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Mitigation of High Solar Irradiance and Heat Stress in Kiwifruit during Summer via the Use of Alleviating Products with Different Modes of Action—Part 2 Effects on Fruit Quality, Organoleptic, and Phytochemical Properties at Harvest and after Storage

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Abstract: In Greece, kiwifruit is grown in areas characterized by high temperatures during the summer months, with high solar radiation, especially during the period of shoot growth and fruit maturation. Therefore, the impact of heat stress is crucial. The objective of the present study was to evaluate the effect of pre-harvest alleviating products' application in the field on the yield and fruit quality attributes of 'Hayward' kiwifruit before and after storage. To achieve this, the osmoprotectant BlueStim (glycine betaine), the reflectant Purshade (calcium carbonate 62.5% *w*/*w*), and the antioxidant Sun Protect were applied by foliar spraying. Fruits produced under the influence of BlueStim exhibited high soluble sugars, total phenols, total flavanols, total flavonoids, and FRAP antioxidant capacity at harvest, while the production per vine increased by almost 17% compared to control. After storage, fruits produced from vines pre-harvest treated with BlueStim showed increased concentrations of soluble sugars, ascorbic acid, total organic acids, total flavonoids, and antioxidant capacity. Fruits produced from vines treated with Purshade presented high concentrations of soluble sugars, total phenols, FRAP and DPPH antioxidant capacity, total soluble solids, and malic acid, while Sun Protect application resulted in increased fruit firmness and total phenols as well. Therefore, the applied treatments alleviated, to some extent, the negative impact of heat stress on fruit quality, with variable effects on the measured quality parameters.

Keywords: antioxidant; bioactive compounds; calcium carbonate; glycine betaine; reflectant; tocopherol

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1. Introduction

Kiwifruit (*Actinidia* spp.) is perfectly acclimatized to wet and warm conditions [1], in frost-free areas characterized by abundant rainfall (reaching 1200–1800 mm year^{-1}), and moderate light intensity [2]. However, due to its economic value along with high agricultural industry demands, kiwifruit cultivation has been expanded in the last decades in the Mediterranean basin, which is less well-suited to its particular requirements. The Mediterranean-type climate is characterized by low rainfall, excessive heat load during the summer months, and high daily radiation levels during the growing season [3]. These specific environmental conditions, which all exhibit additive and interactive restricting effects on kiwifruit physiology, impose a negative impact on both productivity and fruit quality [4,5].

Climatic factors and especially temperature determine fruit quality in various plant species. The previous studies of Greer [6], Hopkirk et al. [7], and Seager et al. [8] suggested that high temperatures during flowering and fruit development affect the kiwifruit vegetative growth and fruit quality. Excessive heat during fruit maturation reduced the fruit's dry mass and soluble solids levels, the starch–sugar conversion and fruit ripening

were delayed, and the flesh color was lighter [7,9]. Furthermore, high temperature during starch accumulation resulted in increased fruit firmness and acidity at harvest, while it dramatically reduced the carbohydrate and vitamin C content. In the following season, growth and flowering were also severely reduced. On the other hand, exposing vines to high temperatures during fruit cell division had minimal long-term effects [9].

Richardson et al. [9] found that high temperatures enhanced the translocation of carbohydrates from fruits, meristems, and perennial organs toward excessive vegetative growth. Consequently, the growth performance in the following season is negatively affected. Concerning carbohydrates, the same authors showed that fruit inositol concentration increased in vines subjected to elevated temperatures during fruit starch accumulation (50–120 days after anthesis) [9]. Furthermore, fruit sucrose and hexose concentrations were reduced in response to increased temperature, regardless of the developmental stage. On the other hand, heat load not only affected fruit commercial maturity and quality attributes, but also influenced postharvest storage behavior [7], and consequently, marketable fruit quality.

Considering that in Greece kiwifruit cultivation is located in areas characterized by high solar irradiance, accompanied by high summer temperatures, where shoot and fruit growth occur, the subject is even more crucial. Kiwifruit vines are subjected, especially during the summer months, to severe heat stress, thus causing several physiological changes in plants [10,11], which consequently impose a negative impact on fruit quality characteristics, both at harvest and after storage. Modern agriculture aims at the production of the highest possible yields of products rich in bioactive compounds with proven functional properties, which are all jeopardized under such harsh conditions.

In response to these challenges, the use of tolerant cultivars has been the proposed solution [12]. Karavolias et al. [13] proposed the use of gene editing, as a prominent new approach, to help plants tolerate the effects of climate change in agriculture, a method that can be individually implemented or in combination with other climate-smart solutions. The use of biotechnological tools and the prediction of extreme weather events can also be used to implement the most effective cultivation techniques [12]. However, plant breeding to create new cultivars or improve old ones in fruit trees is time demanding, compared to annual species. Furthermore, genetically modified plants are not eligible for cultivation in all countries [14]. In contrast, the study and the understanding of the physiological and biochemical responses of plants to any external stimulus constitutes an important tool for the implementation of cultivation practices that improve plant tolerance against these stimuli. To tolerate stressful conditions, plants may have to alter their metabolism to accumulate various compounds acting as osmolytes (sugar alcohols, proline, glycine betaine, etc.) or synthesize tocopherols, phenolic compounds, ascorbic acid, etc., which act as antioxidant agents to avoid oxidative damage [15]. Therefore, the adaptation of innovative cultivation practices for the short-term alleviation of the negative effects of abiotic stress factors is the most efficient and immediate solution.

Various amelioration agents have been used to mitigate the negative impacts of abiotic stresses on plants. Among those, glycine betaine has been effectively used in many plant species such as tomatoes, sugarcane, marigolds, olives, etc. [16,17], as an alleviating agent against heat and high irradiance stress. It has been found to improve stomatal conductance, photosynthesis, chlorophyll content, and plant water status [16,18,19], to reduce DNAse and RNAse activities [20], and to enhance soluble sugar accumulation, phenolic biosynthesis in leaves and fruits, as well as yield parameters [16,21].

Particle film technology is a relatively new approach to counteract the negative impacts of heat stress [22]. The application of particle films has been associated with photoprotective effects and a significant increase in photosynthetic rate, stomatal conductance, and in transpiration, while, at the same time, it reduces the leaf heat load under stress conditions [22–25]. Foliar application of calcium-based films, such as calcium carbonate, has been reported to improve plant performance and alleviate environmental stress [24,25]. In mandarins, it improved the fruit weight and diameter, the juice percentage, total soluble solids (TSS), ascorbic acid, total antioxidants, total phenolics, total flavonoids, and carotenoids

contents [26]. At the same time, it also enhanced the antioxidant enzymes' activity and free radical scavengers, and consequently, increased plant tolerance and improved yield in wheat [27].

Antioxidant compounds are also another approach by which to mitigate the negative impacts of abiotic stress. α-Tocopherol (vitamin E), a lipophilic antioxidant, is involved in different physiological processes, including plant growth and development, membranes stabilization, reactive oxygen species (ROS) scavenging, protection of photosystem II (PSII) from photoinactivation, and membrane lipids from photooxidation [28,29]. It has been found to contribute to plant stress tolerance [30], while decreased levels of α-tocopherol favor oxidative damage [31].

