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Exploring Hydroxytyrosol-Enriched Commercial Formulations: Effects on Tomato Yield and Quality Under Greenhouse Conditions

Ulises Navarro-Zapata [1,†], Alejandro Martinez-Moreno [1,†], Vicente Martinez [1], Huertas María Diaz-Mula [2], Cristian Hernández-Adasme [1], Antonio Frutos-Tortosa [1], Francisco Garcia-Sanchez [1,*] and Teresa Carmen Mestre [1]

[1] Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Campus Universitario de Espinardo, 30100 Murcia, Spain; unavarro@cebas.csic.es (U.N.-Z.); martinezamoreno@gmail.com (A.M.-M.); criherna@ug.uchile.cl (C.H.-A.)

[2] Department of Applied Biology, Universidad Miguel Hernandez, Avenida de la Universidad, s/n. 03202 Elche, 03202 Alicante, Spain; h.diaz@umh.es

* Correspondence: fgs@cebas.csic.es; Tel.: +34-968-396-200 (ext. 4455367)

† These authors contributed equally to this work.

Highlights

- Hydroxytyrosol-enriched commercial formulations increased tomato yield and marketable production.
- Preharvest application improved fruit firmness and substantially extended tomato shelf life.
- The formulations modified fruit sugars, organic acids, amino acids, and mineral composition.
- Fruit mineral enrichment accompanied improvements in fruit quality and postharvest performance.
- Valorization of olive oil processing by-products offers a promising route for sustainable horticultural biostimulants.

Abstract

Agro-food processing by-products represent valuable sources of bioactive compounds with potential applications in sustainable crop production. This study evaluated the agronomic and postharvest responses of greenhouse-grown tomato to two commercial formulations enriched in hydroxytyrosol (Formula A and Formula B) and derived from olive oil processing by-products. Plants were grown in a soilless coco-peat system, and the formulations were applied manually to the root system according to their respective manufacturer-recommended regimes. The substrate bag was considered the experimental unit (n = 6 per treatment), with the greenhouse block retained in the statistical model. Formula A and Formula B increased total yield by 9.6% and 15.7%, respectively, and marketable yield by 14.5% and 21.0%. Both formulations increased fruit firmness, whereas total soluble solids remained unaffected. Shelf life increased from 23 days in control fruit to 32 and 38 days following Formula A and Formula B application, respectively. Antioxidant-related responses were treatment-dependent: Formula B increased DPPH radical-scavenging capacity and total and reduced vitamin C, whereas lycopene increased significantly with Formula A and showed an intermediate response with Formula B; total polyphenols and anthocyanins were unaffected. The treatments also modified fruit primary-metabolite profiles, including sugars, organic acids, and amino acids, and both formulations increased fruit Ca, K, and N concentrations at the final sampling. Overall, these findings demonstrate the potential of hydroxytyrosol-enriched commercial formulations to improve

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tomato productivity and selected postharvest-quality traits while providing a valorization route for olive oil processing by-products. However, because the formulations differed in composition, hydroxytyrosol concentration, dose, and application frequency, the specific contribution of hydroxytyrosol cannot be isolated from that of the complete formulations.

Keywords: hydroxytyrosol; yield; fruit quality; shelf life; agro-industrial waste

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a major crop and one of the most widely consumed vegetables worldwide. According to the latest statistics from the Food and Agriculture Organization [1], global tomato production exceeded 190 million tons in 2023. Spain produced approximately 4.0 million tonnes of tomatoes in 2023. Tomatoes are considered one of the most important protective foods because of their high nutritional value and richness in essential nutrients and antioxidant phytochemicals linked to a reduced risk of chronic diseases [2]. They are rich in carotenoids, such as β-carotene, lycopene, and lutein, and serve as sources of vitamins and phenolic compounds [3]. These bioactive compounds contribute to the antioxidant properties of tomatoes and their health benefits. Moreover, they play a crucial role in determining the final nutritional and commercial quality of tomatoes, which is influenced by genetic, environmental, agronomic, and postharvest factors [4].

Biostimulants are substances and/or microorganisms capable of enhancing nutrient uptake, increasing plant tolerance to abiotic stress, and improving crop quality when applied in small amounts [5]. In recent years, the use of biostimulants in agriculture has garnered significant research attention. Their biological activity is attributed to the presence of indigenous hormones, peptides, phenolic compounds, saccharides, and other organic components in their composition. Commercial biostimulant formulations are primarily based on seaweed extracts, protein hydrolysates, and humic substances [6].

Another interesting source of biostimulants is by-products derived from the agro-food sector. These by-products are usually used for composting, combustion, animal feed, and soil amendment. However, they are rich in bioactive substances, such as phenolic compounds, which could contribute to their revalorization [7]. Alperujo is the main by-product generated during olive oil extraction using a two-phase centrifugation system [8]. It is stored in large open-air ponds for several months until the residual oil is extracted using physical or chemical methods in oil-extracting plants [9]. While this by-product has long been an environmental problem, it could now be a source of bioactive molecules in agriculture as natural pesticides, biostimulants, or plant protectants, providing an alternative to harmful agrochemicals [10]. The main phenolic compounds detected in fresh alperujo are hydroxytyrosol-4-glucoside, hydroxytyrosol, and the dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (HyEDA). Hydroxytyrosol is a well-known health-promoting compound with antioxidant properties [11]. From a technological perspective, hydroxytyrosol is highly useful; however, its synthesis is expensive, and it is not commercially available in large quantities. Therefore, the possibility of recovering these compounds from natural matrices, such as alperujo, is an important goal [10].

Phenolic compounds possess antioxidant and biostimulant properties and can intervene in the natural physiological processes of plants [12]. Plants have evolved different phytochemicals as antioxidant defenses to maintain their growth and metabolism. Antioxidant compounds, such as phenols, vitamin C, and lycopene, are involved in the defense

mechanism in plants and prevent cell damage [13]. Phenols are important signaling molecules, and it has been established that at adequate concentrations, they can produce several positive effects in plants, even when they are exogenously applied or present in plant biostimulant formulations [14]. Moreover, their activity in plant defense against phytopathogens has been explored for application as biopesticides in agriculture [15]. Accordingly, olive-mill by-products have been proposed as potential sources of biostimulants or soil amendments [10], and their phenolic fraction may contribute to plant responses.

