference at the probability level of 1%. But in 2018, due to the stability of the number of canes, the intensity of pruning was reduced and became stable. Vine vigor and viticultural practice of the grapes may also impact on grape anthocyanin concentration. The vigor parameters including main shoot length and total shoot length were positively correlated to yield; were negatively correlated with berry anthocyanin production (Baluja *et al.*, 2012). The low number of shoots per canopy volume can lead to a reduction in the intensity and even to a complete cessation of various metabolic processes (Jones, 1992) which was concluded in

the current work (data was not shown).

4. CONCLUSIONS

Phenolic constitutes possess a large quantity of biochemical properties from which grapes are their renowned source with undisputable importance in the daily diet of people worldwide. Whereas, pruning as a critical practice if adopted un appropriately like pruning all mature canes by local grape growers, heavy exploitation of stored nutritional material of the tree and therefore, reduction in vines vigourity, as well as quality, would be the aftermath. The results obtained are reliable proof that the pruning program can negative or positively influence phenolic compound concentration in grape. More, the grape cultivars show different sensitivity to the type of viticultural practice which was observed in 'Sahebi' and 'Halagho'. These findings are valuable to improve viticultural practices in grape production, allowing the improvement of grape quality to forecast the potential crop before pruning. Nonetheless, applying this pruning program on the same cultivars with other training systems further needs to be investigated. Additionally, the locality is another crucial factor that understands its effectiveness on these two cultivars is essential. Ultimately, the present work expands the knowledge of appropriate pruning and management on two Iranian red grape cultivars for grape growers.

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Table 1: Peel phenolic content of ‘Sahebi’ and ‘Halagho’ grapes pruned during the years 2017 and 2018

Peel phenolic content (mg/g DW)					
		2017		2018	
Treatment	Number of buds per cane	Halagho	Sahebi	Halagho	Sahebi
control	Moderately pruned	43.81 a	28.80 d	19.10 e	12.00 e
	4	42.51 a	27.71 d	20.5 e	14.40 e
Severely pruned	6	31.44 c	26.85 d	23.90 e	12.10 e
	8	38.15 ab	29.10 d	21.30 e	12.20 e
Lightly pruned	4	39.76 ab	27.45 d	20.90 e	14.50 e
	6	34.00 bc	26.59 d	25.50 e	14.00 e
	8	43.27 a	28.51 d	21.20 e	14.20 e

Meanes with the same letters in each column and row show no significant difference in Duncan's multiple range test at $p \leq 5\%$.

Table 2: Pulp phenolic content in 'Sahebi' and 'Halagho' grapes pruned during the years 2017 and 2018

		Pulp phenolic content (mg/g DW)			
		2017		2018	
Treatment	Number of buds per cane	Halagho	Sahebi	Halagho	Sahebi
Control	Moderately pruned	4.05bc	2.65de	1.38fg	0.61g
Severely pruned	4	3.68bcd	3.29cd	0.94fg	0.79fg
	6	3.27cd	2.71de	1.42fg	0.72fg
	8	5.14a	2.70de	1.10fg	0.81fg
Lightly pruned	4	3.29cd	2.89cde	0.84fg	0.84fg
	6	3.05cde	2.65de	1.86ef	0.80fg
	8	4.56ab	2.63de	0.80fg	1.17fg

Meanes with the same letters in each column and row show no significant difference in the Duncan's multiple range test at $p \leq 5\%$.

Table 3: Total phenolic content of the whole berry in 'Sahebi' and 'Halagho' grapes pruned during the years 2017 and 2018

		Total phenol content in the whole berry (mg/g DW)			
		2017		2018	
Treatment	Number of buds per cane	Halagho	Sahebi	Halagho	Sahebi
Control	Moderately pruned	6.23efgh	4.60i	7.74bcde	7.25cdef
Severely pruned	4	5.50hi	4.70i	8.37bc	8.12bc
	6	5.63ghi	4.48i	10.00a	7.28cdef
	8	5.30hi	4.62i	7.02cdefg	7.50bcde
Lightly pruned	4	6.38efgh	4.65i	7.62bcde	7.41bcdef
	6	5.57ghi	5.94fghi	8.86ab	7.92bcd
	8	6.42defgh	4.75i	7.60bcde	8.36bc

Meanes with the same letters in each column and row show no significant difference in Duncan's multiple range test at $p \leq 5\%$.

Table 4: Peel anthocyanin content in 'Sahebi' and 'Halagho' grapes pruned during the years 2017 and 2018

Peel anthocyanin content (mg/g DW)					
		2017		2018	
Treatment	Number of buds per cane	Halagho	Sahebi	Halagho	Sahebi
Control	Moderately pruned	0.93 a-c	0.95 a-c	0.03 f	0.03 f
	4	1.07 a	0.78 b-e	0.04 f	0.04 f
	6	0.88 a-d	0.62 de	0.03 f	0.11 f
Severely pruned	8	1.00 ab	0.63 de	0.03 f	0.04 f
	4	0.69 c-e	0.97 ab	0.03 f	0.04 f
	6	1.09 a	0.55 e	0.04 f	0.03 f
Lightly pruned	8	0.94 a-c	0.74 b-e	0.03 f	0.04 f

Meanes with the same letters in each column and row show no significant difference in Duncan's multiple range test at $p \leq 5\%$.

Table 5: Pulp anthocyanin content in 'Sahebi' and 'Halagho' grapes pruned during the years 2017 and 2018

Pulp anthocyanin content (mg/g DW)					
		2017		2018	
Treatment	Number of buds per cane	Halagho	Sahebi	Halagho	Sahebi
Control	Moderately pruned	0.48 c	0.51 c	0.007 d	0.001 d
	4	0.62 bc	0.55 c	0.003 d	0.011 d
	6	0.86 a	0.52 c	0.006 d	0.002 d
Severely pruned	8	0.49 c	0.70 b	0.004 d	0.043 d
	4	0.48 c	0.55 c	0.001 d	0.005 d
	6	0.49 c	0.51 c	0.001 d	0.006 d
Lightly pruned	8	0.51 c	0.51 c	0.003 d	0.003 d

Meanes with the same letters in each column and row show no significant difference in Duncan's multiple range test at $p \leq 5\%$.

Table 6: Total anthocyanin content in 'Sahebi' and 'Halagho' grapes pruned during the years 2017 and 2018

		Total anthocyanin content (mg/g DW)			
		2017		2018	
Treatment	number of buds per cane	Halagho	Sahebi	Halagho	Sahebi
control	moderately pruned	0.51 a	0.33 c	0.031 d	0.024 d
Severely pruned	4	0.38 a-c	0.40 a-c	0.030 d	0.031 d
	6	0.48 ab	0.34 bc	0.030 d	0.092 d
	8	0.43 a-c	0.37 bc	0.023 d	0.026 d
Lightly pruned	4	0.48 ab	0.35 bc	0.027 d	0.026 d
	6	0.34 bc	0.36 bc	0.028 d	0.026 d
	8	0.38 a-c	0.28 c	0.025 d	0.028 d

Means with the same letters in each column and row show no significant difference in Duncan's multiple range test at $p \leq 5\%$.