Taking these into account, using products with different modes of action, such as an osmolyte (glycine betaine), a reflectant (calcium carbonate), and an antioxidant (tocopherol), can be a potential solution for reducing the negative effects of heat load and high solar irradiance in kiwifruit vines. The objective of the present study was to evaluate the impact of the foliar application of the aforementioned compounds on the quality traits (physiological, organoleptic, and functional characteristics) and yield parameters of 'Hayward' kiwifruit cultivar, the most important green-flesh kiwifruit cultivar in the world. Furthermore, as several changes take place during the postharvest ripening of the fruit, i.e., the modification of the cell wall and softening of the fruit, the conversion of starch to sugars, etc. [32], it was deemed necessary to examine how these pre-harvest applications may affect the marketable fruit quality after the storage period too.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experiment was conducted during two successive years, i.e., in 2018 and 2019, in a 5-hectare kiwifruit orchard located in Agrinio county, (Neapolis village, 38°38′04.1″ N 21°19′08.8″ E), Western Greece. During the first year, measurements were taken at harvest regarding fruit yield per vine, fruit weight, total soluble solids concentration and firmness, and the same (apart from yield) after storage (data not presented). Based on these results, the trial was repeated in 2019 including the analyses of fruit phytochemicals, which are presented here. A 15-year-old orchard of kiwifruit plants of cv. 'Hayward' with a trunk height of 1.8 m, trained as a pergola and planted at distances of 2.0 × 4 m was selected as the trial site. The soil is classified as Fluvisol, and was characterized as loam, with 7.25 pH, 1.76% *w*/*w* organic matter, 0.310 mS cm^{-1} electrical conductivity, and 3.05% *w*/*w* $CaCO_3$. The mean temperature, as well as the highest temperature recorded per month of the 2019 growth period, were as follows: 26.3 °C and 37.8 °C in June, 27.5 °C and 38.3 °C in July, 28.8 °C and 38.8 °C in August, 24.4 °C and 34.1 °C in September, and 19.9 °C and 30.9 °C in October, respectively (data collected by a nearby station). Approximately 83 mm of precipitation occurred during the trial period (from first spray application till harvest). The climate of the area is characterized as a warm temperate climate with a dry, hot summer (Csa) based on Köppen climate classification. The applied cultivation practices (irrigation, fertilizing, pruning, weeding, spraying against fungi and pests) were the same for all the vines of the orchard.

The experiment was arranged as a completely randomized design with four vines of four replications per treatment (a total of 16 vines per treatment, 64 vines in total), where only the two central vines were used, with the two vines at the edges serving as a buffer zone.

2.2. Treatments

A total of three alleviating products were examined during the two-year experimentation period (data presented here are from the 2019 period), to study their effect on the physiological and phytochemical properties of the fruits. Three applications were performed for all treatments on 4 July, 6 August, and 22 August during the first year and on 4 July, 7 August, and 21 August during the second year (the data presented hereafter).

Treatments comprised of the untreated control and the foliar spray application of (a) the osmoprotectant BlueStim 50% SP (glycine betaine, Hellafarm S.A., Paiania, Greece) (at a dose rate of 600 g 100 L^{-1} water), (b) the reflective limestone Purshade (calcium carbonate 62.5% *w*/*w*, Hellafarm S.A., Paiania, Greece) (at a dose rate of 3 L 1000 L^{-1} water), and (c) the antioxidant commercial product Sun Protect (a mixture of UV-absorbing compounds such as α-tocopherol, phenolic acids (ferulic acid), and boron) (at a dose rate of 120 mL 100 L^{-1} water). In all solutions, an adjuvant was added at a dose rate of 10 mL 100 L^{-1} to improve the efficiency of spray application. Approximately 0.90 L of the spray solution of the aforementioned alleviating products was applied per vine. All applications were performed based on the good agricultural practices' guidelines.

2.3. Sampling and Physiological Properties Determination

In late October 2019, all fruits per vine at commercial maturity index were harvested and the yield per vine was measured with a commercial balance at the field. Then, at least 25 fruits per plot were randomly sampled from the harvested fruits, placed into labeled plastic bags, and immediately transferred via a portable freezer to the laboratory for further analysis. At the laboratory, the weight of each fruit along with its diameter and length were determined with an electronic balance (Kern 470, Kern and Sohn, Ziegelei 1, 72336 Balingen, GmbH, Germany) and a digital caliper (Starrett, 727 Series, Athol, New England, Massachusetts, United States). Firmness was measured at the two opposite sides of each fruit with a penetrometer with a conical tip, after peeling a small part of the fruit skin using a sharp knife (Turoni 53205 fruit pressure tester) (T.R. Turoni srl, via Copernico, 26, 47122 Forlì (FC), Italy). The dry matter percentage of eight fruits per plot was determined after drying in an oven at 70 °C to constant weight. The rest of the fruits were peeled and homogenized in a household homogenizer. The pulp was then placed in 50 mL tubes and stored in a freezer at −25 °C until the biochemical analyses.

The rest of the harvested fruits were stored in the cold rooms of the Agricultural Co-operative of Agrinio 'AC Neapolis' under 0.5 °C and 95% humidity for approximately 107 days. A sample of these fruits was then sent to the Laboratory of Pomology at the Agricultural University of Athens. On at least 100 fruits per treatment, randomly collected, the physiological properties, firmness, and dry matter percentage were measured. Finally, fruits were homogenized as previously described to study the post-storage fruit quality characteristics, as affected by the application of the alleviating products.

2.4. Determination of Organoleptic Characteristics

Total soluble solids (TSS), total titratable acidity (TA), and pH were determined in kiwifruit juice as described by Roussos et al. [11]. TSS was expressed as °Brix and TA as % *w*/*v* of citric acid in the juice.

2.5. Soluble Sugars Determination

The frozen pulp (2 g) was extracted twice with 4 mL of HPLC-grade water in a microwave according to the method of Roussos et al. [33]. Sucrose, glucose, fructose, and inositol separation was conducted in a Waters 510 isocratic pump (Waters, 34 Maple St, Milford, MA 01757, United States) with a refractive index detector (Hewlett Packard HP1047A) (Agilent, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, United States), in an Adamas Amino 5μ column (250 × 4.6 mm) (Sepachrom, Via Trento, 33, 20017 Rho MI, Italy), equilibrated at 35 °C, with acetonitrile: water (80:20) as a mobile phase, at a flow rate 1.0 mL min^{-1}. Total sugar concentrations were estimated by summing the concentrations of the individual sugars detected by HPLC. Each sample was analyzed twice and the final concentrations were expressed as g 100 g^{-1} fresh weight.

The sweetness index (SI) of the fruit was calculated as:

SI = 1.00 × (glucose concentration) + 1.35 × (sucrose concentration) + 2.3 × (fructose concentration) + 0.685 × (inositol concentration) [11].

2.6. Organic Acids Determination

Organic acids were analyzed by HPLC (Shimadzu Nexera X2) equipped with a diode array detector (DAD) (SPDM20A, Shimadzu, Kyoto, Japan) after the frozen pulp was extracted twice with 3% w/v of meta-phosphoric acid in water as described by Roussos et al. [11]. Three organic acids (citric, malic, and ascorbic acid) were isocratically identified by the DAD detector at 200 nm, with 0.02% (v/v) formic acid in water as a mobile phase, at a flow rate of 1.0 mL min^{-1}, in a Kinetex C18 EVO column (250 mm 4.6 mm). The total organic acid concentration was calculated by summing the concentrations of the individual acids and was expressed as g 100 g^{-1} fresh weight.