Although the role of well-established biostimulant categories, such as seaweed extracts, protein hydrolysates, and humic substances, has been extensively studied for enhancing tomato growth, yield, and stress tolerance, the agronomic and postharvest performance of commercial formulations enriched in hydroxytyrosol remains insufficiently characterized. In particular, integrated studies evaluating yield and storage responses alongside fruit composition and primary-metabolite profiles in greenhouse-grown tomato remain limited. The novelty of the present study therefore lies in the integrated evaluation of two distinct commercial formulations derived from olive-processing by-products in greenhouse-grown tomato, rather than in demonstrating a new hydroxytyrosol-specific regulatory mechanism. Specifically, the study evaluated yield, shelf life, physicochemical quality, antioxidant-related traits, mineral composition, and fruit primary-metabolite profiles.

We hypothesized that application of the complete commercial formulations could improve tomato yield and selected postharvest-quality attributes. Because the formulations differed in composition and application regimen, the study was not designed to isolate the specific effect of hydroxytyrosol.

2. Materials and Methods

2.1. Plant Growth Conditions

The experiment was carried out in a polycarbonate greenhouse (462 m^2) in the experimental field of CEBAS-CSIC, Santomera (Murcia, Spain), during the spring–summer season. The greenhouse was equipped with automated climate and irrigation control. During the experiment, the temperature inside the greenhouse was set to a maximum of 28 °C. Once this temperature was exceeded, the greenhouse climate control system set in motion a series of actions to activate or deactivate the opening of the zenithal windows, the "air cooling" system, and shade nets to control the temperature. Fertigation was performed using an automatic drip irrigation system with three emitters (2 L h^{-1}) placed in each substrate bag. The nutrient solution (NS) used for irrigation was ½ Hoagland solution, according to [16].

Tomato plant seedlings (*Solanum lycopersicum* L. cultivar Jawara) from Syngenta (Basel, Switzerland) were germinated in 60 × 40 cm trays with rockwool and transplanted into 27 L bags (16 cm × 18 cm × 100 cm) containing cocopeat substrate (Pelemix, Murcia, Spain).

The experiment comprised two greenhouse blocks and three treatments (Control, Formula A, and Formula B). Each treatment was represented by three substrate bags per block and three plants per bag, giving 27 plants per block, 54 plants in total, 18 plants per treatment, and six bags per treatment. Within each block, the three bags assigned to a treatment were grouped, and the position of the three treatment groups was randomized. Each plant had its own drip emitter; the three emitters serving plants in one bag originated from the same fertigation line. Because the plants within a bag shared the substrate and root-zone environment, the substrate bag was defined as the experimental unit. Plants were grown for 155 days after transplanting (DAT).

2.2. Commercial Formulations and Application Regimes

The formulation matrix was obtained from the by-products of the olive oil extraction process.

Formula A: Commercial product "DUREXOL" (Fyneco, SL., Murcia, Spain). The supplied manufacturer information declared 3.2% (*w*/*w*) alginic acid, 4.5% (*w*/*w*) water-soluble K2O, and 1.1% (*w*/*w*) water-soluble P2O5, with pH 5.5 and a density of 1.1. The commercial description also referred generally to phenolic antioxidant compounds obtained from valorized olive-processing residues, polysaccharides, and other plant-derived compounds. Cytokinin content was not declared in the available technical information. Hydroxytyrosol concentration in Formula A was analytically determined by the CEBAS-CSIC metabolomics service and was approximately 2.8 mg mL^{-1}.

Formula B: Commercial product "KRONOX" (Fyneco, SL., Murcia, Spain). The available product information described Formula B as a formulated antioxidant concentrate from the matrix obtained from the liquid phase of alperujo. Hydroxytyrosol concentration in Formula B was analytically determined by the CEBAS-CSIC metabolomics service and was approximately 6.4 mg mL^{-1}. The product is no longer marketed.

Formula A and Formula B were applied manually to the root zone of each plant and were not delivered through a treatment-specific fertigation line. Control plants received the same application water volume as treated plants.

Ten manual applications were performed for Formula A (at 15-day intervals) and 20 applications for Formula B (at 7-day intervals). The doses were 0.3 mL L^{-1} and 3.3 mL L^{-1}, respectively, following the respective commercial recommendations. Plants treated with Formula A received a cumulative total of 0.3 mL product $plant^{-1}$, whereas those treated with Formula B received a cumulative total of 6.6 mL $plant^{-1}$. Because the products differed in composition, hydroxytyrosol concentration, product dose, application frequency, and total number of applications, their comparison is not an equivalent-dose test of hydroxytyrosol.

2.3. Crop Productivity

The fruits were harvested at the red maturity stage. To determine the total and marketable fruit yields, all fruits from each plant were counted, weighed, and visually inspected. For statistical analysis, each production variable was averaged across the three plants in each bag, yielding six independent bag-level observations per treatment (n = 6). Tomato yield was expressed as the total yield per plant (kg $plant^{-1}$) and the marketable yield per plant (kg $plant^{-1}$). The number of fruits was recorded as the total number of fruits per plant, and fruit size was calculated as the average fruit weight per plant. Fruits were classified as marketable if they exceeded 50 g in fresh weight and showed no symptoms of blossom-end rot (BER) or cracking.

2.4. Measuring the Concentration of Mineral Nutrients in Leaf Tissues

The concentrations of Na, K, Mg, Ca, P, S, Fe, Cu, Mn, Zn, and B in the leaves were measured as previously described by [17]. Briefly, one composite leaf sample was obtained from each substrate bag. Each composite sample comprised young, fully expanded leaves collected from all three plants growing in that bag, giving six biological replicates per treatment. Leaves were oven-dried and ground before elemental analysis. For elemental determination, approximately 100 ± 0.1 mg of dry, ground leaf sample was digested at 200 °C with 5 mL of HNO3 (65%, *w*/*v*) and 3 mL of H2O2 (33%, *w*/*v*), followed by a 1:20 dilution. Elements were quantified by ICP-OES using a Thermo iCAP 6500 DUO instrument (Thermo Fisher Scientific, Waltham, MA, USA) at the CEBAS-CSIC ionomics service. Total N was determined from approximately 50 ± 0.1 mg of solid sample enclosed

in a tin capsule using a LECO CHN 628 elemental analyzer (LECO Corp., St. Joseph, MI, USA). Macroelement and microelement concentrations were expressed as g 100 g^{-1} DW and mg 100 g^{-1} DW, respectively.

