**Does Gibberellic Acid (GA3) Modify Antioxidant Potential by Affecting Content of Biologically Active Compounds**

M. Kapłan1, A. Najda2, K. Klimek3, A. Borowy1

(1) Department of Department of Pomology and Nurseries, University of Life Science, 58 Leszczyński Street, 20-068 Lublin, Poland, e-mail: magdalena.kaplan@up.lublin.pl

(2) Department of Vegetable Crops and Medicinal Plants, University of Life Science, 58 Leszczyński Street, 20-068 Lublin, Poland

(3) Department of Applied Mathematics and Informatics, University of Life Science, 28 Głęboka Street, 20-612 Lublin, Poland

*\*Corresponding author: E-mail address:* *magdalena.kaplan@up.lublin.pl*[Tel: 48 81 5247158]

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**Gibberellic acid (GA3) is a plant growth regulator widely used in cultivation of seedless grape varieties to increase their yield. Hormonization treatment has beneficial effects on yield size and quality, yet its influence on a level of biologically active compounds and grape antioxidant activity has not been studied extensively yet. Inflorescences of eleven-year-old grape vines ‘Einset Seedless’ trained according to the single Guyot system were sprayed with GA3 at 100, 200 or 300 mg/l concentration once (7 days after full bloom), twice (7 and 14 days after full bloom) or three times (7, 14 and 21 days after full bloom). Fruits harvested on 25 September were immediately examined for acidity, a content of extract, biologically active substances and antioxidant capacity using the DPPH test. Besides, correlations occurring between some parameters measured were calculated. There was found a negative effect of hormonization on a content of extract, flavonoids, ascorbic acid, while no effect on anthocyanin level. The GA3 application was shown to increase anthocyanin content but its impact was not significant. Antioxidant activity measured by the DPPH test was dependent on gibberellic acid concentration and the number of treatments; it tended to decrease with the increasing number of GA3 sprays.**

INTRODUCTION

Grape consumption is strongly correlated with the reduced risk factor for developing chronic diseases, such as cardiovascular disorders and cancer (Arts & Hollman, 2005; Erdman et al., 2007; Leifert & Abeywardena, 2008). That results from, among others, the presence of biologically active compounds like polyphenols displaying powerful antioxidant effects along with anti-inflammatory, anti-carcinogenic and anti-platelets properties. Besides, polyphenols help dilate blood vessels, boost the immune system and have neuroprotective role attributed mainly to their ability to modulate and induce signaling pathways (Crozier et al., 2010; Dohadwala & Vita, 2009; Frankel, 1999; Pezzuto, 2008; Stevenson & Hurst, 2007; Vislocky & Fernandez, 2010; Xia et al., 2010). Polyphenols inactivate free radicals, chelate divalent metal ions and thus, lower their oxidant potential (Scalbert et al., 2005).

Qualitative and quantitative composition, distribution and antioxidant activity of polyphenols in grapes are quite variable and depend on a species, cultivar, location in berry (skin, pulp, seeds, juice), climate-soil conditions (exposition to light, temperature, soil type), agrotechnical practices (irrigation, nutrient availability, plant growth regulators application, harvest time, berry maturity, yield and berry size) and finally, post-harvest conditions and storage-processing techniques (Jiang et al., 2006; Kim et al., 2003; Liang et al., 2011; Montealegre et al., 2006; Orak, 2007; Peña-Neira et al., 2004; Xia et al., 2010). Polyphenols are responsible for the major sensory attributes of products and beverages of plant origin being the determinants of their appearance (color) and taste, i.e. flavor, bitterness, astringency and aroma (Es-Safi et al., 2007; Tomás-Barberán & Espin, 2001).

