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Chapter

The Effects of Different Substrates with Chemical and Organic Fertilizer Applications on Vitamins, Mineral, and Amino Acid Content of Grape Berries from Soilless Culture

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Abstract

Due to its advantages, soilless cultivation has been used for both early- and late-maturing grape varieties. High nutritional and energy value is one of the strongest features that make the grape an effective component of agriculture and the human diet. Therefore, it was thought that it would be useful to determine the nutrient content of the berries in a soilless culture study carried out on the Early Cardinal grape variety. One-year-old vines were trained to a guyot system and grown in 32-liter plastic pots containing four different solid growing media, namely, zeolite, cocopeat, and zeolite+cocopeat (Z + C) (1:1 and 1:2, v:v). A total of three different nutrient solutions (Hoagland, Hoagland A (adapted to the vine) and organic liquid worm fertilizer (OLWF)) were applied to the plants. Grapevines were given different solutions starting from the bud burst. Z + C (1:1) substrate mixture giving the highest values of 14 amino acids, vitamins, and most macro- and microelements. Hoagland and Modified Hoagland nutrient solutions mostly gave higher values than OLWF for the properties studied. In general, it was observed that there were no significant losses in terms of mineral, vitamin, and amino acid composition in soilless grape cultivation.

Keywords: grapevine, phytochemicals, fertilization, vermicompost, zeolite, cocopeat

1. Introduction

Grapes (*Vitis vinifera* L.) are the most produced fruit in the world. The total grape area and its production globally are 7.4 million ha and 77.8 million tons, respectively, in 2018 [1]. About 36% of the total is consumed for fresh, 7% for dried, and 57% for winemaking. Five countries represent 50% of the world's vineyards. Turkey is in the fifth position in vineyard areas in the world in 2018 with a total surface of 448,000 ha, after Spain, China, France, and Italy. It is the sixth in total grape

production (3.9 million tons) among the major grape producers that after China, Italy, USA, Spain, and France; fourth in table grapes (2.2 million tons, 56.1%), and first in dried grape production (396,825 tons, 40.7%), about fortieth in wine grape production among the grape-growing countries. In Turkey, the grapes used for winemaking are 124,800 tons (3.2%) [1].

Soilless culture techniques are primarily applied in ornamental plants and vegetables in the world and Turkey [2, 3]. In recent years, this technique is also used to overcome some problems due to its various advantages in grape cultivation [2, 4–6]. No need for tillage and soil preparation, protection from soil pathogens, effective use of water and nutrient solutions, reduction of spraying, obtaining more quantity and quality products per unit area, production of new or traditional grape varieties in a more extended period according to market demands, and control of harvest time are among some advantages of soilless cultivation [2, 4, 7].

In the world and Turkey, when it is considered together with the cultivation of greenhouse grapes for early grape ripening or late harvest, grape cultivation in soilless culture is considered an important cultivation method due to its advantages. This technique may be used for both early- and late-maturing grape varieties. According to our current information, no producer grows grapes commercially in soilless culture in Turkey. Studies on the subject are still carried out in horticulture departments of some agriculture faculties and viticulture research institutes.

Depending on the research purposes, different varieties, substrate mixtures, containers and nutrient solutions [2, 4, 7–15] were used in the grape cultivation experiments in the soilless culture system.

In the studies conducted by Tangolar et al. [6], the effect of substrates on the grape yield and quality of the berries in vines grown in the open and under the greenhouse was determined. The study that examined the yield, cluster, and berry properties of Early Sweet variety determined that perlite:peat (2:1) and cocopeat substrates gave better results. Tangolar et al. [16] also researched Early Sweet and Trakya Ilkeren cultivars to determine the effects of three different media, namely perlite:peat (2:1), cocopeat and pumice, and two different modified Hoagland nutrient solutions on shoot diameter as well as the nutrient element and chlorophyll levels of the leaves and grape yield and quality characteristics. The study found a significant difference between media and nutrient solution application for some characteristics examined.

Achieving a good quality in grapes is an essential goal wherever it is grown; one of the important components that make up the quality is the phytochemical content of the berries. Grapes contain a number of phytochemicals beneficial for human health, as well as amino acids, proteins, vitamins, and minerals [17–26]. So, berries are efficiently used to increase the nutritional and energy value of the human diet.

Some studies [27] have shown that magnesium, calcium, zinc, and vitamins such as B and C are related to people's cognitive performance. Clinical findings have revealed that extreme deficiencies of one or more of these nutrients are not uncommon, even in developed countries. These deficiencies may affect cognitive performance, especially in vulnerable groups such as the elderly and those exposed to occupational pressures and difficult living conditions.

Key et al. [28] noted that dietary science is increasingly recognized for its ability to prevent and support disease prevention and new technologies and therapies to improve modern medical practice. Researchers noted that dietary studies help discover specific dietary patterns that promote healthy brain aging and moderate the involvement of nervous systems known to facilitate cognitive performance in later life [28].

The composition of grape berries in different grape cultivars grown open field is affected by different factors such as variety, stress conditions, biostimulants, irrigation, fertigation, pruning, and others [26, 29–49].

In spite of this, the studies conducted in the world and Turkey found no study of the effects of the different substrates and nutrition solutions on the biochemical content of berries obtained from varieties grown in soilless culture. So, this subject is thought to have not been sufficiently investigated yet.

Because of these, it has been seen beneficial to examine the effects of substrates and nutrition solutions on the biochemical contents, which are essential for human health. Therefore, this study was designated to evaluate the amino acid, mineral, and vitamin content of berries from Early Cardinal table grape cultivar grown in different soilless culture medium and plant nutrient solutions.