2.5. Parameters Evaluated in Fruits

2.5.1. Fruit Quality Traits

When tomatoes were harvested from the fourth cluster at 137 days after transplanting (DAT), two representative red-ripe fruits were collected from each substrate bag for quantitative analysis of fruit quality parameters. Thus, 12 fruits were sampled per treatment (two fruits per bag), representing six biological replicates (bags) (n = 6). The two fruits from each bag were treated as subsamples, and one bag-level value was used in the statistical analysis. Initially, the fruits were subjected to analyses that required intact fruit. Subsequently, tomatoes were sectioned into portions and processed according to the requirements of the different analyses: one fraction was used to obtain fresh juice for physicochemical analyses (pH, electrical conductivity, total soluble solids, and total acidity); another fraction was used for dry matter determination, while material intended for mineral analysis was prepared as described below; and the remaining portions were frozen and stored for fresh biochemical analyses described below. These fruits were exclusively used for quality and compositional analyses and were not included in the shelf-life evaluation.

For shelf-life assessment, an independent set of six ripe and marketable fruits per treatment was harvested at 137 DAT, with one fruit representing each substrate bag and three fruits from each block. Fruits were stored in the dark on trays, without wrapping or packaging, at 8–10 °C; relative humidity was not controlled. Fruits were evaluated and weighed generally every 4 days. Three trained panelists independently scored each fruit on a 0–5 scale for firmness loss, spots or surface depressions, and decay, where 0 indicated no defect and 5 an unacceptable defect. The three scores were averaged for each fruit, and commercial quality was considered lost when the mean score reached $\geq$4 for any attribute. The results are expressed as the number of days maintaining commercial quality.

Fruit size was determined using the equatorial and longitudinal diameters with a Vernier caliper and expressed in mm. The shape index was calculated as the ratio of the equatorial diameter to the longitudinal fruit diameter. Fruit firmness was determined using an analog penetrometer (Fruit Pressure Tester, Bertuzzi, Busto Arsizio, Italy) equipped with an FT 011 tool. Firmness was expressed in N cm^{-2} as the mean of three measurements per fruit. The dry matter (DM) was calculated after weighing fresh fruit samples cut into portions and drying them in an air oven for 15 days at 60 °C. The results are expressed as the percentage of DM. The pH and electrical conductivity (EC) of tomato juice were determined using a digital multimeter (HACH HQ40D two-channel digital multimeter (Hach, Loveland, CO, USA)) with a precision of $\pm$ 0.01 for pH and $\pm$ 0.01 dS m^{-1} for EC. The results were expressed in units of pH and dS m^{-1}. The total soluble solids (TSS) and total acidity (TA) of the tomato juice were measured according to [3]. The results are expressed in terms of ºBrix and g L^{-1}, respectively. The maturity index (MI) was calculated as the ratio of total soluble solids (TSS) to total acidity (TA).

2.5.2. Mineral Analysis in Fruit and Relative Ca Distribution

For the analysis of minerals in the fruits (Ca, K, Mg, Na, P, S, B, Cu, Fe, Mn, Mo, and Zn), 0.1 g of dried fruit was analyzed as described by [17]. Fruit mineral concentrations were determined at 137 and 155 DAT. The two fruits collected from each substrate bag were pooled to obtain one composite sample, resulting in six biological replicates per treatment. Fruit samples were oven-dried and ground to a fine powder. Mineral elements were determined following the same acid digestion and ICP-OES procedure described

above for leaf samples. At 155 DAT, fruits, leaves, and stems were collected separately, and the total Ca content of each organ was calculated as the product of its Ca concentration and corresponding dry biomass. The relative distribution of Ca among the measured aboveground organs was subsequently calculated by expressing the Ca content of each organ as a percentage of the summed Ca content of fruits, leaves, and stems. Roots were not included in this calculation.

2.5.3. Analysis of Antioxidants and Bioactive Compounds in Tomato Fruit

Antioxidant capacity and the concentrations of vitamin C, lycopene, total polyphenols, and anthocyanins were determined in portions of the fruits sampled at 137 DAT. Samples were frozen at −80 °C, ground under liquid nitrogen, and stored again at −80 °C until analysis. Six bag-level biological replicates were analyzed per treatment.

The total antioxidant capacity was measured by determining the free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, based on previously published methods [18], with slight modifications. Extraction of 200 mg of ground fresh tomato was carried out in 1.5 mL of methanol–water (80:20) in a magnetic homogenizer (IKA 3000, Staufen im Breisgau, Germany) with the aid of dry ice. The absorbance was measured at 515 nm using a UV-VIS spectrophotometer (Thermo Fisher, MA, USA). The results were expressed as the percentage decrease at 30 min with respect to the initial absorbance (% inhibition).

Lycopene content was measured according to the methodology proposed by [19], with slight modifications. Extraction was performed on a sample of 200 mg of ground fresh tomato with 2 mL of extractant (500 µL of 0.05% (*w*/*v*) butylated hydroxytoluene (BHT) in acetone, 500 µL of 95% (*v*/*v*) ethanol, and 1 mL of hexane) in a magnetic homogenizer cooled with dry ice. The lycopene content was quantified using the following equation:

$$\text{Lycopene content}\left({}^{\text{mg}}/_{100\ \text{g}_{\text{tomato}}}\right) = \frac{\text{A}_{503} \times 0.0312 \times 5}{100\ \text{g tomato}}$$

where A_{503} is the absorbance at 503 nm, 0.0312 is the molar extinction coefficient of lycopene in hexane, and 5 is the conversion factor for the path length of the 1 cm cuvette at 0.2 cm of the plates used (1/0.2 = 5).

The total and reduced vitamin C contents were measured as previously described by [20], with some modifications. Fresh tomato tissue (500 mg) was homogenized in liquid nitrogen using a magnetic homogenizer (Bullet Blender 5E Gold; New York, NJ, USA). The sample was extracted using 2 mL of 6% trichloroacetic acid (TCA). After the process described by the cited author, the absorbance was recorded at 525 nm (A525). A calibration curve was prepared using an ascorbic acid standard. The results are expressed as mg of vitamin C (total or reduced) per 100 g of fresh weight (mg 100 g^{-1} FW).