Seedless grape varieties have been on the rise in the world grape market because of their high quality and consumers` preferences, they enjoy increasing popularity not only as table grapes but as raisins as well (Artés-Hernández et al., 2006). One of the most promising and commercial seedless cultivars which can be grown successively in cool climate areas, e. i. in Poland, proves to be ‘Einset Seedless’, a pink grape with a unique strawberry-like flavor. This variety can be used for raisin production or fresh consumption as table grapes. However, a natural berry size of `Einset Seedless` variety (± 2÷3 g) is not large enough for table grape use and thus represents a problem for commercialization. To overcome this hardship and improve grape size and quality, plant growth regulators (gibberellic acid GA3 most often) are applied globally (Dimovska et al., 2014; Harrell & Williams, 1987; Kapłan et al., 2017; Nampila et al., 2010). Gibberellic acid promotes cell division, enhances earlier blooming and increases fruit size and yield. The effect of GA3 application relies on a variety, concentration and application time (Dimovska et al., 2014; Kapłan et al., 2017; Khan et al., 2009; Nampila et al., 2010). The earlier studies of the present authors showed a positive response of this cultivar to gibberellic acid treatment considering fruit set, the size of clusters and berries (Kapłan, 2011; Kapłan et al., 2017). These findings are of utmost importance currently because modern table grape production is fully expected to conform with the requirements of the market demanding improved grape quality, that is aiming at uniform repeatable clusters, equal berry size, shape and uniform skin color as well as increased resistance to transportation. Besides, an important attribute of grape berry quality proves to be the absence of seeds (Dimovska et al., 2014).

The objective of the present studies was to determine parameters affecting a content of biologically active compounds and antioxidant activity in grapes of `Einset Seedless` variety subject to concentration and gibberellic acid number of applications.

MATERIALS AND METHODS

**Plant materials**

The field experiment assessed the effect of GA3 concentration and the number of treatments on a level of chosen secondary metabolites of grapes. The inflorescences of 11-year-old `Einset Seedless` grapevines under the single Guyot training system were treated with GA3 spray at three concentration levels 100, 200 or 300 mg/l once (7 days after full bloom), two times (7 and 14 days after full bloom) or three times (7, 14 and 21 days after full bloom). The solution contained 99% of gibberellic acid and an adhesive and wetting SILWET Gold agent at 0,015% concentration, i. e. 150 µl. The solution was prepared immediately before the treatment. The clusters were treated with a hand pump sprayer covering the pedicels and berries thoroughly. The untreated grapes constituted the control. The analyzed fruits were obtained from the `NOBILIS Vineyard` (50o39`N; 21o34`E) located in the Sandomierska Upland in south-eastern Poland. The own-rooted vines of `Einset Seedless` were planted in spring 2003 at the 2,0 x 1,0 m spacing (5000 units × ha-1) on loess soil. The grapevines were pruned in single Guyot style.

 The studies were carried out in the laboratory for Vegetable and Herbal Material Quality at the Department of Vegetable Crops and Medicinal Plants, the University of Life Sciences in Lublin. The research material comprised `Einset Seedless` grape variety (‘Fredonia’ × ‘Canner’, Reisch et al., 1986) subjected to the hormonization with gibberellic acid GA3.

**Chemicals**

All reagents and solvents were analytical grade chemicals from Merck (Darmstadt. Germany), Sigma Chemical Co. (St. Louis. MO. USA) or POCh (Gliwice, Polska). GA3 from Acros OrganicsTM (Thermo Fisher Scientific Geel. Belgium) and SILWET Gold from Chemtura Europe Limited (Warsaw, Poland).

**Physicochemical analyses**

Fruit extract content was measured on the harvest day using the refractometer Abbe WAY 2W (EnviSense Poland)while squeezing the juice from 100 representative berries collected from each combination. In order to determine biologically active compounds and antioxidant activity, grapes were transported to the laboratory on the harvest day, stored at 8 C for 16 hours in the cooler and finally, underwent the chemical analyses. Titratable acidity (TA) was determined in accordance to the Polish Norm PN-90/A/75101/02.