2. Materials and methods

2.1 Trial conditions

This research was carried out in a greenhouse at the Department of Horticulture, Faculty of Agriculture, the University of Cukurova, which was conducted under a 21 m, 9 m, and 3 m in length, width, and height greenhouse covered with UV plastic with a thickness of 0.4 mm. During the research, no heating process was done in the greenhouse.

2.2 Plant material

As plant material, own-rooted Early Cardinal grape (*V. vinifera* L.) cv. grown in soilless culture was used. To produce plant material, cuttings from Early Cardinal grapes (*V. vinifera* L.) grown were planted in perlite pools on January 15, 2018, and irrigated immediately after planting. Rooting of cutting occurred after approximately 90 days at a satisfactory level. Well-rooted cuttings were selected and transplanted into 32-liter plastic pots containing four different solid growing media, namely, zeolite, cocopeat, zeolite+cocopeat (Z + C) (1:1, v:v), and Z + C (1:2, v:v). A total of three different nutrient solutions were applied to the rooted cuttings: two chemical nutrient solutions (Hoagland (H) and Hoagland A (HA- adapted to the vine) and organic liquid worm fertilizer (OLWF) (**Table 1**). Nitrogen, phosphorus, potassium, magnesium, sulfur, and boron concentrations in the modified Hoagland solution were reduced between 3.2% (phosphorus) and 76.5% (sulfur) compared with Hoagland, and on the other hand, iron 2, manganese 6, zinc 20, and molybdenum 5 fold have been increased. With the same amount of solution in volume, more N, P, Mg, Zn, Cu, Mn, and Fe were given than Hoagland A and Hoagland through OLWF. The pots were placed in the greenhouse with a distance of 1.50 m between rows and 0.60 m in rows. After planting, a well-irrigation was performed to saturate the cultivation media.

One-year-old vines entered the resting period at the end of the first year were pruned and trained to a guyot system to prepare for the crop year, on January 31, 2019. About 20 buds were left per vine. The number of clusters of the vines was equal to 12 clusters by removing the excessive clusters on May 24, 2019, after the berry set. Grapevines were given different solutions within the second vegetation year, starting from the bud burst.

The pH value of the tap water used in the experiment was 7.68, and the EC value was 0.813 mS cm⁻¹. The amount of water given to the plants varied between 1 and

Element	Formula	Hoagland A (mg kg ⁻¹)	Hoagland (mg kg ⁻¹)	Organic liquid worm fertilizer
N	K ₂ (NO ₃) ₂	150	210	5%
P	H ₃ PO ₄	30	31	0.49%
K	K ₂ SO ₄	175	235	1.47%
Mg	MgSO ₄ .7H ₂ O	20	48	0.78%
S	CaSO ₄ .H ₂ O	15	64	Not detected
Fe	Fe-EDDHA	5	2.5	5257 ppm
Mn	MnSO ₄ .H ₂ O	3	0.5	565 ppm
B	H ₃ BO ₃	0.4	0.5	Not detected
Cu	CuSO ₄ .5H ₂ O	0.02	0.02	58 ppm
Zn	ZnSO ₄ .7H ₂ O	1	0.05	152.5 ppm
Mo	(NH ₄) ₆ Mo ₇ O ₂₄ .4 H ₂ O	0.05	0.01	Not detected
pH				5.28
Total dry matter				13%
Humic-fulvic acid				38%

Table 1.

Composition and formula of chemical and organic nutrient solutions used in the trial.

3 L pot⁻¹ per day according to the water-holding capacity of the growth medium. The total amount of nutrients applied per plant in the first and crop year of the experiment is shown in **Table 2**.

2.3 Biochemical analysis

When the total soluble solids (TSS) reached about 12–14%, five cluster samples were taken from each of the three replicates of treatments on July 1, 2019. After removing from the clusters, stored berries at –20°C before the phytochemical analysis were analyzed in the Department of Genetic and Bio-Engineering, Faculty of Engineering, University of Yeditepe.

2.3.1 Mineral elements

Macro and micronutrient element analyses were carried out using samples of berries. Phosphorus (P) was determined vanadomolibdo phosphoric acid yellow color method as reported by Bremner [50]. Potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) concentrations of the berries were analyzed by atomic absorption spectrophotometer [51].

2.3.2 Amino acids

1 g fresh sample was treated with 0.1 N HCl, homogenized with ultra turrax, and incubated at 4°C for 12 hours. Supernatants were filtered through 0.22-µm filters after

Element	Hoagland A		Hoagland		Organic liquid worm fertilizer	
	2018	2019	2018	2019	2018	2019
N (g)	12.75	21.00	17.85	29.39	37.40	59.90
P (g)	2.55	4.20	2.64	4.34	3.67	5.87
K (g)	14.87	24.50	19.97	32.89	10.99	17.61
Mg (g)	1.89	15.91	4.53	37.47	5.83	9.34
Zn (mg)	84.92	139.86	4.165	6.86	114.07	182.70
Cu (mg)	1.70	2.80	1.70	2.80	43.38	69.48
B (mg)	85.0	140.00	106.25	175.00	Not detected	Not detected
Mn (mg)	255.0	420.00	42.5	70.00	422.62	676.87
Mo (mg)	0.43	0.70	0.09	0.14	Not detected	Not detected
Fe (mg)	474.8	777.9	235.5	387.8	3932.2	6297.9

Table 2.