2.7. Phenolic Compounds and Antioxidant Capacity Determination

Phenolic compounds were extracted from the frozen pulp according to Roussos et al. [11]. Total phenols, total *o*-diphenols, total flavanols, and total flavonoids were determined based on the method of Roussos et al. [11] in the methanolic supernatants. The results were expressed as mg equivalent gallic acid (GAE), caffeic acid (CAE), catechin (CtE), and CAE per g fresh weight (FW), respectively.

The antioxidant capacity was determined based on the ABTS 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and FRAP (Ferric Reducing Antioxidant Power) assays as described by Roussos et al. [11] in the same methanolic fraction as phenolic compounds, and were expressed in μmol Trolox equivalents (TE) per g fresh weight.

2.8. Statistical Analysis

Data analysis was performed using the statistical software Statgraphics Centurion XV (Statgraphics Technologies, Inc., The Plains, VA, USA) and JMP13 (SAS Institute, Cary, North Carolina, United States). Data were evaluated as one-way ANOVA and significant differences were determined according to Tukey's HSD test at $\alpha = 0.05$. Principal component analysis (PCA) after varimax rotation was also performed to describe the effects of the alleviation products on fruit quality attributes, by a reduced number of factors, separately at harvest and after the storage period.

3. Results

3.1. Effect of Alleviating Products on Fruit Physiological, Quality, and Organoleptic Characteristics

Sun Protect application significantly increased mean fruit weight at harvest, followed by the control (Table 1). On the other hand, BlueStim application resulted in the lowest mean fruit diameter and length (Table 1). Fruit firmness, as well as dry matter percentage, did not exhibit any difference due to alleviating product applications. The total yield per vine was significantly enhanced under the use of BlueStim with control treatment, resulting in a significantly lower yield. It is noteworthy that BlueStim treatment resulted in an almost 16.9% higher production per vine when compared with the control treatment, while Purshade and Sun Protect increased the production by about 7.9% and 9.3%, respectively. After storage, no differences were detected concerning fruit biometric parameters and dry matter percentage (Table 2). On the other hand, fruit firmness exhibited the highest value under the Sun Protect application, followed by Purshade.

Table 1. Alleviating products' application effect on kiwifruit mean weight (g), diameter (mm), length (mm), firmness (N), dry matter percentage (%), and total yield per vine (Kg) at harvest.

Treatment	Weight	Diameter	Length	Firmness	Dry Matter	Total Yield/Vine
	***	***	***	ns	ns	***
Control	131.47 ab	57.66 a	75.96 a	27.94 a	16.67 a	39.69 b
BlueStim	117.91 c	54.97 b	72.69 b	28.94 a	16.19 a	46.38 a
Purshade	124.86 bc	56.95 a	73.68 b	26.52 a	16.23 a	42.81 ab
Sun Protect	137.78 a	57.93 a	76.91 a	30.15 a	16.28 a	43.38 ab

Means within the same column followed by the same letter do not significantly differ according to Tukey's HSD multiple range test at $\alpha = 0.05$. Abbreviations: ns, not significant; ***, $p < 0.001$.

Table 2. Alleviating products' application effect on kiwifruit mean weight (g), diameter (mm), length (mm), firmness (N), and dry matter percentage (%) after storage at 0.5 °C for 107 days.

Treatment	Weight	Diameter	Length	Firmness	Dry Matter
	ns	ns	ns	**	ns
Control	124.13 a	55.55 a	74.20 a	11.81 b	16.95 a
BlueStim	112.90 a	54.08 a	72.71 a	11.86 b	17.28 a
Purshade	118.98 a	55.84 a	72.37 a	13.01 ab	17.26 a
Sun Protect	135.18 a	56.20 a	72.23 a	15.71 a	17.08 a

Means within the same column followed by the same letter do not significantly differ according to Tukey's HSD multiple range test at $\alpha = 0.05$. Abbreviations: ns, not significant; **, $p < 0.01$.

The organoleptic characteristics of the fruits (pH value, TA, and TSS:TA ratio) did not differ among treatments both at harvest and after storage, as indicated in Tables 3 and 4. However, at harvest, TSS content was found to be lower in fruits from vines treated with BlueStim (Table 3) and highest under the control, while the other treatments did not significantly differ.

Table 3. Alleviating products' effect on kiwifruit juice pH, total soluble solids (TSS) (°Brix), titratable acidity (TA) (g citric acid 100 g^{-1} fresh weight), and the ratio of total soluble solids: titratable acidity (TSS:TA) at harvest.

Treatment	pH	TSS	TA	TSS:TA
	ns	**	ns	ns
Control	3.48 a	7.16 a	2.40 a	3.09 a
BlueStim	3.39 a	6.55 b	2.22 a	2.96 a
Purshade	3.42 a	6.85 ab	2.60 a	2.72 a
Sun Protect	3.53 a	6.75 ab	2.21 a	3.06 a

Means within the same column followed by the same letter do not significantly differ according to Tukey's HSD multiple range test at $\alpha = 0.05$. Abbreviations: ns, not significant; **, $p < 0.01$.

Table 4. Alleviating products' effect on kiwifruit juice pH, total soluble solids (TSS) (°Brix), titratable acidity (TA) (g citric acid 100 g^{-1} fresh weight), and the ratio of total soluble solids: titratable acidity (TSS:TA) after storage at 0.5 °C for 107 days.

Treatment	pH	TSS	TA	TSS:TA
	ns	ns	ns	ns
Control	3.39 a	13.98 a	1.90 a	7.47 a
BlueStim	3.24 a	13.60 a	1.71 a	7.95 a
Purshade	3.33 a	14.08 a	1.82 a	7.77 a
Sun Protect	3.26 a	14.10 a	1.87 a	7.58 a

Means within the same column followed by the same letter do not significantly differ according to Tukey's HSD multiple range test at $\alpha = 0.05$. Abbreviations: ns, not significant.

3.2. Effect of Alleviating Products on Kiwifruit Soluble Sugars and Organic Acids

The major carbohydrate found in fruits at harvest was fructose followed by glucose, sucrose, and inositol, in that order (Figure 1). Fruits from vines treated with BlueStim presented high glucose, sucrose, and fructose concentrations at harvest (Figure 1A–C). Furthermore, both BlueStim and Purshade enhanced total sugar concentration (Figure 1D,E), while no significant difference among treatments was detected concerning the fruit sweetness index at harvest. After the storage period, glucose and fructose concentrations were higher in the fruits from vines treated with BlueStim (Figure 1A,B), while the concentrations of sucrose, inositol, and total sugars were higher in the fruits of BlueStim and Purshade treatments compared to the control and Sun Protect treatments (Figure 1C–E).