**Determination of L-ascorbic acid**

The fresh and comminuted grape fruits (5 g) were extracted twice for 30 min with 2.5 ml 4.0% (m/V) L-cysteine and 10.0 ml water by sonification. All aqueous extracts were combined and diluted with water to 25 ml. The samples were analyzed Using high performance liquid chromatography. Analyses were done with an LaChrom-Merck HPLC system with a photodiode array detector (DAD L–7450), and all separations were on a Lichrospher 100 RP18 column (250.0×4.0 mm, 5.0 μm; Merck). The mobile phase consisted of 0.0272 g L−1 KH2PO4 adjusted to pH 2.40 with H3PO4, applied in isocratic elution for 30 min. The flow rate was adjusted to 1.0 ml/min. The detection wavelength was set to DAD at λ=254.0 nm. 20.0 μL samples were injected. All separations were performed at 24.0°C. Peaks were assigned by spiking the samples with standard compounds and comparing the UV spectra and retention times (ascorbic acid 5.66 min) (Najda, 2017). Calibration curves were obtained from 5 concentrations of each external standard (0.01–1.40 mg mL−1). The regression coefficient (R2) of the calibration curve for ascorbic acid (Y = 85.231 x = 18.787). The RSD values for the repeatability (n=4) of standard solution were 0.40% (0.01 mg ml−1 ascorbic acid). The limits of quantitation (LOQ) and detection (LOD) of ascorbic acid were 0.16 and 0.04 mg L−1, respectively. All solvents used were HPLC grade (Merck). Reference standards were obtained from Sigma-Aldrich.

**Total phenolic acids estimation** was carried out according to Arnov method (Polish Pharmacopoeia, 2002).

One milliliter of sample was mixed with 5 ml of distilled water. 1 ml 0.5 M HCl, 1 ml of Arnov reagent and 1 ml 1M NaOH, and subsequently adjusted to 10 ml with distilled water. The absorbance was measured at 490 nm. The total phenolic acid content was expressed as caffeic acid equivalent (CAE).

**Anthocyanins estimation** **by means of colorimetry**

 Samples of raw material (1.0 g) were extracted with 50 ml HCl (1mol ∙ dm3 ) and heated in water bath for 1 hour. The obtained extract was hydrolyzed with 20 ml n-buthanol, and then two 10 ml n-buthanol portions were added as a solution. Anthocyanin extracts were rinsed in 50 ml flask with n-buthanol. The absorbance was measured immediately at 533 nm (Miłkowska & Strzelecka, 1995).

The percentage of anthocyanins, as delphynidyn chloride. was calculated from the expression:

|  |  |
| --- | --- |
| P = | A×V×F |
| m |

where:

P – total anthocyanins (mg ∙ 100g-1), A – absorbance at 533 nm, V - value of buthanol phase (50 ml), F - coefficient for delphinidyn chloride (2,6), m - mass of sample to be examined (mg).

**Determination of antiradical activity (AA)**

A 0.1 ml aliquot of the methanol extract prepared above, was mixed with 3.9 ml of an 80% ethanolic 0.6 mM DPPH solution. The tubes were vortexed for 15 s and allowed to stand for 180 min. as described by Cai, Sun, & Corke (2003). After this. the absorbance of the mixture was measured at λ = 517 nm wavelength using the HITACHI UV–Vis spectrophotometer (UV–Vis model U-2900. Shimadzu. Kyoto. Japan). Most tested compounds react completely within 180 min in this condition. Reaction time for vitamin C is less than 1 min due to its fast oxidation. Ethanol (80%) was used as a blank solution. and DPPH solution without test samples (3.9 ml of DPPH + 0.1 ml of 80% ethanol) served as the control. All tests were performed in triplicate. The antiradical activity of the test samples was expressed as the median effective concentration for radicalscavenging activity (EC50): TP (mg) of antioxidant (test sample) required for a 50% decrease in absorbance of DPPH radicals. and inhibition (%) of DPPH absorbance = (*A*control *– A*test) x 100/*A*control. A plot of absorbance of DPPH vs. concentration of antioxidant was made to establish the standard curves (dose-response curves) and to calculate that EC50. *A*control is the absorbance of the control (DPPH solution without the test sample). and *A*test is the absorbance of the test sample (DPPH solution plus 0.1 ml of 5 μM test compound). Ascorbic acid served as a standard. The results of the assay were expressed relative to an ascorbic acid equivalent.