The amount of nutrients given per plant by different nutrient solutions in 2 years.

samples were centrifuged at 1200 rpm for 50 minutes (Millex Millipore). The supernatants were then transferred to a vial, and the amino acids were analyzed in HPLC as described by Antoine et al. [52] and Kitir et al. [53]. Readings from Zorbax Eclipse-AAA 4.6150 mm and 3.5 m columns (Agilent 1200 HPLC) were taken at 254 nm, and the amino acids were identified by comparing them to standards of O-phthalaldehyde (OPA), fluorenylmethyl-chloroformate (FMOC), and 0.4 N borate. The following solutions were used in the mobile phase chromatography system: Phase A: 40 mM NaH₂PO₄ (pH: 7.8) and Phase B: acetonitrile/methanol/water (45/45/10 v/v/v), after a 26-minute derivation process in HPLC, aspartate, glutamate, asparagine, serine, glutamine, histidine, glycine, arginine, alanine, tyrosine, cysteine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, thionine, and proline.

A 50 mg frozen berry sample was crushed using liquid nitrogen and extracted with 4.5 mL of 3-sulfosalicylic acid, and then filtered through a Whatman filter paper (#2) for proline measurement. In a test tube, 2 mL of the filtrate were mixed with 2 mL acid-ninhydrin and 2 mL glacial acetic acid for 1 hour at 100°C, stopped the reaction with an ice bath, and the filtrates were analyzed. The concentration of proline was measured spectrophotometrically at 520 nm [54].

2.3.3 Vitamins

2.3.3.1 Vitamin A

Berry samples were ground for vitamin A (Retinol). Berry samples were extracted with a mixture of n-hexane and ethanol. 1% BHT was added and kept in the dark environment for 1 day. At the end of this period, centrifugation was conducted at 4000 rpm (+4°C) for 10 min. The obtained supernatant was filtered with the help of Whatman filter paper and added 0.5 mL of n-hexane. Drying was then performed using nitrogen gas. The residue in the tubes was dissolved in a methanol + tetrahydrofuran mixture. Analyses were carried out in Thermo Scientific Finnigan Surveyor

model high-performance liquid chromatography (HPLC) and in amber glass vials on Tray, and autosampler using PDA array detector [55, 56].

2.3.3.2 Vitamin B

A total of 10 g of samples were weighed and homogenized. The samples were then transferred to a conical flask with 25 mL of extraction solution. A shaking water bath at an ambient temperature of 70°C was used to sonicate the solution for 40 minutes. Following sonication, the sample was cooled and filtered to make a volume of 50 mL with extraction solution. The extraction solution was again filtered with filter trips (0.45 µm), and 20 µl aliquots solution was injected into the HPLC by using an auto-sampler. A reversed-phase C-18 analytical column (STR ODS-M, 150 mm 4.6 mm ID, 5 m, Shimadzu Corporation, Japan) separated the B complex vitamins. At 40°C, the mobile phase consists of a 9:1 (v/v) combination of 100 mM sodium phosphate buffer (pH: 2.2) containing 0.8 mM sodium-1-octane sulfonate and acetonitrile. The flow rate was constant at 0.8 mL/min using a PDA detector with a 270 nm absorption rate. The peak area of the corresponding chromatogram was used to calculate B vitamins using the following equation [57]:

$$\text{B vitamins (mg100 g}^{-1}\text{)} = \text{Concentration of standard} \\ \times (\text{Area of sample} / \text{Area of Standard}) \\ \times \text{Dilution factor}$$

2.3.3.3 Vitamin C

Plants were sliced, frozen in liquid nitrogen, and kept at a temperature of –80°C until the analyses were completed. The extraction solution was combined with 2.5 ml of frozen crushed plant material (3% MPA and 8% acetic acid for MPA-acetic acid extraction and 0.1% oxalic acid for oxalic acid extraction). The mixture was titrated with indophenol solution (25% DCIP and 21% NaHCO₃ in water) until light, but the distinct rose-pink color appeared and persisted for more than 5 seconds [58].

2.4 Experimental design and statistical analysis

The study was designed according to the “Randomized Complete Blocks” with three replicates in 12 treatments. For each application and replicate, approximately 500 g of the berry samples were taken and analyzed for the compounds to be studied. Data obtained from the study were subjected to variance analysis using the SAS-based JMP statistical package programmer. The least significant difference (LSD) test was used to separate different groups at a 5% significance level.

3. Results and discussions

Besides bodywork, vitamins, and minerals, protection of the body from diseases, blood formation, bone, dental health, etc., are required for functions. Each food contains different amounts of various vitamins and minerals. Its richest sources are fresh vegetables and fruits [59].

As shown in **Table 3**, there were significant differences among the substrates related to macro- and microelements of berries except for boron. Considering, P, K, Ca, Mg, Mn, and Cu concentrations of berries were higher in Z + C (1:1) than the

other substrates. However, zeolite, cocopeat, and Z + C (1:1) for Na, Cocopeat, and Z + C (1:1) for Fe, and zeolite for Zn concentrations gave higher values than the other applications. Phosphorus, Mg, Fe in Hoagland; K in Hoagland A; calcium, Na, and Mn in Hoagland and Hoagland A, and zinc in OLWF fertilizers were recorded have higher concentrations than those of the others.