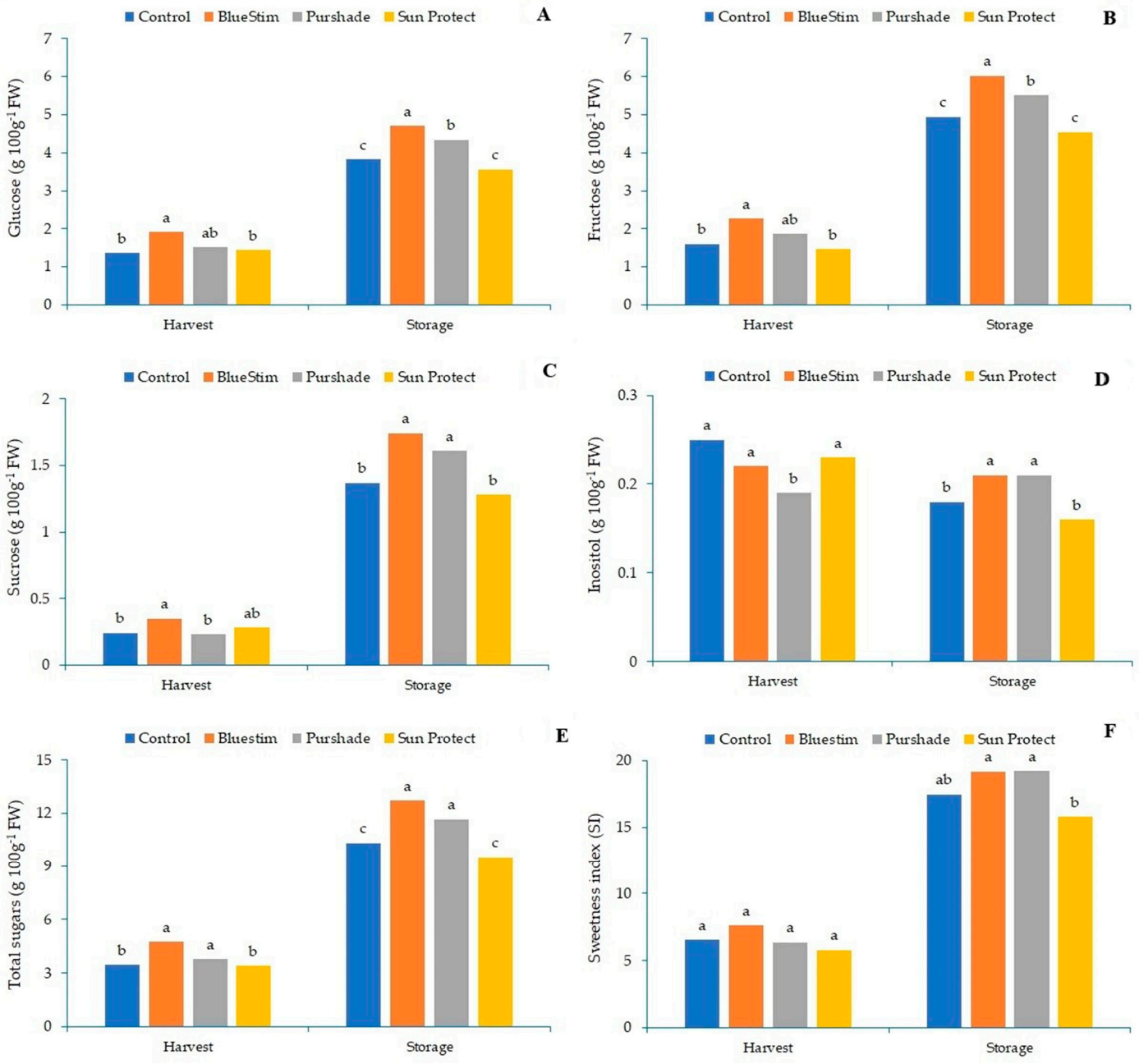

Figure 1. Effect of alleviating products on kiwifruit soluble sugars concentration ((**A**), glucose, (**B**), fructose, (**C**), sucrose, (**D**), inositol, (**E**), total sugars) and sweetness index (**F**) at harvest and after storage at 0.5 °C for 107 days. Treatment columns with the same lower case letter above, within the same production event (harvest or storage), do not significantly differ according to Tukey's HSD multiple range test at $\alpha = 0.05$.

There were no significant differences among treatments regarding fruits' organic acids concentration at harvest (Figure 2). On the other hand, after storage, malic acid concentration was highest under the influence of Purshade and Sun Protect (Figure 2A), while citric acid did not exhibit any significant change due to alleviating product application (Figure 2B). Furthermore, fruits from the BlueStim-treated vines exhibited the highest concentration of ascorbic acid (Figure 2C) and total organic acids after storage, along with Sun Protect (Figure 2D), while the lowest amount of total organic acids was detected in the fruits of the control plants.