The total polyphenol content was determined according to [21], with slight modifications. A sample of 250 mg of tomato powder was extracted with 1.5 mL of methanol–water (1:4) and 2 mM sodium fluoride. Quantification was performed through interpolation with a standard line of gallic acid between 0 and 50 mg 100 mL^{-1} dissolved in 10% ethanol. The results are expressed as mg 100 g^{-1} FW.

The total anthocyanin content was determined using the methodology described by [22], with modifications. Tomato powder (300 mg) was extracted in 1 mL of 95% ethanol:1.5N HCl (85:15). The absorbance of the supernatant was determined at 530 and 657 nm. The actual absorbance of anthocyanins was calculated using the following equation:

$$ABS_{530real} = ABS_{530sample} - \left(ABS_{657sample} - ABS_{657blank}\right) - ABS_{530blank}$$

The results were obtained by interpolation with a standard line of cyanidin-3-glucoside (0.001–0.075 mM) and expressed in mg 100 g^{-1} FW.

2.5.4. Metabolite Profiling

Metabolite profiling was performed using ^{1}H NMR to determine the profiles of sugars, organic acids, and amino acids. The freeze-dried and finely homogenized samples were extracted following the protocol described by [23]. The analysis was performed on a 500 MHz Bruker spectrometer (Bruker Biospin, Rheinstetten, Germany) with a 5 mm Prodigy BBO N2 broadband cryo-probe. The NMR equipment provided a frequency graph of the detected signal. The resulting spectra were sent to the Chenomx NMR Suite version 8.3 processor (Chenomx, Edmonton, AB, Canada), and metabolite quantification was performed according to [17].

2.6. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA). The substrate bag was considered the experimental unit (n = 6 per treatment). Before ANOVA, model residuals were checked for normality and homogeneity of variance. Data were analyzed using a randomized-block ANOVA including Treatment and Block as fixed factors, with Block retained in the model irrespective of its statistical significance. Treatment means were compared using Tukey-adjusted pairwise comparisons at $p \leq 0.05$. Significance levels are indicated as $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***), and ns for non-significant differences.

3. Results

3.1. Yield Parameters

The application of Formula A and Formula B showed a significant increase in total tomato yield compared to the control (9.6% and 15.7%, respectively) (Figure 1A). This increase was primarily caused by the increase in tomato size, which was significantly higher in formulation-treated plants (Figure 1B), rather than fruit number, which remained unaffected by the treatments (Figure 1C). The marketable yield also significantly increased in the formulation-treated plants. Formulas A and B increased yield by 14.5% and 21.0%, respectively, compared to that of the control. This was associated with a higher average fruit size in treated plants, reaching 125.8 and 127.9 g, compared to 116.9 g in the control (Figure 1D,E). In addition, application of the formulations resulted in a greater number of marketable fruits per plant (Figure 1F).

3.2. Shelf Life

Tomato fruits from plants treated with Formula A and Formula B showed significantly longer shelf lives during storage than the control (Figure 2; $p < 0.001$). At 8–10 °C, shelf life averaged 23 days in the control, 32 days with Formula A, and 38 days with Formula B, corresponding to increases of 37.6% and 64.5%, respectively. All treatment means differed in Tukey-adjusted comparisons.