**Total flavonoids estimation**

Studied material was investigated for total content of flavonoids. using modified Christ and Müller method, calculated for quercetin QE (Polish Pharmacopoeia, 2014).Absorbance was measured at 425 nm on HITACHI U-2900 spectrophotometer.

The content of flavonoids was calculated from the equation:

|  |  |  |
| --- | --- | --- |
| X = | 8.75×A | where m (g) was the amount of fresh mass  |
| m |

**Tannin estimation**

The amount of tannin estimation was determined usingPharmacopoeia procedure (2014). The content of tannins was expressed as fresh and dry weight percentage.

**Statistical analysis**

The results obtained in this study were analyzed statistically using the one-way analysis of variance and Tukey`s confidence intervals. The inference was based on the significance level p<0,05. The estimation of correlations occurring between the qualitative parameters of grapes was done by counting of Pearson`s correlation coefficients. There were employed multidimensional analysis techniques to show the similarities in the groups in such a way that the homogeneous objects could be found in the same cluster. The results of the cluster analysis were graphically shown in the dendrogram. All the statistical analyses were made using SAS Enterprise Guide 5.1. software.

RESULTS AND DISCUSSION

 Sugars are one of the major components determining fruit quality and responsible for their sweetness. The sugar/organic acid ratio in fruit plays the most important role for the final flavor of grapes (Topalovic & Mikulic-Petkovsek, 2010). The statistical analysis indicated significant influence of GA3 concentration and application number on total extract content, vitamin C level and total acidity of `Einset Seedless` grape variety (Table 1). It was found that irrespective of GA3 concentration and number of treatments, a content of extract and vitamin C in the hormonized fruits was significantly lower compared to the control, except for the grapevines subjected to this treatment once. The significantly lowest level of both estimated parameters was recorded in the fruits treated with GA3 three times and 200 mg/l GA3. The studies of Al-Atrushy (2016) showed that the increasing number of applications and concentration of gibberellic acid increased significantly the total sugar level. Dimovska et al. (2014) applied GA3 two and three times at 5, 10 and 20 mg/l concentration and did not observe any significant effect on extract content in grapes of ‘Flame Seedless’.

/insert table 1/

A vitamin C content in `Umran` variety grapes after 10 mg/l GA3 application was lower than in the control, whereas at higher concentrations, that is 30 and 50 mg/l GA3, the hormonization had most beneficial impact on the parameter under study (Rachna & Sukhdev Singh, 2013). A vitamin C content after two applications of GA3 in seven seedless grape varieties was found to increase depending on a variety by 10-27%, yet it has not been confirmed in the present studies (Gougoulias & Masheva, 2010). Awad and Al-Qurashi (2012) used gibberellic acid in the cultivation of date palm of ‘Barhee’ variety and showed a positive influence of the hormonization on vitamin C level in fruits of the studied species. Application of 100 and 150 mg/l GA3 significantly increased a content of the investigated parameter as compared to the control and the application dose 50 mg/l GA3.