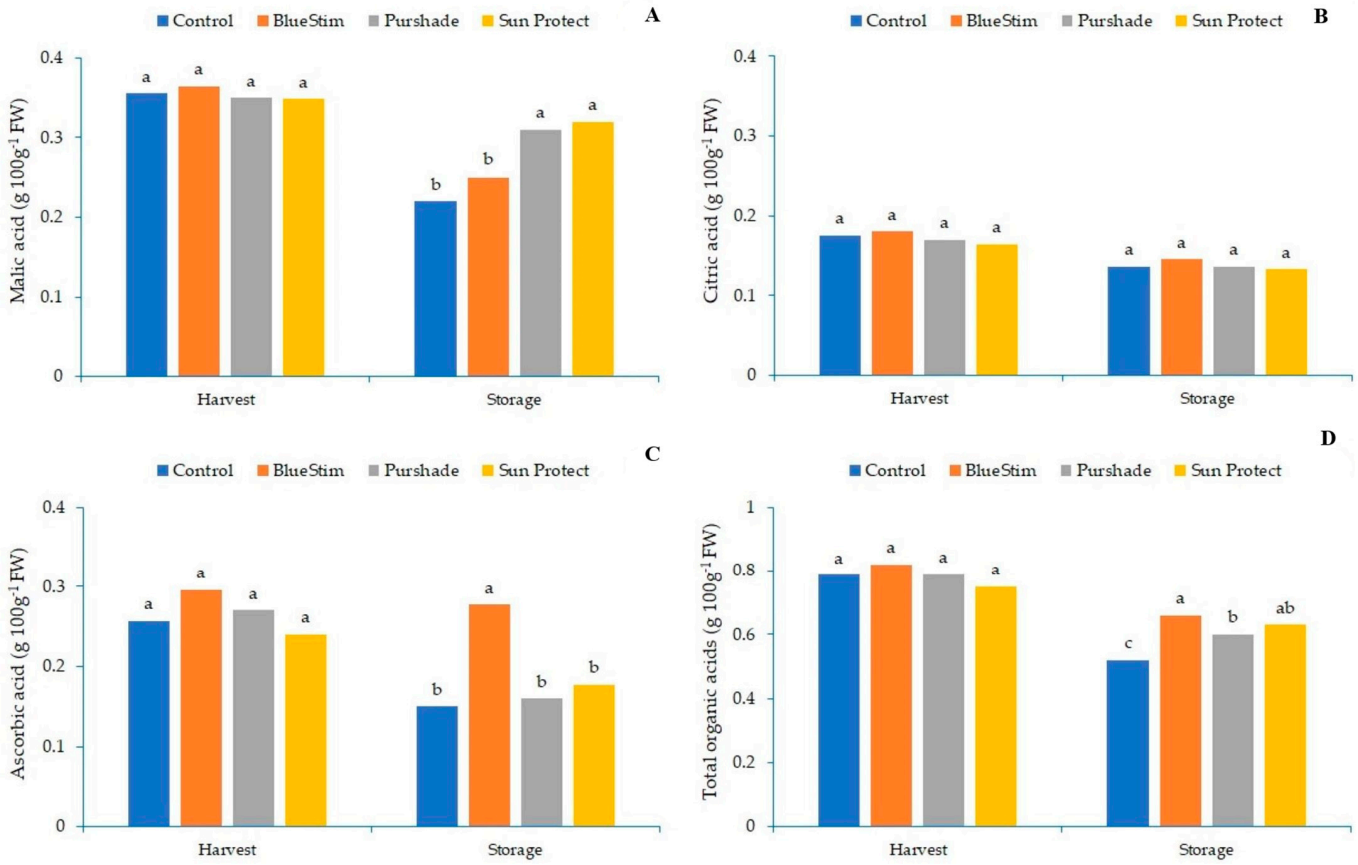

Figure 2. Effect of alleviating products on kiwifruit organic acids concentration ((**A**), malic acid, (**B**), citric acid, (**C**), ascorbic acid and (**D**), total organic acids) at harvest and after storage at 0.5 °C for 107 days. Treatment columns with the same lower case letter above, within the same production event (harvest or storage), do not significantly differ according to Tukey's HSD multiple range test at $\alpha = 0.05$.

3.3. Effect of Alleviating Products on Kiwifruit Phenolic Compounds and Antioxidant Capacity

At harvest, the application of BlueStim and Sun Protect resulted in the highest concentration of total phenols, followed by Purshade (Figure 3A), while total o-diphenols concentration did not differ among treatments (Figure 3B). Fruits from vines treated with BlueStim presented the highest total flavanols concentration among treatments (Figure 3C). Furthermore, the application of the alleviating products resulted in a higher concentration of total flavonoids in the fruits (Figure 3D) than the control treatment. After storage, the pre-harvest application of Sun Protect significantly increased the fruits' total phenols concentration compared to other treatments (Figure 3A). On the other hand, the application of the alleviating products had no significant effect on the total o-diphenols, total flavanols, and total flavonoids concentration (Figure 3B–D).

The antioxidant capacity of the fruit was significantly affected by the various treatments, both at harvest and after storage (Table 5). More specifically, fruits derived from the vines treated with Purshade presented the highest antioxidant capacity (by all assays), both at harvest and after storage, compared to both control and Sun Protect treatments. BlueStim application, on the other hand, also exhibited increased FRAP antioxidant capacity at harvest, while Sun-Protect-treated fruits exhibited the lowest antioxidant capacity throughout the experimentation period.

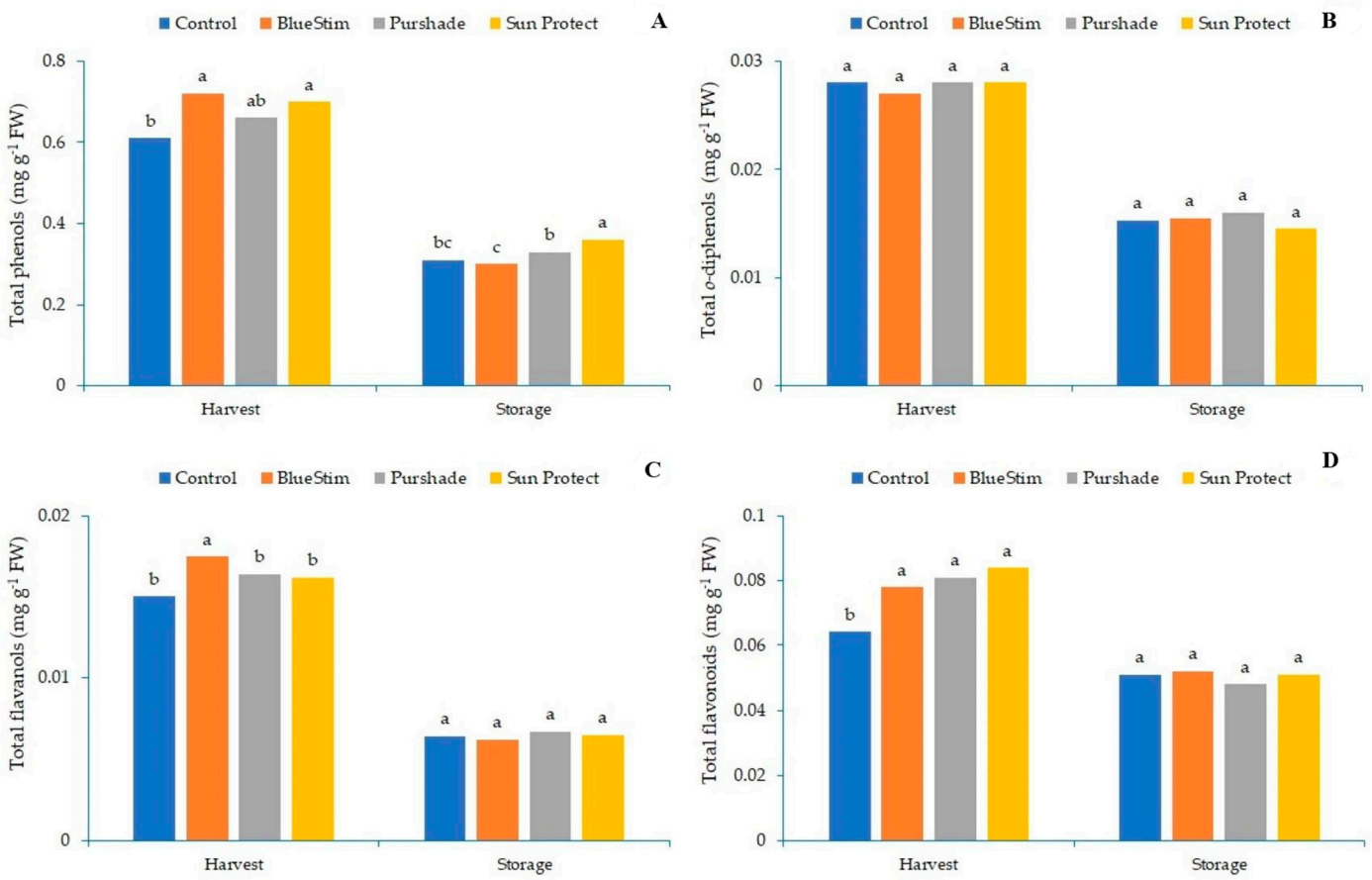

Figure 3. Effect of alleviating products on kiwifruit phenolic compounds concentration ((**A**), total phenols, (**B**), total o-diphenols, (**C**), total flavanols and (**D**), total flavonoids) at harvest and after storage at 0.5 °C for 107 days. Treatment columns with the same letter above, within the same production event (harvest or storage), do not significantly differ according to Tukey's HSD multiple range test at $\alpha = 0.05$.

Table 5. Alleviating products' effect on kiwifruit antioxidant capacity (μmol equiv. Trolox g^{-1} fresh weight) at harvest and after storage at 0.5 °C for 107 days.

