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Closing the loop in agriculture: Evaluation of the effects of fermented tomato-based biostimulant on *Solanum lycopersicum* (L.) growth and fruit quality

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ABSTRACT

Food waste is a global concern, and its valorization through biostimulant production offers a sustainable and circular approach to agriculture. This study evaluated the effects of a novel fermented tomato-based biostimulant, obtained from unsold green and red tomatoes fermented with *Weissella cibaria* 4253, on the growth and fruit quality of indeterminate mini plum tomato plants (*Solanum lycopersicum* L., cv. Solarino) cultivated in a soilless greenhouse system. The biostimulant was applied weekly at three concentrations (100, 200, and 300 mL·L^{-1}) and compared to untreated controls during a 16-week experiment in a commercial greenhouse in Emilia-Romagna, Italy. Vegetative parameters, physiological traits, including chlorophyll content, SPAD, Fv/Fm, and fruit characteristics were monitored. Fruits from different truss positions (lower, medial, upper) were analyzed for morphological, physico-chemical, and biochemical traits, including firmness, total soluble solids, titratable acidity, total polyphenol content, antioxidant activity, and lycopene content. FTB application did not significantly affect vegetative growth, but improved several fruit quality parameters. The 100 mL·L^{-1} dose increased total yield by 23 % and fruit weight by 8 %, while the 300 mL·L^{-1} dose enhanced °Brix, sugar/acid ratio, antioxidant activity (up to 20 %), and improved fruit texture, particularly in upper trusses. Lycopene content increased in treated plants over the harvest season, while untreated plants showed a decline. Heatmap and BIOLOG analyses confirmed treatment-specific effects on fruit quality and substrate microbial activity. The potential of an experimental biostimulant derived from fermented unsold tomato fruits to enhance crop performance while reducing food waste was demonstrated, supporting circular agricultural practices.

1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) estimates that one-third of all produced foods (approximately 1.3 billion tons), intended for human consumption, is lost or wasted annually (Gustavson et al. 2011; Ishangulyyev et al. 2019). Combined with the current challenges posed by climate change and a growing global population, these losses underscore the urgent need to adopt sustainable agricultural practices (Yang et al. 2023). Despite the increasing global demand for food production, waste continues to pose a significant challenge across all stages of the agri-food supply chain. At the same time, consumer preferences are increasingly shaped by health, ethical, and environmental considerations, with a growing demand for products that offer both nutritional benefits and sustainability (Mounaimi et al. 2024). Since the agri-food sector is one of the major drivers of global environmental challenges (Eslami et al. 2024), enhancing agricultural sustainability is a critical objective. In this context, biostimulants have gained attention as a promising solution, with extensive research highlighting their potential to sustainably enhance plant performance (Ertani et al. 2015; Szparaga et al. 2019; Shukla et al. 2022; Jiang et al. 2024). According to the European Biostimulant Industry Council (EBIC), biostimulants— defined under European regulation n. 2009/201— are "substances or microorganisms that, when applied to plants or the rhizosphere, stimulate natural processes to improve nutrient uptake,