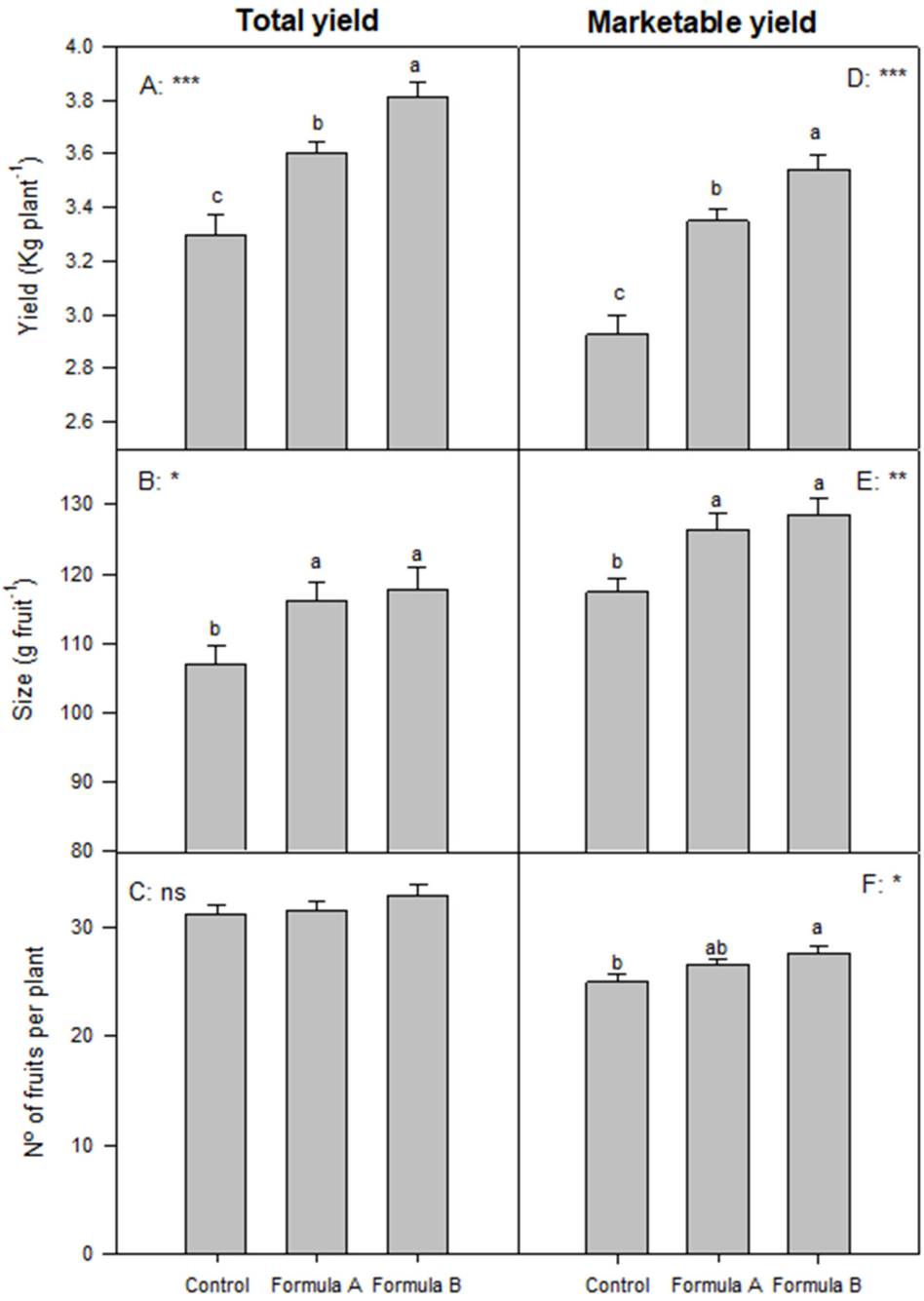

Figure 1. Production parameters: yield (kg plant^{-1}), mean fruit mass (g fruit^{-1}) and fruit number per plant for total (**A**–**C**) and marketable (**D**–**F**) production after application of Formula A or Formula B. Data are bag-level means ± SE (n = 6). Different letters indicate Tukey-adjusted pairwise differences at $p \leq 0.05$. *, **, and *** indicate overall treatment effects at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively; ns, not significant.

3.3. Fruit Quality Parameters

At 137 DAT, fruit firmness was significantly higher in both Formula A- and Formula B-treated fruits than in the control (4.9 and 5.2 vs. 4.0 N cm^{-2}, respectively), with no significant difference between Formula A and Formula B (Table 1). No significant differences were observed in the other quality parameters analyzed.

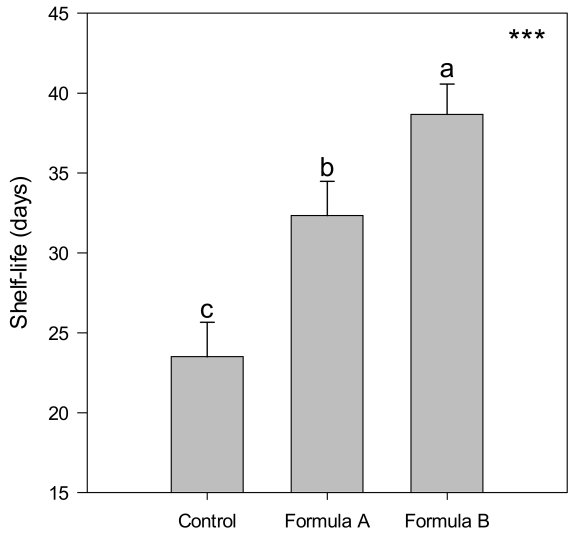

Figure 2. Shelf life of ripe tomato fruit harvested at 137 DAT after preharvest application of Formula A or Formula B. Data are means ± SE for six independent fruits per treatment, with one fruit representing each substrate bag (n = 6). Different letters indicate Tukey-adjusted differences at $p \leq 0.05$; *** indicates an overall treatment effect at $p \leq 0.001$.

Table 1. Fruit quality parameters of red-ripe tomatoes sampled at 137 DAT after preharvest application of Formula A or Formula B. Dry matter (DM), electrical conductivity (EC), total soluble solids (TSS), and maturity index (MI).

	Control	Formula A	Formula B	Significance
Equatorial diameter (mm)	66	70	68	ns
Longitudinal diameter (mm)	58	61	58	ns
Shape index	1.1	1.2	1.2	ns
Firmness (N cm^{-2})	4.0 ^{b}	4.9 ^{a}	5.2 ^{a}	**
DM (%)	5.5	5.4	5.6	ns
pH	4.7	4.7	4.7	ns
EC (dS m^{-1})	3.9	3.9	3.5	ns
TSS (°Brix)	4.8	5.2	4.9	ns
Acidity (g L^{-1})	2.7	2.7	2.5	ns
MI	1.7	1.9	2.0	ns

Values are means of six biological replicates (bags); two fruits per bag were subsamples. Within a row, different letters indicate Tukey-adjusted differences at $p \leq 0.05$. ** indicate treatment effects at $p \leq 0.01$; ns, not significant.

3.4. Antioxidants in Fruit

The Formula B-treated tomatoes showed a significantly higher antioxidant capacity than the control fruits (Figure 3A). Lycopene concentration was 36% higher under Formula A than in the control, whereas Formula B showed an intermediate 21% increase that did not differ significantly from either Formula A or the control (Figure 3D). The vitamin C content was notably affected by the applied treatments. The total vitamin C concentration in the Formula B-treated fruits (66.3 mg 100 g^{-1} FW) was significantly higher than that in the control fruit (56.3 mg 100 g^{-1} FW) (Figure 3E). Similarly, the reduced vitamin C concentration was significantly enhanced under Formula B compared to the control (29%). No significant differences were observed in the total polyphenol and anthocyanin concentrations (Figure 3B,C).