Treatment	Harvest			Storage		
	FRAP	DPPH	ABTS	FRAP	DPPH	ABTS
	***	***	***	*	*	*
Control	4.40 c	2.90 b	6.43 b	1.69 b	1.44 b	3.32 b
BlueStim	5.32 a	3.01 b	6.75 b	1.99 ab	1.64 ab	3.53 ab
Purshade	5.61 a	3.31 a	7.70 a	2.10 a	1.96 a	3.75 a
Sun Protect	4.88 b	2.89 b	6.40 b	1.72 b	1.57 b	3.41 b

Means within the same column followed by the same letter do not significantly differ according to Tukey's HSD multiple range test at $\alpha = 0.05$. Abbreviations: ABTS, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, Ferric Reducing Antioxidant Power; *, $p < 0.05$; ***, $p < 0.001$.

3.4. Overall Effects of Alleviating Products Treatments on Kiwifruit Quality Characteristics and Biochemical Compounds

PCA was used to examine the overall effects of the alleviating products on the measured parameters. Eight components were produced, both during harvest and after the storage period, with eigenvalue above 1.0. The first two principal components (PC) described almost 45% of the variability of the original data both at harvest and after storage. At harvest, PC1 was comprised of glucose, fructose, total sugars, and total flavanols concentration, while PC2 was comprised of total organic acids concentration. Fruits produced

under the influence of BlueStim were located at the right side of PC1 (Figure 4), characterized by high values of glucose, fructose, total sugars, and total flavanols concentration, while fruits from the control and Sun Protect treatments were located at the negative side of PC1, clearly separated from those of BlueStim.

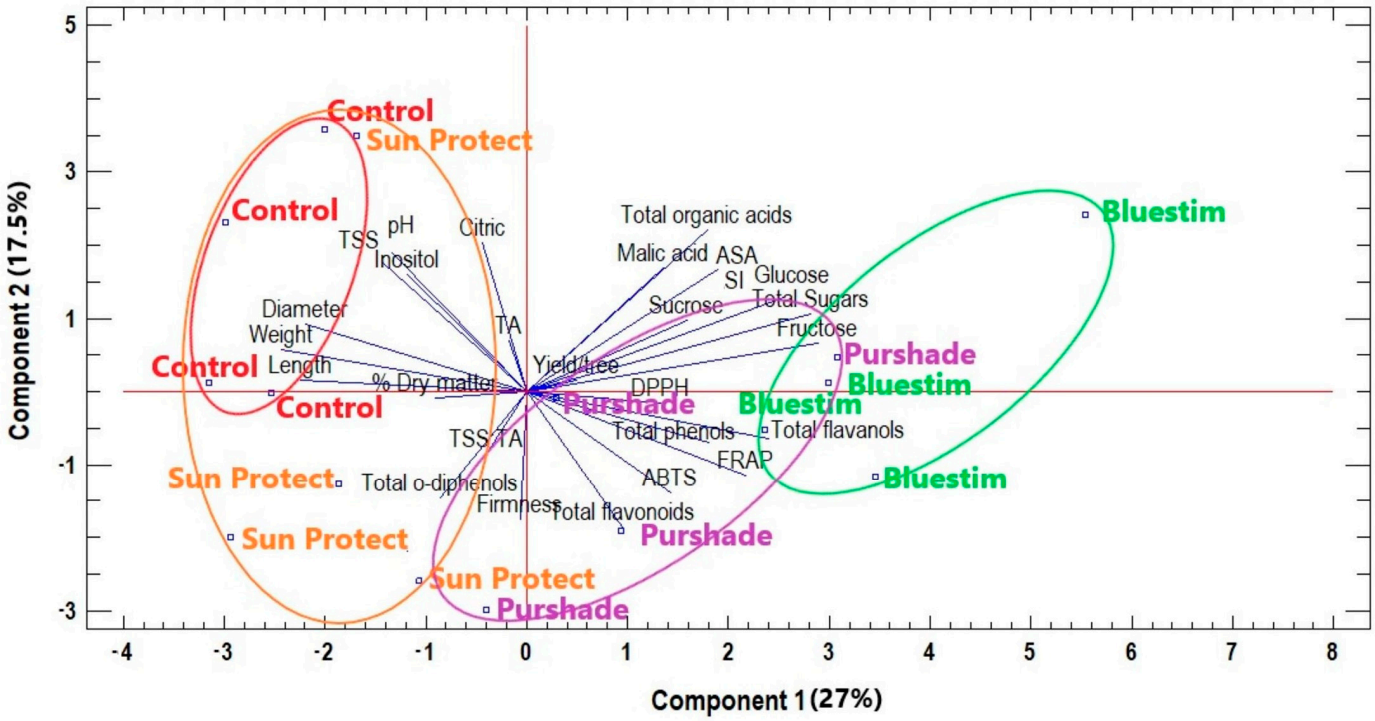

Figure 4. Biplot of the principal components analysis of data collected at harvest. Abbreviations: ABTS, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); ASA, ascorbic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, Ferric Reducing Antioxidant Power; SI, sweetness index; TA, titratable acidity; TSS, total soluble solids; TSS:TA, ratio of total soluble solids:titratable acidity.

After storage, PC1 was comprised of fructose, glucose, sucrose, inositol, and total sugars concentration, while PC2 was comprised of total phenols concentration, FRAP and DPPH antioxidant capacity, total soluble solids, and malic acid concentration. Fruits produced under BlueStim influence were located at the positive side of PC1 and the negative side of PC2, indicating high concentrations of fructose, glucose, sucrose, inositol, and total sugars, and low values of total phenols concentration, FRAP and DPPH antioxidant capacity, total soluble solids, and malic acid concentration (Figure 5). Fruits produced under the influence of Purshade were located at the positive side of both PC1 and PC2, indicating that they were characterized by high concentrations of fructose, glucose, sucrose, inositol, total sugars, total phenols concentration, FRAP and DPPH antioxidant capacity, as well as TSS and malic acid concentrations. Fruits from the control treatment and Sun Protect shared a common area at the negative side of PC1, clearly separated from the fruits produced under BlueStim and Purshade influence.