Laszlo and Saayman (1990) and Topalovic and Mikulic-Petkovsek (2010) found that grape acidity correlates with their taste which results from the presence of the tartaric and malic acid whose content reaches as much as 90% in grapes. Total acidity in fruits varied between 0.2 and 0.4% and it differed significantly between the combinations under study. It was shown that the grapes treated with 100 and 300 mg/l GA3 and with gibberellic acid solution applied once and twice displayed significantly higher total acidity as against the control. Wholly different relationships were reported by Al-Atrushy (2016) who in his study on grapes subjected to hormonization (irrespective of concentration and number of treatments) indicated significantly lower acidity than the control. Similarly, Dimovska et al. (2014) applied GA3 at 20 mg/l concentration and, irrespective of treatment number, the application had significantly decreased the acidity level. Rachna and Sukhdev Singh (2013) assessed the gibberellic acid influence on a content of chosen biologically active compounds in fruits of the *Zizyphus mauritiana* Lamk. cv. ‘Umran’ and observed unfavorable effect of the hormonization at 50 mg/l GA3 application rate on total acidity at the harvest. Whereas the studies of Kok (2017) did not show significant influence of GA3 applied in combination with a biostimulant on the acidity of grapes of ‘Cardinal’.

/insert table 2/

Antioxidant capacity of grape material is associated with the presence of secondary metabolites, i.e. phenolic acids, anthocyanins, flavonoids and tannins. A phenolic acids level in the fruits studied depended significantly on GA3 concentration and the number of applications (Table 2). It was found that the GA3 applied at 100 and 200 mg/l concentrations as well as single and three times spray significantly increased phenolic acid content in ‘Einset Seedless’ grapes.

Anthocyanins are a group of the most important phenolic components of dark grape cultivars as they directly affect the intensity of berry skin coloration which is the key quality attribute determining the grape market value and consumers acceptance (Kok, 2017). Besides, anthocyanins with their strong antioxidant properties are involved in protection against fungal and bacterial infections. Notably, anthocyanin synthesis occurs mostly in grape berry skin only (Doshi et al., 2015). Hormonization was found to increase the anthocyanin content, yet it was not significant (Table 2). This research finding was confirmed by Dimovska et al. (2014) whose studies also did not show any significant effect of hormonization on these compounds content in ‘Flame Seedless’ grape berries. Different results were reported by Gougoulias and Masheva (2010) after GA3 was applied twice and caused a 30% rise in anthocyanin content in ‘Kishmish Tjurkmenski’ fruit. A vast body of studies indicates that a level of anthyocanins and tannins largely relies on a cultivar, species, maturity degree of fruit, climate and site of fruit production (Mattivi et al., 2002; Mazza, 1995; Muñoz-Espada et al., 2004; Yang et al., 2009).

 Antioxidant potential of extracts of fruits analyzed was determined by the DPPH method and ranged from 56.272 up to 83.652 uM TE/g, it significantly depended on the number of treatments and concentration (Table 2). The treatments applied had significantly positive effect on the parameter under study in most combinations. It was found that control grapes and those after 100 and 300 mg/l GA3 application displayed significantly higher antioxidant activity as compared to grapes treated with 200 mg/l GA3. The number of hormonization treatments also had significant impact on the parameter analyzed as compared to the control combination, increasing number of applications has significantly decreased antioxidant potential. Tian et al., (2011) demonstrated that 100 mg/l GA3 applied twice affected unfavourably a level of antioxidant capacity measured by the DPPH assay in ‘Muscat Hamburg’ fruit. The authors highlighted the opposite relationship studying the plant anatomical parts, that is leaf, stem and tendril. Gougoulias and Masheva (2010) noted beneficial influence of hormonization that increased the antioxidant potential in seedless grape cultivars by 16 – 42%.

Flavonoid content in the grapes under investigation varied between 0.083 and 0.103 mg/of cyanidin 3-glucoside equivalents/100 grapes and it differed significantly between the combinations assessed. The grapes subjected to hormonization had significantly less flavonoids than the control (Table 2). It was observed that the rising concentration of gibberellic acid promoted a significant increase of flavonoid level. Fruits after three hormonization treatments displayed the significantly lowest flavonoid content among the hormonized and control grapes. The obtained results are consistent with those reported by Tian et al. (2011) who noted that GA3 application decreased total flavonoid content substantially in grape pulp and skin. However, contrary results were obtained by Gougoulias and Masheva (2010) while assessing fruit of ‘Trakijska perla’ with amber yellow coloration of grape berry and ‘Kishimish Tjurkmenski’ violet - red coloring after GA3 treatment applied twice. The authors observed an increase in flavonoid content by 10 and 12%, respectively as against the control.