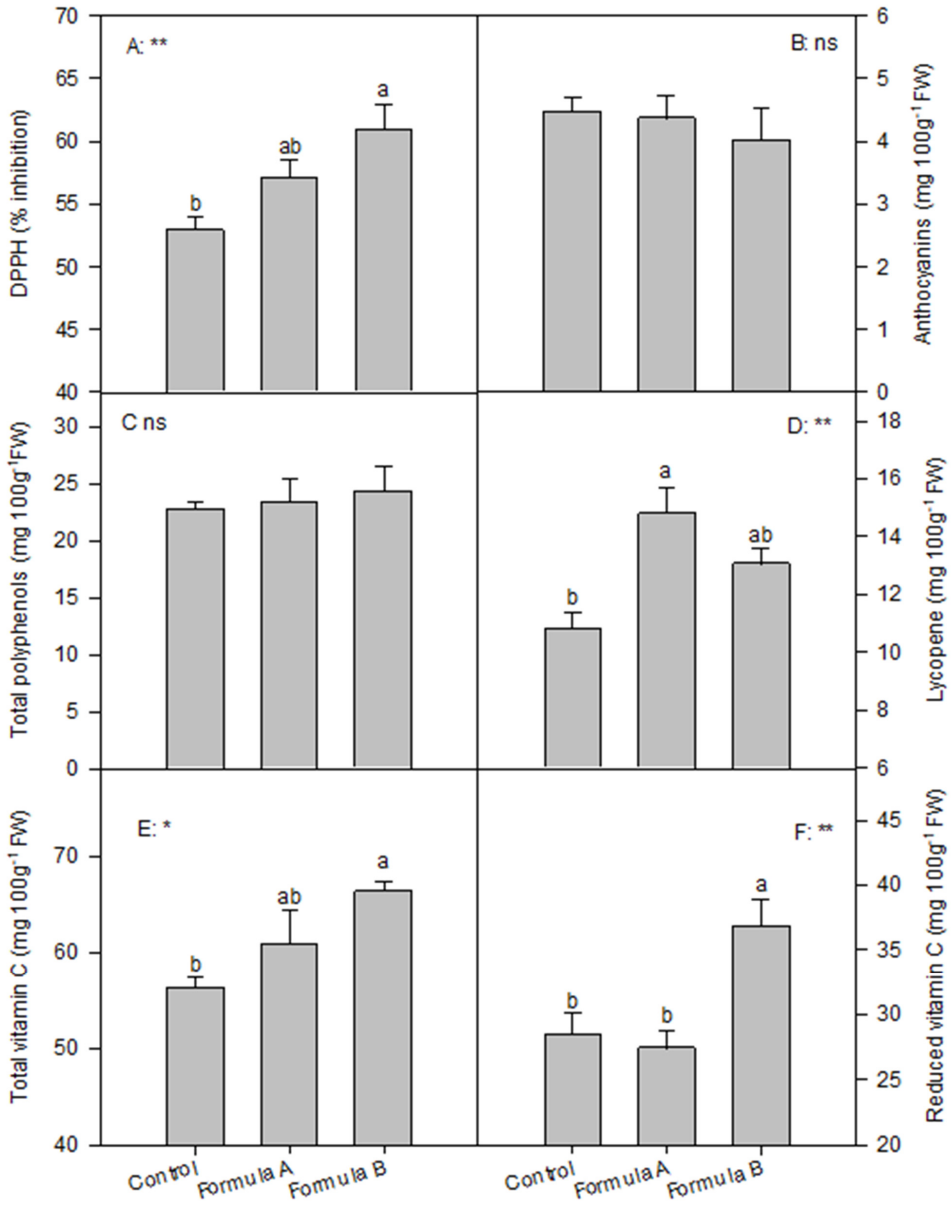

Figure 3. DPPH radical-scavenging capacity (**A**), anthocyanins (**B**), total polyphenols (**C**), lycopene (**D**), total vitamin C (**E**), and reduced vitamin C (**F**) in ripe tomato fruit sampled at 137 DAT after preharvest application of Formula A or Formula B. Concentrations are expressed as mg 100 g^{-1} FW except for DPPH inhibition (%). Data are bag-level means ± SE (n = 6). Different letters indicate Tukey-adjusted differences at $p \leq 0.05$. *, ** indicate treatment effects at $p \leq 0.05$, $p \leq 0.01$, respectively; ns, not significant.

3.5. Metabolites

The application of the commercial formulations significantly altered the metabolic profiles of tomato fruits at 137 DAT (Table 2). Both formulations induced significant changes in sugar metabolism. Formula A and Formula B significantly increased the fructose concentration by 12.8 and 12.2%, respectively, compared to that of the control. The glucose concentration followed a similar trend, with Formula A showing the highest increase (15.7% higher than that of the control). In contrast, both formulations significantly reduced sucrose concentration relative to that of the control. Organic acid metabolism was also differentially affected by the treatments. For example, malate concentration was significantly augmented under Formula A, followed by Formula B by 40.3 and 21.6%, respectively, compared to

the control. Conversely, Formula B significantly diminished 2-oxoglutarate concentration compared to the control and Formula A, whereas citrate levels remained unchanged. Amino acid profiles revealed treatment-specific responses. In particular, Formula B significantly increased glutamine levels by 66.9% compared with those in the control group. In contrast, both treatments reduced the glutamate and asparagine concentrations. No significant differences were observed in the other quantified metabolites.

Table 2. Concentrations of primary metabolites in ripe tomato fruit at 137 DAT determined by ^{1}H NMR-based profiling (mg 100 g^{-1} FW).

	Control	Formula A	Formula B	Significance
Fructose	1480 b	1670 a	1660 a	*
Glucose	1400 b	1620 a	1510 ab	**
Citrate	363	341	326	ns
Glutamate	183 a	114 b	120 b	**
Aspartate	66.0	62.2	54.7	ns
GABA	50.2	51.8	43.2	ns
Glutamine	48.0 b	42.2 b	80.1 a	**
Malate	45.4 c	63.7 a	55.2 b	***
Asparagine	30.9 a	27.2 ab	22.9 b	*
Sucrose	51.4 a	39.3 b	24.8 c	***
2-oxoglutarate	21.9 a	23.8 a	12.9 b	*
Pyroglutamate	14.3	10.1	10.9	ns
Alanine	7.97	7.12	6.13	ns
Threonine	7.02	6.30	5.68	ns
Choline	7.01	6.25	6.27	ns
Phenylalanine	3.56	4.28	2.98	ns
Proline	3.06	3.07	2.40	ns
Tyrosine	2.87	3.25	2.35	ns
Leucine	2.09	2.42	2.10	ns
Isoleucine	1.95	2.13	1.97	ns
Trigonelline	1.23	1.19	1.31	ns
Valine	1.12	1.27	1.15	ns

Values are means of six biological replicates (bags). Within a row, different letters indicate Tukey-adjusted differences at $p \leq 0.05$. *, **, and *** indicate treatment effects at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively; ns, not significant.

In contrast, the sugar/organic acid ratio significantly increased by 15.5% and 19.7% with Formula A and Formula B, respectively, compared to that in the control (Figure 4).