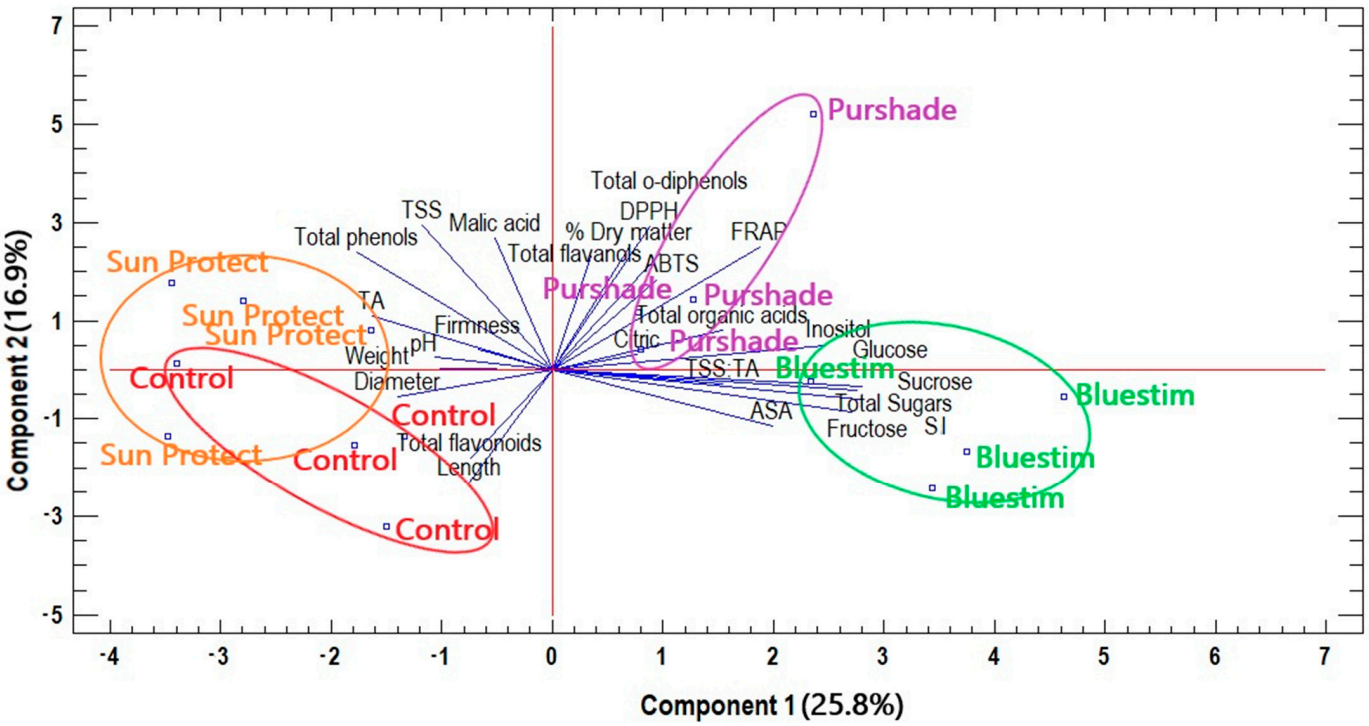

Figure 5. Biplot of the principal components analysis of data collected after storage at 0.5 °C for 107 days. Abbreviations: ABTS, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); ASA, ascorbic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, Ferric Reducing Antioxidant Power; SI, sweetness index; TA, titratable acidity; TSS, total soluble solids; TSS:TA, the ratio of total soluble solids: titratable acidity.

4. Discussion

During the last decade, climate change has been increasingly experienced as extreme weather conditions have become more frequent. Perennial plants and, in this specific trial, the kiwifruit vine, as sessile organisms, are not able to escape these extreme conditions, occurring throughout the growing season. High temperatures and solar irradiance, especially during the summer months, can negatively affect plant growth and physiology, causing various physiological and biochemical alternations [11]. These changes may result in growth retardation, yield loss, and low fruit quality, affecting its organoleptic and phytochemical characteristics, and the perceived quality at harvest, or even after the storage period. To alleviate the effects of environmental stresses on both fruits and plants, ameliorating agents, such as glycine betaine, particle films, polyamines, silicon, salicylic acid, and jacmonic acid, etc., have been used with various efficacy [34].

PCA analysis of the raw data indicated that the applied treatments mitigated, to some extent, the negative impact of heat stress on several fruit quality attributes, with different effects on the measured parameters. Sun Protect treatment resulted in increased mean fruit weight at harvest, without significant difference from the control treatment, but with better performance than BlueStim and Purshade. Similar results have been also reported for apple fruits treated with Sun Protect compared to the control treatment [35]. However, the vines treated with BlueStim exhibited the highest yield, almost 17% higher compared to the control treatment. Previous studies have also shown that glycine betaine (BlueStim) application can improve fruit yields under heat stress in various species [36–39]. It has been also reported that under stress conditions, glycine betaine maintains the proper turgor pressure in plants and prevents water loss through the leaves, which can lead to increased growth and yield as well [39].

Sawicki et al. [40] reported that stress conditions caused a premature fruit drop during development, therefore resulting in a decreased number of fruits on woody plants at

harvest. According to Wang et al. [4], premature kiwifruit drop can occur under hot summer conditions. Considering the mean yield per vine and the mean fruit weight, it is obvious that BlueStim application alleviated the heat stress impacts by increasing the overall number of fruits per vine (probably by inhibiting the premature drop of fruits due to heat stress). However, the increased yield observed was accompanied by a decrease in the mean fruit weight and size. Several authors have found that crop load and fruit growth are negatively correlated in several species [11,41] due to the limitations imposed on the assimilates' distribution among fruits. This lower supply of fruits with assimilates can cause size reduction, as has been reported in the present study as well.

Apart from fruit size and vine yield, dry matter accumulation, firmness, TSS, and organic acids are some of the primary factors determining fruit quality and storability [42]. In the present study, the dry matter was beyond the lower allowable limit for harvest (above 15%) and did not differ among treatments. This was also found regarding fruit firmness at harvest. The kiwifruits were harvested at the commercial maturity stage when the TSS content reached the minimum marketing value at harvest, that of 6.2 °Brix [43]. Therefore, kiwifruits of the control treatment exhibited the highest TSS content, while the lowest content was detected in fruits of vines treated with BlueStim. Similar results have been reported for kiwifruit [11] and other species, such as apples [44], peaches [45], and citrus [46], under stress-alleviating treatments. Xu et al. [42] suggested that the reported increased fruit load resulted in lower TSS content of fruits, as was also the case for the BlueStim application in the present trial. However, it is important to consider that TSS measures a group of substances such as organic acids, soluble amino acids, sugars, minerals, and others [47]. As total sugars concentration increased under BlueStim treatment and organic acids levels remained unchanged, it is safe to assume that carbohydrates are not the reason for the lower TSS levels determined under Bluestim influence. Thus, the lower TSS levels of fruits treated with BlueStim could be ascribed to a decrease in components other than sugars and organic acids found in the fruits.

During storage, TSS content was doubled, reaching on average 14 °Brix, which is generally considered acceptable by consumers [48], without any significant difference among treatments. According to Rivera-López et al. [49], Tavarini et al. [50], and Mahmoudi et al. [51], the detected increase in TSS during the storage period could be attributed to the starch breakdown into soluble sugars, hydrolysis of cell wall polysaccharides, and increased activity of sucrose–phosphate synthase leading to sucrose biosynthesis. TA and pH of fruit juice exhibited a small reduction during storage. Similarly, fruit firmness decreased by about 50%. Notably, the pre-harvest application of Sun Protect resulted in the highest fruit firmness after storage. Therefore, it is fair to assume that the antioxidant properties of Sun Protect (a-tocopherol and phenolic acids) possibly delayed the oxidation process in fruits, preventing the degradation of cell membranes and maintaining their integrity, thus preserving the fruit's texture, as well as the firmness loss.

In a previous study by Ntanos et al. [47], the beneficial effects of BlueStim on enhancing the photosynthetic activity under heat stress conditions in kiwifruit vines were highlighted. The same authors supported the assumption that, in many plants, glycine betaine enhances the partitioning of photosynthetic products towards reproductive and growing organs [52,53], supporting their growth with energy and carbon skeletons. These earlier findings could justify the increased soluble sugars (except for inositol) and total sugars detected in the present study under the BlueStim application, demonstrating once again its role in mitigating the heat stress impact.