Macrominerals presented in **Table 3** determined that the potassium contents of berries were higher than those of the others, ranging from 234 mg 100 g⁻¹ for Z + C

Sources of variation	Macroelements (mg 100 g ⁻¹)				
	P	K	Ca	Mg	Na
Substrate					
Zeolite	17.7 c ^y	213 b	48 b	13.7 d	2.7 a
Cocopeat	19.1 b	208 c	47 b	17.9 b	2.4 a
Z + C (1:1) ^x	21.0 a	234 a	51 a	20.0 a	2.4 a
Z + C (1:2)	15.4 d	193 d	39 c	16.7 c	1.9 b
LSD 5%	0.4	5	2	0.8	0.3
<i>p-value</i>	<0.0001	<0.0001	<0.0001	<0.0001	0.0011
Fertilizer					
Hoagland A	19.3 b	227 a	49 a	16.8 b	2.6 a
Hoagland	19.8 a	223 b	50 a	18.1 a	2.6 a
OLWF	15.8 c	186 c	40 b	16.3 b	1.9 b
LSD 5%	0.4	4	1	0.7	0.3
<i>p-value</i>	<0.0001	<0.90001	<0.0001	<0.0001	<0.0001
Interaction					
Zeolite × Hoagland A	2.52 a	3.35a	0.67 a	1.61de	0.43 a
Zeolite × Hoagland	1.63 ef	1.92 f	0.46 d	1.25 g	0.29 b
Zeolite × OLWF	1.15 i	1.13 j	0.31 g	1.24 g	0.08 e
Cocopeat × Hoagland A	1.38 h	1.45 i	0.36 f	1.41 f	0.16 d
Cocopeat × Hoagland	2.31 c	2.24 d	0.55 b	1.97b	0.28 b
Cocopeat × OLWF	2.06 d	2.54 c	0.50 c	1.98b	0.27 b
Z + C (1:1) × Hoagland A	2.40 b	2.48 c	0.57 b	2.20a	0.26 bc
Z + C (1:1) × Hoagland	2.34 bc	2.87 b	0.56 b	2.26a	0.27 b
Z + C (1:1) × OLWF	1.55 g	1.67 h	0.39 e	1.56e	0.20 cd
Z + C (1:2) × Hoagland A	1.40 h	1.81 g	0.36 f	1.49ef	0.19 d
Z + C (1:2) × Hoagland	1.65 e	1.88 fg	0.41 e	1.77c	0.19 d
Z + C (1:2) × OLWF	1.57 fg	2.10 e	0.40 e	1.74 cd	0.19 d
LSD 5%	0.7	8	3	1.3	0.6
<i>p-value</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^xZ + C: Zeolite+Cocopeat, OLWF: Organic liquid worm fertilizer,

^yMean separation within columns by LSD multiple range test at 0.05 level.

Table 3.

The effect of different substrates and nutrient solution applications on the level of macro elements in berries.

(1:1) substrate and 186 mg 100 g⁻¹ for OLWF fertilizer. Followed calcium content of grapes was found between 51 mg 100 g⁻¹ for Z + C (1:1) substrate and 40 mg 100 g⁻¹ for OLWF fertilizer. Among the macroelements, sodium gave the lowest amount.

Considering trace elements, the highest iron content (0.362 mg 100 g⁻¹) is obtained from Z + C (1:1), whereas the lowest level of iron (0.255 mg 100 g⁻¹) was found in zeolite. The zinc content of grape berries was in the range of 0.299 and 0.184 mg 100 g⁻¹, whereas the manganese content of grape berries was in the range of 0.235–0.178 mg 100 g⁻¹. Copper and boron microminerals varied between 0.147 and 0.105 and 0.481 and 0.329 mg 100 g⁻¹, respectively. The substrate × fertilizer interaction was significant for all elements except Cu and B (**Tables 3 and 4**).

In the study by Abdrabba and Hussein [35], calcium, magnesium, potassium, phosphorus, and iron values were determined as 120, 31, 154, 39, and 5 mg 100 g⁻¹ as the average of pulp, seed, and peel, respectively, and these minerals useful for the human body have been deemed necessary.

Similarly, the values given in Kral et al. [59] for Ca, K, Mg, Na, Cu, Fe, Mn, and Zn; in Cantürk et al. [60] for Ca, K, Mg, Na, P, Cu, Fe, Mn, B, and Zn; in Abdrabba and Hussein [35] for Ca, K, Mg, P, and Fe; in Anonymous [61] for Ca, K, Mg, Na, and Fe; in Olsen and Ware [62] for Ca, K, Mg, Na, P, Fe, Mn, B, and Zn were found to be quite close to the values given in **Table 3** for the specified elements.

For this reason, it was concluded that there were no significant losses in terms of mineral levels of grapes grown under soilless culture conditions.

Vitamins, like minerals, are micronutrients that play an essential role in fulfilling metabolic functions, producing new cells, and repairing damaged cells.

There were found significant differences among substrates and fertilizers in terms of vitamin contents of berries analyzed in the study. The higher vitamin A, B1, B2, B6, and C values were analyzed in berries of plants grown in Z + C (1:1) substrate mix and berries of applications using Hoagland solution (**Table 5**). The higher values obtained from vitamin A, B1, B2, B6, and C were 39.21, 65.12, 167.06, 95.19, and 15.21 mg 100 g⁻¹, respectively. The substrate × fertilizer interaction was significant for all vitamins examined (**Table 5**).

According to the Bourre [63] and Key et al. [28], nutrients such as vitamins, minerals, and amino acids play a crucial role in ensuring proper brain function. Vitamins protect against inflammation and reactive oxidative species. Minerals function as cofactors for enzymes, prevent lipid peroxidation, and promote energy production. Amino acids serve as precursors to neurotransmitters and neuromodulator metabolites responsible for various functions related to attention, mood, arousal, and memory.