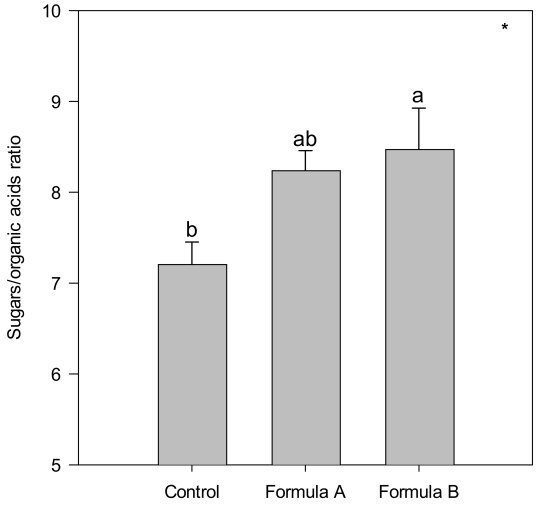

Figure 4. Sugar/organic acid ratio in ripe tomato fruit at 137 DAT, calculated from ^{1}H NMR data as (fructose + glucose + sucrose)/(citrate + malate). Data are bag-level means ± SE (n = 6). Different letters indicate Tukey-adjusted differences at $p \leq 0.05$. * indicate treatment effects at $p \leq 0.05$; ns, not significant.

3.6. Mineral Concentration in Fruit

Fruit Ca, K, and total N concentrations were significantly higher in both Formula A and Formula B than in the control, whereas fruit Na concentration was lower under Formula B than in the control. Mn concentration did not differ significantly among treatments (Table 3). All measured mineral concentrations were within the expected range, with no indication of deficiency or toxicity.

Table 3. Mineral concentrations in tomato fruit at the final sampling (155 DAT).

		Control	Formula A	Formula B	Significance
g 100 g^{-1} DW	Ca	0.157 ^{b}	0.181 ^{a}	0.196 ^{a}	**
g 100 g^{-1} DW	K	2.593 ^{b}	2.958 ^{a}	3.014 ^{a}	***
g 100 g^{-1} DW	Mg	0.121	0.125	0.120	ns
g 100 g^{-1} DW	Na	0.026 ^{a}	0.024 ^{ab}	0.021 ^{b}	*
g 100 g^{-1} DW	P	0.325	0.331	0.321	ns
g 100 g^{-1} DW	S	0.150	0.144	0.134	ns
g 100 g^{-1} DW	N_{total}	1.945 ^{b}	2.243 ^{a}	2.254 ^{a}	**
mg 100 g^{-1} DW	B	0.491	0.574	0.492	ns
mg 100 g^{-1} DW	Cu	0.516	0.480	0.440	ns
mg 100 g^{-1} DW	Fe	6.269	5.936	6.074	ns
mg 100 g^{-1} DW	Mn	1.607	2.068	1.795	ns
mg 100 g^{-1} DW	Zn	2.005	2.017	1.592	ns

Values are means of six composite bag-level samples. Within a row, different letters indicate Tukey-adjusted differences at $p \leq 0.05$. *, **, and *** indicate treatment effects at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively; ns, not significant.

The relative distribution of total measured Ca among fruit, leaves, and stems differed among treatments (Figure 5). Fruit represented 10.25%, 14.43%, and 16.78% of measured aboveground Ca in the control, Formula A, and Formula B, respectively. The corresponding leaf fractions were 68.42%, 63.58%, and 56.45%, whereas the stem fraction was higher with Formula B than with the control or Formula A.

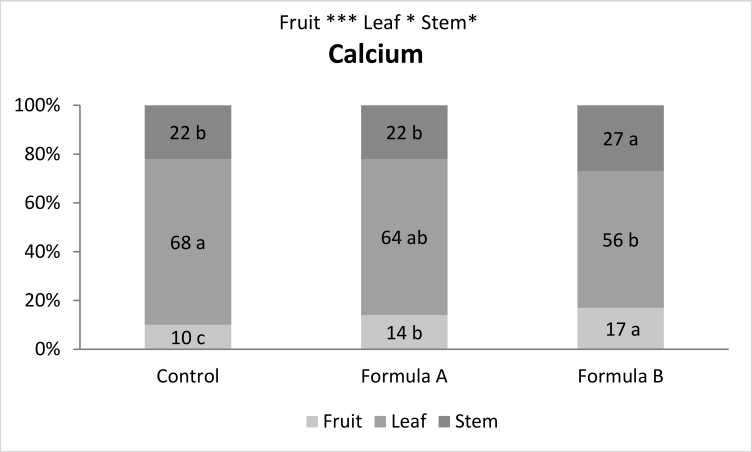

Figure 5. Relative distribution of total measured Ca among fruit, leaves, and stems at 155 DAT. Organ Ca content was calculated from Ca concentration and dry biomass, and each organ was expressed as a percentage of the sum for the three measured aboveground organs. Data are bag-level means ± SE (n = 6). Different letters within an organ indicate Tukey-adjusted differences at $p \leq 0.05$. Roots were not included. * and *** indicate treatment effects at $p \leq 0.05$ and $p \leq 0.001$, respectively.

3.7. Mineral Content in Leaves

No significant differences were observed in the leaf nutrient concentrations with the application of Formula A and Formula B at 155 DAT (Table 4). All values were within the normal range, and no deficiency or toxicity was detected.

Table 4. Mineral concentrations in young, fully expanded tomato leaves at 155 DAT.

		Control	Formula A	Formula B	Significance
g 100 g^{-1} DW	Ca	1.95	1.85	1.50	ns
g 100 g^{-1} DW	K	2.31	2.01	2.03	ns
g 100 g^{-1} DW	Mg	0.352	0.321	0.325	ns
g 100 g^{-1} DW	Na	0.0330	0.0250	0.0210	ns
g 100 g^{-1} DW	P	0.541	0.512	0.545	ns
g 100 g^{-1} DW	S	0.601	0.582	0.551	ns
g 100 g^{-1} DW	N_{total}	3.41	3.20	3.10	ns
mg 100 g^{-1} DW	B	3.13	2.75	2.73	ns
mg 100 g^{-1} DW	Cu	1.22	1.20	1.21	ns
mg 100 g^{-1} DW	Fe	20.6	17.2	15.1	ns
mg 100 g^{-1} DW	Mn	8.95	8.65	8.70	ns
mg 100 g^{-1} DW	Mo	0.971	1.00	0.912	ns
mg 100 g^{-1} DW	Zn	2.23	2.12	2.05	ns

Values are means of six composite bag-level samples; each sample comprised leaves from the three plants in one bag. ns, not significant.