Concerning carbohydrate metabolism after storage, a remarkable increase was observed in soluble sugars and total sugars concentration (Supplementary Materials), as well as in the sweetness index. The postharvest carbohydrate metabolism of kiwifruit is a topic of great interest since, during ripening, the starch content decreases and the levels of sucrose and hexoses increase [54]. By the time the fruit is edible, the starch has been depleted and the sugar content has approximately fivefold compared to that determined at harvest [55]. That was also the case in the present study, as fructose and glucose were detected at double

their concentration at harvest, while an almost sixfold increase was determined in sucrose. Furthermore, according to Macrae et al. [32], the approximately similar levels of fructose and glucose observed after storage, as was also the case in the present study, suggests that these soluble sugars were mainly formed through the synthesis of sucrose and its subsequent hydrolysis. Furthermore, the results presented along with the PCA analysis data showed that BlueStim and Purshade pre-harvest application enhanced the soluble sugar concentration of fruits after storage. Glycine betaine can improve the stress tolerance of fruits, maintaining fruit metabolic processes and delaying their senescence [56]. This leads to a slower breakdown of carbohydrates, resulting in increased levels of sucrose, glucose, and fructose in fruits after storage. Additionally, glycine betaine may stimulate certain metabolic pathways that are involved in the synthesis of the above sugars, further contributing to the increased levels observed. Purshade exhibited similar action, indicating that, irrespective of the mitigating factor used, the successful alleviation of stress will result in increased levels of soluble carbohydrates in the fruits after storage.

Fruits' organic acids concentration did not present any significant differences among treatments at harvest. On the other hand, malic and citric acid were reduced during fruit storage, possibly due to respiration and/or other metabolic processes. Although the respiration rate decreases at low storage temperatures, organic acids breakdown is retained as they consist of one of the main substrates of respiratory metabolism [57]. The reduction of organic acids concentration is closely linked to the decreased TA during storage as well. Furthermore, the present results showed that ascorbic acid concentration decreased during the storage period, as had also been reported in other studies [58]. Ascorbic acid is a water-soluble, non-enzymatic component of the antioxidant system, which can directly contribute to the scavenging of ROS even without the involvement of enzymes [59]. The comparison of the alleviating products showed that the BlueStim-treated fruits exhibited the highest ascorbic acid concentration after storage, indicating a positive correlation between ascorbic acid content and glycine betaine application. The results are consistent with Xu et al. [42] and Habibi et al. [56], who also reported that glycine betaine application, applied either pre- or post-harvest, increased the ascorbic acid content of bananas and orange fruit under refrigerated storage. Moreover, Mahmoudi et al. [51] proposed that, since ascorbic acid degradation is promoted by oxygen, glycine betaine may have reduced the oxygen diffusion and respiration rate, thus preserving the ascorbic acid accumulation and justifying its increased levels found in the present trial after the pre-harvest application of Bluestim.

Phenolic compounds are efficient radical scavengers as well, with high antioxidant properties [60] closely linked to antioxidant capacity. Mansinhos et al. [61] also determined increased phenolic compounds concentration under elevated temperatures through the upregulated PAL activity, thus contributing to the increase in the antioxidant capacity of the tissue or organ they are found in. This was further observed in kiwifruit leaves, where the total concentration of phenolic compounds increased due to heat stress [47]. Nonetheless, lower concentrations have been detected in the fruits of stressed plants as well [62,63]. In the present trial, total phenols and total flavonoids, as well as antioxidant capacity, were lower in the fruits of the control treatment at harvest. This is in agreement with Zahedi et al. [64], who reported a reduction in phenolic compounds and a decrease in antioxidant capacity under stress conditions in strawberries. According to Šamec et al. [63], phenolic compounds play a crucial role in protecting plants from the deleterious effects of abiotic stress, by preventing plant tissues' oxidative damage through their oxidation. Therefore, it could be assumed that the lower concentration of all the measured phenolic compounds in the control treatment is attributed to their consumption by the fruit tissues to overcome stressful environmental conditions.

On the other hand, the applied alleviating factors increased the total phenolic compounds concentration and the antioxidant capacity. This could have occurred by supplying the tissue with antioxidants through Sun Protect components [35], by the direct regulation of the antioxidant mechanisms by glycine betaine (BlueStim) [65], or indirectly through the

reflecting action of Purshade [47,66]. The phenolic content of fruits and vegetables may either increase or decrease, depending on storage conditions [48,67]. A significant increase in the polyphenol content after storage has been observed in kiwifruits [48,50] and in other species as well [68–70]. Kevers et al. [71] also reported that the antioxidant capacity and the level of phenolic compounds increase during storage in various fruits. However, the data of the present study showed a clear decrease in the concentration of the total phenolic compounds and antioxidant capacity during storage. These results are in agreement with Kim et al. [72], who suggested the same for faster phenolic degradation during the storage of hardy kiwi puree. Krupa et al. [73] also found a drastic decline in phenolic compounds in hardy kiwifruit after long-term storage, even if in the short term, i.e., seven days in the storage facility, an increased concentration had been detected. Similarly, Kalt [67] reported that 27% of the total phenolic content of apples was lost after about 6 months of storage at 5 °C in air.

Pre-harvest treatments did not impose any significant effect concerning the phenolic compounds of the fruits after storage, as only total phenols were found to be higher in the fruits of vines treated with Sun Protect. This should be expected since Sun Protect, based on its composition, probably supplied the plants with additional phenolic compounds, thus protecting the in vivo synthesized ones.

Vines treated with Purshade produced fruits with the highest antioxidant capacity after storage. According to Bang et al. [74], Ca increases the phenolics' content and antioxidant capacity in some species by slowing down their degradation, whereas it retains the integrity of membranes, reducing the levels of free radicals and preserving higher levels of antioxidant capacity even under storage conditions [75,76]. Thus, it can be assumed that Purshade not only acts as a stress alleviating factor, reflecting excess sunlight, but also as a nutrient factor, supplying the vine with Ca which translocates into the fruit. Similar results have been obtained by other forms of Ca, such as calcium oxide (CaO) and Si–Ca-based compounds in citrus and cucumber [76,77].

5. Conclusions

The results of the present study showed that the applied products significantly influenced the evaluated physiological and biochemical attributes of the fruits, both at harvest and after storage, thus demonstrating their different modes of action under heat stress conditions. BlueStim (glycine betaine) was found to be the most effective among the applied alleviating agents in reducing the impact of heat stress on fruits' biochemical attributes, as it enhanced the concentration of soluble sugars, total phenols, total flavanols, total flavonoids, and FRAP antioxidant capacity at harvest. Additionally, BlueStim increased the yield per vine by nearly 16.9%, but resulted in smaller fruit size, probably due to intra-vine fruit competition for assimilates. After storage, the pre-harvest application with BlueStim resulted in a higher concentration of bioactive compounds in the fruits, including soluble sugars, ascorbic acid, total organic acids, total flavonoids, and antioxidant capacity. Fruits produced from vines treated with Purshade exhibited high concentrations of soluble sugars, total phenols, total soluble solids, and malic acid, as well as FRAP and DPPH antioxidant capacity. On the other hand, Sun Protect increased fruit firmness and total phenols after storage. Therefore, the applied treatments effectively reduced the negative impact of heat stress on fruit quality, with varying effects on the measured parameters, and therefore, they should be considered valuable tools in farmers' defensive arsenal against harsh summer conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13030701/s1, Figure S1: Percentage of the relative change after storage on the concentration of (a) pH, titratable acidity (TA), total soluble solids (TSS), ratio of total soluble solids:titratable acidity (TSS:TA) and firmness, (b) soluble sugars and sweetness index (SI), (c) organic acids and (d) phenolic compounds and antioxidant capacity (ABTS, DPPH and FRAP).

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