Most vitamins and microelements have been studied concerning brain functioning. For example, it has been reported by Bourre [63] that the use of glucose for energy production occurs in the presence of vitamin B1. This vitamin regulates cognitive performance, especially in the elderly. It has been reported that vitamin B6 is beneficial in treating premenstrual depression. Vitamins B6 and B12, among others, are directly involved in synthesizing certain neurotransmitters. Vitamin B12 delays the onset of signs of dementia and blood abnormalities when administered at an appropriate time before the first symptoms.

Emphasizing the importance of mineral nutrients for healthy brain aging, Key et al. [28] stated in their results that a nutrient regime containing macro- and micronutrients softens the effect of brain structure on cognitive function in old age and supports the effectiveness of interdisciplinary methods in nutritional cognitive neuroscience for a healthy brain. In the article of Çetin et al. [64], different researchers reported

Sources of variation	Microelements (mg 100 g ⁻¹)				
	Fe	Zn	Mn	Cu	B
Substrate					
Zeolite	0.255 c ^y	0.299 a	0.178 c ^y	0.105 b	0.348
Cocopeat	0.353 a	0.184 c	0.208 b	0.131ab	0.448
Z + C (1:1) ^x	0.362 a	0.187 c	0.235 a	0.147 a	0.481
Z + C (1:2)	0.288 b	0.192 b	0.195 b	0.113 ab	0.329
LSD 5%	0.011	0.011	0.016	0.036	NS
<i>p</i> value	<0.0001	<0.0001	<0.0001	0.1082	0.002
Fertilizer					
Hoagland A	0.325 b	0.206 b	0.208 a	0.123	0.399
Hoagland	0.340 a	0.207 b	0.216 a	0.136	0.455
OLWF	0.279 c	0.233 a	0.188 b	0.112	0.351
LSD 5%	0.010	0.009	0.014	NS	NS
<i>p</i> -value	<0.0001	<0.0001	0.001	0.2907	0.3459
Interaction					
Zeolite × Hoagland A	373.26 c	23.36c	257.02 b	111.36	33.55
Zeolite × Hoagland	274.67e	26.09b	161.89 fg	107.69	36.95
Zeolite × OLWF	119.72 g	40.33a	115.50 h	96.29	33.83
Cocopeat × Hoagland A	229.96f	22.09 cd	145.29 g	113.61	38.77
Cocopeat × Hoagland	399.01 b	17.68gh	222.25 cd	159.97	59.94
Cocopeat × OLWF	430.45 a	15.31i	255.55 b	120.14	35.54
Z + C (1:1) × Hoagland A	403.44 b	19.74ef	247.77 bc	177.22	61.79
Z + C (1:1) × Hoagland	404.49 b	17.81gh	290.87 a	135.89	40.02
Z + C (1:1) × OLWF	276.79de	18.58fgh	166.47 fg	126.40	42.59
Z + C (1:2) × Hoagland A	294.99 d	17.26 h	182.54 f	90.53	25.58
Z + C (1:2) × Hoagland	282.14de	21.29de	188.47 ef	142.21	44.89
Z + C (1:2) × OLWF	289.78de	19.16 fg	212.86 de	104.91	28.33
LSD 5%	0.020	0.018	0.028	NS	NS
<i>p</i> -value	<0.0001	<0.0001	<0.0001	0.3888	0.3886

^xZ + C: Zeolite+Cocopeat, OLWF: Organic liquid worm fertilizer.
^yMean separation within columns by LSD multiple range test at 0.05 level,
 NS: Nonsignificant.

Table 4.
 The effect of different substrates and nutrient solution applications on the level of microelements in berries.

that potassium is a very important component of human health. A high-potassium diet lowers blood pressure and reduces cardiovascular disease morbidity and mortality [65]. In addition, potassium intake reduces urinary calcium excretion and decreases the risk of osteoporosis [66]. Ca is the primary element of the bone system, assists in tooth development, helps regulate endo- and exo-enzymes, and plays a significant role in regulating blood pressure [67]. Therefore, it is an essential mineral for human

Sources of variation	A Retinol	B1 Thiamin	B2 Riboflavin	B6 Pyridoxine	C Ascorbic acid
Substrate					
Zeolite	29.95 d ^y	45.39 b	113.76 d	78.50 c	12.49 c
Cocopeat	34.91 b	59.59 a	148.49 b	88.27 b	13.51 b
Z + C (1:1) ^x	39.21 a	65.12 a	167.06 a	95.18 a	15.21 a
Z + C (1:2)	31.65 c	46.02 b	121.29 c	69.74 d	12.14 c
LSD 5%	1.09	5.54	6.59	4.55	0.42
<i>p-value</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Fertilizer					
Hoagland A	34.51 b	55.67 b	140.93 b	84.44 b	13.62 b
Hoagland	36.51 a	60.47 a	153.29 a	91.79 a	14.46 a
OLWF	30.76 c	45.95 c	118.74 c	72.54 c	11.93 c
LSD 5%	0.95	4.80	5.71	3.94	0.36
<i>p-value</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction					
Zeolite × Hoagland A	39.40 b	56.80 bc	144.69 de	93.26 b	15.72 b
Zeolite × Hoagland	28.89 de	49.01 cd	114.02 fg	80.73 c	12.41 d
Zeolite × OLWF	21.54 g	30.37e	82.57 h	61.52 f	9.33 f
Cocopeat × Hoagland A	26.70 f	43.21 d	106.56 g	71.01 de	10.88 e
Cocopeat × Hoagland	39.49 b	74.24 a	187.54 b	109.98 a	15.58 b
Cocopeat × OLWF	38.53 b	61.32 b	151.37 d	83.81 c	14.07 c
Z + C (1:1) × Hoagland A	43.75 a	82.81 a	204.58 a	113.18 a	17.43 a
Z + C (1:1) × Hoagland	43.59 a	63.66 b	172.08 c	94.21 b	16.08 b
Z + C (1:1) × OLWF	30.29 d	48.88 cd	124.53 f	78.14 cd	12.11 d
Z + C (1:2) × Hoagland A	28.19 ef	39.86 de	107.89 g	60.31 f	10.43 e
Z + C (1:2) × Hoagland	34.08 c	54.98 bc	139.50 e	82.23 c	13.76 c
Z + C (1:2) × OLWF	32.67 c	43.22 d	116.47 fg	66.69 ef	12.21 d
LSD 5%	1.89	9.60	11.41	7.88	0.72
<i>p-value</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^xZ + C: Zeolite+Cocopeat, OLWF: organic liquid worm fertilizer.
^yMean separation within columns by LSD multiple range test at 0.05 level.