Tannins occur in grape skin, seeds and pedicels. Their amount in fruit juice (must) and wine is related to grapevine cultural practices, vine load and environmental conditions, maceration procedures and fermentation conditions (Matthew & Nuzzo, 2007). Tannins possess several vital properties that affect the fruit color and color stability, depth of mouth feel and astringency (Weston, 2005). The analysis made showed that hormonization with 300 mg/l GA3 and single application had significant effect on tannin content. Similar relationships were reported by Awad and Al-Qurashi (2012) who treated date palm ‘Barhee’ cv. with 100 and 150 mg/l GA3. However,the available literature does not provide any data on the impact of concentration and the number of GA3 sprays on tannin level in grape berries.

An interaction between gibberellic acid concentration and the number of treatments was found to significantly affect the chosen secondary metabolites in ‘Einset Seedless’ variety grapes, only anthocyanins made an exception.

/insert table 3/

The Pearson`s coefficient indicates a strong correlation between total extract content and concentration of 100 mg/l and a single application GA3, vitamin C level and treatment applied once (Table 3). A strong negative correlation was noted between total extract content and twice application and concentration 200 mg/l GA3; vitamin C and 300 mg/l GA3 concentration and two and three times applied sprays as well as between total acidity and concentration 200 mg/l GA3. A negative correlation was observed between vitamin C content and application at 200 mg/l GA3 and between total acidity and treatment at 300 mg/l GA3 concentration.

/insert table 4/

The analysis of Pearson`s coefficient for the parameters determining antioxidant activity of fruits showed a strong correlation between total phenolic acids and concentration of 100 mg/l GA3 and treatment rate of 300 mg/l GA3, flavonoid level and the number of applications as well as tannin content and single GA3 treatment (Table 4). A strong negative correlation existed between total phenolic acids and concentration 200 mg/l GA3 and treatment applied three times and between the DPPH parameter and 200 mg/l GA3 application and between flavonoid content and single GA3 treatment and applied two times. The correlations were established between anthocyanin level and single GA3 application, DPPH parameter and 300 mg/l GA3 application rate, flavonoid content and 300 mg/l GA3 concentration, tannin level and single GA3 treatment. A negative correlation was found between total phenolic acids and a single application, anthocyanin content and 300 mg/l GA3 application rate, between tannin content and treatment at 200 and 300 mg/l GA3 concentration.

/insert figure 1/

**Fig.1** Branching-Tree diagram for antioxidant activity in ‘Einset Seedless’ grapevines subject to gibberellic acid concentration.

The dendrogram shows two separate clusters, in that one object being a clear outlier (Fig.1). This is the control which differs distinctively from other combinations. They display high similarity at the group-level, these are: group 1 – 100 and 300 mg/l GA3 concentration and group 2 – 200 mg/l GA3 concentration. Both clusters are quite similar to each other.

/insert figure 2/

**Fig. 2**. Branching-tree diagram for antioxidant activity of ‘Einset Seedless’ grapevine subject to number of applications

 The dendrogram (Fig. 2) enabled defining the similarity of the effect of the number of gibberellic acid applications on a level of antioxidant activity in fruit. The obtained results served to define two separate clusters exhibiting some similarities. It was shown that a single gibberellic acid treatment and GA3 applied twice affect antioxidant activity very similarly, whereas at three-times treatment the similarity with the control combination is noted.

/insert figure 3a, 3b/

**Fig. 3.** PCA map visualizing relationship between a content of chosen secondary metabolites subject to concentration (Fig. 3a) and number of GA3 applications (Fig. 3b).