4. Discussion

The yield response to the two commercial formulations is consistent with reports showing that plant-derived biostimulants can improve tomato productivity and fruit quality, although the magnitude and traits affected vary with biostimulant source, cultivar, environment, and application regime [24–27]. Because photosynthetic rate, photosynthetic pigments, chlorophyll fluorescence, and whole-plant biomass were not measured in the present experiment, the increase in fruit production cannot be assigned to enhanced photosynthesis or to a redistribution of carbon towards reproductive sinks. The present data therefore support an agronomic response to the complete formulations rather than a defined mechanism of carbon allocation.

A particularly relevant response from a commercial perspective was the extension of fruit shelf life. Both formulations increased fruit firmness relative to the control, with no significant difference between Formula A and Formula B, whereas TSS and the other routine physicochemical traits remained unaffected. Thus, the treatment response was selective rather than a general improvement in all fruit-quality variables, with firmness and storage longevity showing the clearest practical benefit. Maintenance of firmness is important for handling and marketability, but the measurements performed here do not establish the process by which softening was delayed.

Fruit Ca, K, and total N concentrations at 155 DAT were higher in both treated groups than in the control, whereas leaf concentrations did not differ significantly among treatments. In addition, the proportion of measured aboveground Ca located in the fruit increased, and the leaf fraction decreased, particularly with Formula B, with an accompanying increase in the stem fraction. These results are compatible with a treatment-associated difference in the distribution of Ca among the measured aboveground organs. However, because roots were not included and total nutrient uptake, root biomass, and whole-plant nutrient-use efficiency were not determined, the data should not be interpreted as evidence of improved nutrient uptake or nutrient-use efficiency.

Calcium has a well-established role in cell-wall and membrane stability, while K contributes to osmotic regulation and maintenance of turgor [28–31]. Accordingly, the simultaneous increase in fruit Ca and K concentrations and in firmness provides a plausible physiological association with the improved storage performance. Nevertheless, cell wall composition, pectin cross-linking, pectin methylesterase activity, and fruit water relations were not measured, and therefore a calcium-mediated cell wall reinforcement mechanism cannot be demonstrated from the present data. This distinction is particularly important because firmness did not differ significantly between the two formulations.

The antioxidant-related response was also selective. Formula B increased DPPH radical-scavenging capacity and total and reduced vitamin C relative to the control, whereas lycopene increased significantly with Formula A, while Formula B showed an intermediate response that did not differ significantly from either Formula A or the control. Total polyphenols and anthocyanins were unaffected. Therefore, the results do not support a uniform enhancement of all antioxidant components but rather treatment-specific changes in particular antioxidant traits. Variability in antioxidant responses among tomato studies may reflect differences in genotype, developmental stage, growing environment, dose, and formulation composition [26,32–34].

The NMR profiles likewise indicate specific changes in primary metabolites rather than a global metabolic shift. Fructose increased with both formulations, glucose was highest with Formula A, and sucrose decreased in both treated groups. Malate increased most strongly with Formula A, whereas Formula B produced the largest increase in glutamine together with lower glutamate, asparagine, and 2-oxoglutarate. These coordinated differences are compatible with changes in primary carbon and nitrogen metabolism, but the underlying enzymatic steps cannot be inferred because activities of sucrose-metabolizing enzymes, TCA-cycle enzymes, and nitrogen-assimilation enzymes, as well as metabolic fluxes, were not measured. The amino acid pattern should therefore be interpreted as an association with treatment rather than direct evidence of enhanced nitrogen assimilation [35–37].

The higher sugar/organic acid ratio in treated fruits may have practical relevance for flavor balance because the relative proportions of sugars and acids contribute to tomato taste [38,39]. However, TSS did not differ significantly among treatments, showing that the NMR-derived change in this ratio should not be equated with a generalized increase in soluble solids or sweetness. Likewise, the metabolite profile was determined at harvest and does not demonstrate that treated fruits maintained a more active metabolism throughout storage.

Interpretation of the differences between Formula A and Formula B must also consider that they were not equivalent hydroxytyrosol treatments. Formula A contained declared alginic acid, K2O, and P2O5 and was described as containing polysaccharides and other plant-derived phenolic compounds, whereas Formula B was a different antioxidant concentrate. In addition, the products differed in hydroxytyrosol concentration, product dose, application interval, and cumulative number of applications. Consequently, the greater response of Formula B for some traits, notably shelf life, cannot be attributed to hydroxytyrosol concentration alone or considered evidence of a hydroxytyrosol dose–response. Synergistic or confounding effects of other formulation components and of the application regime remain possible.

Overall, the concurrent improvements in yield, marketable production, firmness, and shelf life, together with selective changes in mineral composition, antioxidant-related traits, and primary metabolites, demonstrate the agronomic and postharvest potential of the tested commercial formulations under the greenhouse and nutrient regime used in this experiment. They also support the valorization of olive-processing by-products as sources of horticultural inputs. At the same time, the study was not designed to

demonstrate nutritional stress or fertilizer-saving capacity, and it lacks direct measurements of photosynthesis, whole-plant biomass, root nutrient uptake, cell wall properties, enzyme activities, gene expression, and metabolic fluxes. These limitations restrict mechanistic interpretation and define the measurements required to test the physiological hypotheses generated by the present results.

5. Conclusions

The present study shows that two distinct hydroxytyrosol-enriched commercial formulations derived from olive-processing by-products can improve tomato agronomic and postharvest performance under the greenhouse conditions tested. Both formulations increased total and marketable yield and fruit firmness and extended shelf life, while their effects on fruit composition were selective: changes were detected in Ca, K, and N, several primary metabolites, and some antioxidant-related traits, whereas TSS, total polyphenols, and anthocyanins were not significantly affected. These findings support the practical potential of the formulations for improving marketable production and postharvest longevity and provide a route for valorizing agro-industrial by-products.

However, the two products differed in composition, hydroxytyrosol concentration, dose, and application schedule; therefore, the observed responses cannot be assigned specifically to hydroxytyrosol or interpreted as an equivalent-dose comparison. The study also does not establish the physiological mechanisms responsible for the responses. Future work should compare purified hydroxytyrosol with composition-matched controls under equivalent application regimes, test dose responses across cultivars and environments, and include direct measurements of photosynthesis, whole-plant nutrient uptake and biomass allocation, cell wall properties, and relevant metabolic activities. Such experiments will be necessary to distinguish hydroxytyrosol-specific effects from those of the complete formulations and to define their agronomic range of application.

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