Table 5.

The effect of different substrate and nutrient solution applications on vitamins (mg 100 g⁻¹).

health. Zn and Fe deficiency in the diet programs is a common problem and a matter of great concern, especially in developing countries where people trust vegetarian diets more. Zn is involved with the immune system, and Fe is concerned with hemoglobin, myoglobin, and cytochrome [68]. They are also recognized to be potential antioxidants [69]. Mg is essential to all living cells, where they play a major role in manipulating important biological polyphosphate compounds such as ATP, DNA, and RNA. Also, more than 300 enzymes require magnesium ions to function [70].

In the study, the effects of applications on 20 amino acids in grapes were evaluated. For all amino acids examined in **Table 5**, the differences between treatments were statistically significant. The highest values were found from Z + C (1:1) application in 14 amino acids (**Table 6**), namely aspartate, glutamate, proline, arginine, glutamine, histidine, alanine, cystine, methionine, tryptophan, phenylalanine, isoleucine, leucine, and lysine. In Z + C (1:1), Z + C (1:2), and cocopeat applications for valine; in Z + C (1:1) and zeolite for serine; and in cocopeat and Z + C (1:2) applications for glycine were the highest values. Apart from these, the highest tyrosine and asparagine in Zeolite were detected. Among nutrient solutions, Hoagland for aspartate, glutamate, alanine, and phenylalanine amino acids; Hoagland and Hoagland A for proline, arginine, glutamine, tyrosine, methionine, tryptophan, isoleucine, and leucine; Hoagland and OLWF nutrient solutions for histidine; Hoagland A for glycine, thionine, cystine, valine, lysine, asparagine and serine amino