The PC sum (PC1 and PC2 components)of the total variation of traits for the GA3 concentrations reached 81.1%, in that 52.47% for PC1 and 28.63% for PC2 (Fig.3a). The PC1 was responsible for a level of flavonoids, vitamin C, anthocyanins and phenolic acids, whereas PC2 for the potential of sugars, tannins, acidity and DPPH. The control displayed a high content of extract, flavonoids, while low of anthocyanins and phenolic acids. The PCA analysis shows differences between the application rates, that is fruits subjected to 100 mg/l GA3 treatment had a high vitamin C content. The application rates 200 and 300 mg/l GA3 contributed to high tannin level and noteworthy, 300 mg increased acidity as well.

 The sum PC of total variation for the analyzed number of GA3 applications was 84% (60.73 and 23.7%, respectively). The PC1 denotes a phenolic acid level, while PC2 – the other secondary metabolites as well as a level of vitamin C and extract. The control fruits were characterized with a high content of flavonoids, vitamin C and extract while low of the remaining components. The hormonization spray, irrespective of the number of applications, affected beneficially a level of phenolic acids, anthocyanins, DPPH and fruit acidity.

CONCLUSIONS

The astudies assessed the chosen biologically active compounds of ‘Einset Seedless. cv. grapes subject to gibberellic acid concentration and the numer of applications. The hormonization treatment had an adverse effect on a content of extract, vitamin C and flavonoids in grapes. The GA3 application was found to increase anthocyanin level, yet its impact was not significant. Antioxidant activity determined by the DPPH assay depended on concentration and the number of treatments and with the increasing application number the analyzed parameter was shown to decrease significantly. Gibberellic acid at 100 and 300 mg/l application rates has significantly increased DPPH level compared to the control and 200 mg/l concentration. The single GA3 treatment and applied three times and application rates 100 and 200 mg/l were shown to have significant influence on phenolic acid content. A level of tannins after a single GA3 treatment and 300 mg/l concentration increased significantly.

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Table 1. Effect of hormonization on extract content, vitamin C level and total acidity in `Einset Seedless` grapevine.

|  |  |  |  |
| --- | --- | --- | --- |
| Combination | Solid content(oBx) | Ascorbic acid(mg/100g) | Total acidity(%) |
| Treatment(mg/l GA3) | Control | 19.63 ± 0.12 D | 72 ± 1 D | 0.2 ± 0.1 A |
| 100 | 19.46 ± 0.38 C | 47 ± 22 C | 0.3 ± 0.0 B |
| 200 | 18.78 ± 1.35 A | 31 ± 4 A | 0.2 ± 0.1 A |
| 300 | 19.00 ± 1.02 B | 41 ± 13 B | 0.3 ± 0.1 B |
| Mean | 19.08 ± 0.92 | 39 ± 13 | 0.3 ± 0.1 |
| p-value | **<0.0001** | **<0.0001** | **0.0001** |
| Number of applications | Control | 19.63 ± 0.12 C | 72 ± 1 D | 0.2 ± 0.1 A |
| 1 | 19.40 ± 0.35 C | 41 ± 12 B | 0.4 ± 0.1 B |
| 2 | 19.08 ± 1.07 B | 51 ± 19 C | 0.3 ± 0.1 B |
| 3 | 18.76 ± 1.33 A | 27 ± 2 A | 0.2 ± 0.1 A |
| Mean | 19.08 ± 0.92 | 39 ± 11 | 0.3 ± 0.1 |
| p-value | **<0.0001** | **<0.0001** | **0.0003** |
| Treatment × Number of applications | p-value | **<0.0001** | **<0.0001** | **0.0021** |

\* Mean values marked with the same letters do not differ significantly at *P* < 0.05; NS: not significant.