Sources of Variation	Aspartate	Glutamate	Proline	Arginine	Glutamine
Substrate					
Zeolite	14,930 c ^y	10,637 d	28,607 c	34,258 c	20,750 c
Cocopeat	16,289 b	14,849 b	33,667 b	39,258 b	24,768 b
Z + C (1:1) ^x	17,718 a	15,751 a	37,901 a	42,880 a	27,569 a
Z + C (1:2)	13,867 d	12,257 c	34,200 b	35,427 c	22,018 c
LSD 5%	5529	774	1290	2222	1668
<i>p-value</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Fertilizer					
Hoagland A	16,172 b	13,440 b	34,041 a	39,771 a	24,293 a
Hoagland	16,725 a	15,096 a	34,020 a	38,911 a	25,437 a
OLWF	14,206 c	11,585 c	32,720 b	35,186 b	21,599 b
LSD 5%	470	670	1117	1924	1445
<i>p-value</i>	<0.0001	<0.0001	0.0342	0.0001	<0.0001
Interaction					
Zeolite × Hoagland A	20,134 ab	14,265 c	42,259 c	51,443 a	26,212 bc
Zeolite × Hoagland	13,650 efg	12,818 de	22,751 ij	28,563 ef	19,198 ef
Zeolite × OLWF	11,005 i	4828 g	20,810 j	22,769 g	16,841 f
Cocopeat × Hoagland A	12,168 h	10,323 f	23,521 i	26,383 fg	18,822 ef
Cocopeat × Hoagland	18,646 cd	18,030 a	32,766 f	40,354 c	28,919 ab
Cocopeat × OLWF	18,052 d	16,195 b	44,713 b	51,038 a	26,562 b
Z + C (1:1) × Hoagland A	19,396 bc	17,604 a	36,692 e	46,293 b	31,632 a
Z + C (1:1) × Hoagland	20,511 a	16,144 b	51,120 a	54,359 a	30,277 a
Z + C (1:1) × OLWF	13,248 fg	13,505 cd	25,890 h	27,989 f	20,799 de
Z + C (1:2) × Hoagland A	12,990 gh	11,568 ef	33,693 f	34,966 d	20,506 de
Z + C (1:2) × Hoagland	14,091 ef	13,390 cd	29,442 g	32,367 de	23,354 cd
Z + C (1:2) × OLWF	14,520 e	11,814 e	39,465 d	38,948 c	22,193 d
LSD 5%	940	1341	2234	3849	2889
<i>p-value</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Sources of variation	Histidine	Glycine	Thionine	Alanine	Tyrosine
Substrate					
Zeolite	1895 d	2190 b	5423 a	22,905 c	2724 a
Cocopeat	3454 b	2510 a	5598 a	26,921 b	2535 bc
Z + C (1:1) ^x	3752 a	2200 b	4870 b	30,365 a	2632 ab
Z + C (1:2)	3113 c	2560 a	5699 a	25,722 b	2455 c
LSD 5%	243	150	289	1855	138
<i>p-value</i>	<0.0001	<0.0001	<0.0001	<0.0001	0.0034
Fertilizer					
Hoagland A	2892 b	2710 a	6197 a	26,486 ab	2807 a
Hoagland	3149 a	2130 c	4904 b	27,826 a	2689 a
OLWF	3119 a	2260 b	5091 b	25,123 b	2264 b
LSD 5%	211	130	250	1607	120
<i>p-value</i>	0.073	<0.0001	<0.0001	0.0079	<0.0001
Interaction					
Zeolite × Hoagland A	2314 fg	141.2 e	4365 ef	29,162 cd	4232 a
Zeolite × Hoagland	1313 h	169.9 d	4589 e	20,585 fg	2817 c
Zeolite × OLWF	2059 g	346.6 ab	7314 bc	18,968 g	1124 g
Cocopeat × Hoagland A	2360 fg	367.8 a	7761 ab	20,839 fg	1900 f
Cocopeat × Hoagland	3648 c	157.1 de	3686 gh	28,825 cd	2623 cd
Cocopeat × OLWF	4355 b	227.4 c	5348 d	31,100 bc	3082 b
Z + C (1:1) × Hoagland A	3761 c	337.4 b	7120 c	32,508 b	2561 d
Z + C (1:1) × Hoagland	4904 a	150.8 de	3484 h	35,810 a	3072 b
Z + C (1:1) × OLWF	2592 f	170.7 d	4005 fg	22,776 f	2263 e
Z + C (1:2) × Hoagland A	3134 de	235.6 c	5541 d	23,435 ef	2535 d
Z + C (1:2) × Hoagland	2732 ef	372.2 a	7856 a	26,085 de	2243 e
Z + C (1:2) × OLWF	3472 cd	160.0 de	3699 gh	27,646 d	2589 cd
LSD 5%	422	260	501	3214	239
<i>p-value</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Sources of variation	Cysteine	Valine	Methionine	Tryptophan	Phenylalanine
Substrate					
Zeolite	3846 ab ^y	1526 b	6339 c	5409 c	7410 d
Cocopeat	3675 b	1728 a	7544 b	5845 b	9456 b
Z + C (1:1) ^x	3995 a	1892 a	8232 a	6663 a	10,707 a
Z + C (1:2)	3272 c	1805 a	6697 c	5886 b	8196 c
LSD 5%	177	170	599	329	595
<i>p-value</i>	<0.0001	0.0015	<0.0001	<0.0001	<0.0001
Fertilizer					
Hoagland A	3986 a	1818 a	7405 a	6213 a	9070 b
Hoagland	3822 b	1655 b	7501 a	6018 a	9796 a
OLWF	3283 c	1740 ab	6702 b	5621 b	7961 c
LSD 5%	153	147	519	285	515

Sources of variation	Cysteine	Valine	Methionine	Tryptophan	Phenylalanine
<i>p-value</i>	<0.0001	0.0930	0.0079	0.0010	<0.0001
Interaction					
Zeolite × Hoagland A	6100 a	2834 b	9659 b	8966 a	9836 de
Zeolite × Hoagland	3273 f	934 e	5259 f	4190 g	7250 gh
Zeolite × OLWF	2164 j	810 e	4099 g	3071 h	5146 i
Cocopeat × Hoagland A	2525 i	920 e	4934 fg	3689 g	6936 h
Cocopeat × Hoagland	3975 d	1410 d	8014 c	5578 e	11,157 bc
Cocopeat × OLWF	4523 c	2854 b	9685 b	8268 b	10,276 cd
Z + C (1:1) × Hoagland A	4098 d	1454 d	8261 c	6291 d	12,360 a
Z + C (1:1) × Hoagland	5093 b	3214 a	10,906 a	9520 a	11,623 ab
Z + C (1:1) × OLWF	2794 h ₁	1007 e	5528 f	4178 g	8139 fg
Z + C (1:2) × Hoagland A	3220 fg	2065 c	6766 de	5906 de	7150 gh
Z + C (1:2) × Hoagland	2945 gh	1062 e	5827 ef	4785 f	9157 ef
Z + C (1:2) × OLWF	3650 e	2288 c	7496 cd	6967 c	8285 f
LSD 5%	307	294	1038	571	1031
<i>p-value</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Sources of Variation	Isoleucine	Leucine	Lysine	Asparagine	Serine
Substrate					
Zeolite	4933 c	9161 c	7862 c	9618 a	16,332 a
Cocopeat	5582 ab	10,046 b	9003 b	7140 c	14,232 b
Z + C (1:1) ^x	6111 a	11,322 a	9860 a	8111 b	15,996 a
Z + C (1:2)	5119 bc	9917 bc	9350 ab	8500 b	14,284 b
LSD 5%	531	790	658	754	1060
<i>p-value</i>	0.0006	0.0001	<0.0001	<0.0001	0.0003
Fertilizer					
Hoagland A	5717 a	10,580 a	9411 a	9851 a	16,941 a
Hoagland	5528 a	10,270 a	8620 b	7332 b	15,112 b
OLWF	5064 b	9485 b	9024 ab	7844 b	13,580 c
LSD 5%	460	684	570	653	918
<i>p-value</i>	0.0214	0.0092	0.0297	<0.0001	<0.0001
Interaction					
Zeolite × Hoagland A	7633 a	14,380 ab	14,573 b	20,483 a	28,776 a
Zeolite × Hoagland	3996 ef	7216 de	4845 fg	5060 fg	12,623 fg
Zeolite × OLWF	3170 f	5889 e	4168 g	3310 h	7599 j
Cocopeat × Hoagland A	3672 ef	6456 e	4777 fg	3636 h	9376 ij
Cocopeat × Hoagland	5610 bc	10,072 c	7385 e	5030 fg	13,807 ef
Cocopeat × OLWF	7463 a	13,609 b	14,846 b	12,755 c	19,514 c
Z + C (1:1) × Hoagland A	6440 b	11,145 c	7614 e	5672 f	15,296 de
Z + C (1:1) × Hoagland	7999 a	15,692 a	16,718 a	14,686 b	22,072 b