Table 2. Hormonization and antioxidant activity of grapes `Einset Seedless`.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Combination | Phenolic acid(mg 100/g FM) | Total anthocyanin(mg 100/g FM) | DPPH(µM TE/g FM) | Total flavonoids(mg 100/gFM) | Tannins(%) |
| Treatment(mg/l GA3) | Control | 0.051 ± 0.000 B | 9.930 ± 0.501 A | 66.386 ± 0.081 B | 0.103 ± 0.001 D | 0.0643 ± 0.007 A |
| 100 | 0.055 ± 0.006 C | 10.220 ± 0.507 A | 82.063 ± 1.524 C | 0.083 ± 0.005 A | 0.0680 ± 0.009 A |
| 200 | 0.056 ± 0.006 C | 10.434 ± 0.439 A | 56.272 ± 9.681 A | 0.090 ± 0.004 B | 0.0745 ± 0.017 A |
| 300 | 0.048 ± 0.004 A | 10.163 ± 0.549 A | 83.652 ± 1.403 D | 0.098 ± 0.005 C | 0.1514 ± 0.182 B |
| Mean | 0.053 ± 0.005 | 10.272 ± 0.498 | 73.996 ± 4.203 | 0.090 ± 0.005 | 0.098 ± 0.069 |
| p-value | **<0.0001** | **0.4944** | **<0.0001** | **<0.0001** | **0.0242** |
| Number of applications | Control | 0.051 ± 0.000 A | 9.930 ± 0.501 A | 66.386 ± 0.081 A | 0.103 ± 0.001 C | 0.0643 ± 0.007 A |
| 1 | 0.053 ± 0.010 B | 10.353 ± 0.509 A | 76.390 ± 8.524 D | 0.091 ± 0.004 B | 0.1629 ± 0.176 B |
| 2 | 0.051 ± 0.002 A | 10.384 ± 0.480 A | 75.424 ± 11.610 C | 0.092 ± 0.008 B | 0.0673 ± 0.014 A |
| 3 | 0.055 ± 0.006 C | 10.080 ± 0.493 A | 70.172 ± 19.922 B | 0.088 ± 0.009 A | 0.0637 ± 0.013 A |
| Mean | 0.053 ± 0.006 | 10.272 ± 0.494 | 73.996 ± 13.375 | 0.090 ± 0.007 | 0.098 ± 0.068 |
| p-value | **<0.0001** | **0.3844** | **<0.0001** | **<0.0001** | **0.0689** |
| Treatment × Number of applications | p-value | **<0.0001** | **0.4370** | **<0.0001** | **<0.0001** | **0.0298** |
|  |  |  |  |  |  |  |

\* Mean values marked with the same letters do not differ significantly at *P* < 0.05; NS: not significant.

Table 3. Correlation coefficient for chosen quality parameters defining fruit taste.

|  |  |  |  |
| --- | --- | --- | --- |
| Combination | Solid content | Ascorbic acid | Total acidity |
| Treatment(mg/l GA3) | 100 | **0.7952** | -0.1309 | 0.1598 |
| 200 | **-0.8047** | **-0.4736** | **-0.9607** |
| 300 | -0.0566 | **-0.9977** | **-0.6495** |
| Number of applications | 1 | **0.9389** | **0.7633** | 0.2738 |
| 2 | **-0.8401** | **-0.7832** | 0.2165 |
| 3 | -0.0217 | **-0.9221** | -0.2582 |

Table 4. Correlation coefficient for chosen secondary metabolites

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Combination | Phenolic acid | Total anthocyanin | DPPH | Total flavonoids | Tannins |
| Treatment(mg/l GA3) | 100 | **0.7043** | 0.0803 | 0.1858 | **-0.8238** | -0.2604 |
| 200 | **-0.9707** | -0.1361 | **-0.9577** | **-0.8816** | **-0.6893** |
| 300 | **0.9743** | **-0.6100** | **0.6494** | **0.5386** | **-0.6304** |
| Number of applications | 1 | **-0.5000** | **0.4886** | 0.1306 | **0.7267** | **0.6362** |
| 2 | -0.1218 | -0.4064 | -0.0680 | **0.8895** | -0.1986 |
| 3 | **-0.7777** | -0.2571 | 0.0873 | **0.9834** | -0.0333 |