Sources of Variation	Isoleucine	Leucine	Lysine	Asparagine	Serine
Z + C (1:1) × OLWF	3894 ef	7129 de	5247 fg	3974 gh	10,621 hi
Z + C (1:2) × Hoagland A	5122 cd	10,338 c	10,683 d	9611 e	14,315 ef
Z + C (1:2) × Hoagland	4507 de	8099 d	5531 f	4552 fgh	11,949 gh
Z + C (1:2) × OLWF	5728 bc	11,316 c	11,835 c	11,338 d	16,588 d
LSD 5%	919	1369	1140	1305	1836
<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^xZ + C: Zeolite+Cocopeat, OLWF: organic liquid worm fertilizer.
^yMean separation within columns by LSD multiple range test at 0.05 level.

Table 6.

The effect of different substrate and nutrient solution applications on amino acid content ($\mu\text{g kg}^{-1}$) of Early Cardinal berries.

acids gave the highest values. As can be seen in **Table 6**, substrate × fertilizer interaction was found to be significant for all amino acids.

Proline is reported in many works of literature as an amino acid whose synthesis is increased, especially under abiotic stress conditions such as drought [43, 71]. For this reason, we evaluated that the high increase in proline amino acid in Hoagland A and Hoagland nutrient solutions may be due to the lower amounts of some macro-(N) and microelements (Zn, Cu, Mn, Fe) in these solutions compared with OLWF nutrient solution (**Table 1**). Anjum et al. [72], Liang et al. [73], and Arabshahi and Mobasser [74] indicated that sensitive plants are less able to accumulate solutes, but increases in proline can be found in most organisms (including animals) following water stress [25, 43].

According to the Huang and Ough [29], Canoura et al. [43], Bouzas-Cid et al. [36, 47–49], Sánchez-Gómez et al. [41], Gutiérrez-Gamboa et al. [26, 42, 45, 46], Fernández-Novales et al. [75], and Wu et al. [44], amino acid contents of grape berries are affected by different variety, rootstock, location and fertilization, etc., viticultural practices. For instance, in the study by Gutiérrez-Gamboa et al. [26], the effect of foliar application of a seaweed extract to a Tempranillo Blanco variety on must and wine amino acids and ammonium content was determined. The results suggested that Tempranillo Blanco behaved as an arginine accumulator variety. Biostimulation after seaweed applications at a high dosage to the grapevines increased the concentration of several amino acids in the 2017 season while scarcely affecting their content in 2018.

In the another research by Gutiérrez-Gamboa et al. [46], results showed that of some elicitors and nitrogen foliar applications to Garnacha and Tempranillo grapevines decreased the must amino acid concentration. The treatments applied to Graciano grapevines affected the grape amino acid content. According to the percentage of variance attributable, the variety had a higher effect on the must amino acid composition than the treatments and their interaction. In the study by Fernández-Novales et al. [75], researchers have investigated the use of visible and near-infrared spectroscopy to estimate the grape amino acid content on whole berries of Grenache grape variety. Amino acid values ranged between 0.01 mg L^{-1} (Leucine) and 341 mg L^{-1} (Arginine). In their results, amino acid values obtained in our study varied from $1526 \mu\text{g kg}^{-1}$ (valine in zeolite) to $42,880 \mu\text{g kg}^{-1}$ (arginine in Z + C (1:1)).

These values were close to the values of valine (1.07 mg L^{-1}) given by Fernández-Novales et al. [75] for Grenache and arginine ($38.44\text{--}89.60 \text{ mg L}^{-1}$) given by Valdes

et al. [76] for Tempranillo berries. Arginine and proline amino acids were recorded as the most abundant amino acids in all media and nutrient solutions used in our experiment; valine, glycine, and tyrosine were determined as the amino acids with the lowest values. These results agree with Fernández Novales et al. [75] and Valdes et al. [76] that arginine and proline were also reported as the most abundant amino acids, both of the researches.

From the above statements, it has been concluded that grapes grown in soilless culture will not encounter a significant nutrient loss in terms of amino acids examined in this study. In our study, it has been evaluated that the Z + C (1:1) mixture substrate, which has the higher values for 14 amino acids, including proline as well as arginine, is remarkable in terms of nutrient saving.

4. Conclusions

According to the main results obtained from this study;

- In soilless culture cultivation of table grapes, it has been observed that zeolite and cocopeat media can be used alone, as well as a 1:1 mixture of Zeolite:Cocopeat, where the highest values are obtained.
- Hoagland and modified Hoagland nutrient solutions mostly gave higher values than OLWF for the properties studied. However, since OLWF did not have a significant negative effect, it was considered that it would be appropriate to continue working with this and similar solutions.
- Amino acid, vitamins, and mineral contents of grapes grown in soilless culture conditions were found to be close to the values given in the literature for grapes grown in open field.

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