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enhance nutrient efficiency, increase tolerance to abiotic stress, and improve crop quality". Among the various biostimulants, those derived from food waste have garnered significant interest for their dual role in reducing waste and enhancing the nutritional quality of crops, both in traditional and soilless cultivation systems (Kumar et al. 2023; Dasgan et al. 2024). Fermentation, a process traditionally used to improve the nutritional value, digestibility, and safety of food, has recently emerged as an innovative method to repurpose agricultural and food waste into high-value products (Ricci et al. 2019; Hadj Saadoun et al., 2021), including biostimulants. Fermentation, for example, has already been used as a sustainable strategy for the production of biostimulants, as shown by Bulgari et al. (2023). where agricultural digestate was employed as a substrate for the growth of *Trichoderma* species through solid-state fermentation. Moreover, Nazeer et al. (2024) demonstrated that fermented kiwifruit by-products, processed through lactic acid bacteria (LAB), can serve as an effective and sustainable additive to the nutrient solution, enhancing growth and fruit quality in hydroponically grown strawberries. Similarly, Galaverni et al. (2025) reported that a fermented kiwifruit-based biostimulant improved fruit yield and quality traits in open-field tomato, highlighting the potential of LAB-driven fermentation to transform agri-food waste into value-added biostimulants. Tomato (*Solanum lycopersicum* L.) is the most produced vegetable worldwide, but unfortunately, 25–50 % of tomato fruits are lost throughout the cycle, from harvesting to post-harvest stages such as processing and marketing (Bancal and Ray, 2022). This study seeks to address this issue by evaluating the application of an experimental biostimulant (FTB-Fermented tomato-based biostimulant)—produced from unsold mature green and red ripe tomatoes from the 2023 season—in a soilless tomato cultivation system in Emilia-Romagna, Italy. The region is among the leading national districts for tomato cultivation, accounting for more than one-third of the Italian production (Donati et al. 2019). Given the economic relevance of tomato production in Emilia-Romagna, sustainable innovations in this sector can have significant agronomic and environmental benefits.

Beyond overall yield and crop quality, one crucial aspect of tomato production is the natural variation in fruit characteristics, depending on their position along the plant. In indeterminate tomato varieties, fruits from different trusses (lower, medial, upper) often exhibit distinct qualitative traits, such as variations in sugar content, acidity, and nutrient composition (Biais et al. 2014; Yao et al. 2023; Ávila-Juárez and Miranda-Rodríguez, 2018). These differences, influenced by factors such as resource allocation and fruit development dynamics, are essential when evaluating the impact of agricultural inputs. Therefore, this study will assess the general effects of FTB on plant growth and fruit quality, and investigate how applying it influences these natural quality gradients across different fruit positions. In particular, both vegetative parameters and physico-chemical fruit traits were monitored, including color, texture, total soluble solids (TSS), titratable acidity (TA), electrical conductivity (EC), total polyphenol content (TPC), antioxidant capacity (AO), and lycopene content (LC). By addressing these objectives, the study aims to explore a practical strategy for reducing tomato waste while contributing to broader discussions on sustainable agriculture, circular economy models, and resource efficiency.

2. Materials and methods

2.1. Biostimulant production: sample preparation and selection of core strain

Unsold green and red tomatoes from the 2023 season were collected from Azienda Agricola Anzola, Boretto, Reggio Emilia, Italy. Equal amounts (1:1) of mature green and red ripe tomatoes, cv. “Solarino”, were mixed and homogenized, using a laboratory blender. The resulting mixture was then sterilized in an autoclave by applying heat treatment at 121°C for 20 min.

During this work, 5 microbial strains previously isolated from food and belonging to the University of Parma Culture Collection (UPCCO) of the Department of Food and Drug were employed for the fermentation of the tomato mixture. In particular, the selected strains were *Weissella cibaria* 4253 (Wc4253, Okra, MRS, 37 °C), *Lactiplantibacillus plantarum* 4188 (Lp4188, Pecorino cheese, MRS, 30°C), *Pediococcus acidilactici* 3992 (Pa3992, Grana Padano cheese, MRS, 28 °C), *Lacticaseibacillus zeae* 2240 (Lz2240, Parmigiano Reggiano cheese, MRS, 42 °C), and *Lacticaseibacillus rhamnosus* 1473 (Lr1473, Parmigiano Reggiano cheese, M17, 42 °C). The stock cultures were stored at −80 °C in De Man Rogosa and Sharpe (MRS) broth (Oxoid, Basingstoke, UK) supplemented with glycerol (12.5 % v/v), and before fermentation, they were transferred twice in fresh medium (3 % v/v) and incubated at their optimum growth temperature. Then the strains were transferred once again in fresh broths and incubated at their optimal growth temperature for 15 h, to reach a microbial density of 9 log CFU/mL (colony-forming units per millilitre). Centrifugation (Eppendorf centrifuge 5810 R, Eppendorf, Hamburg, Germany) (12,857 ×g for 10 min at 4◦C) was performed to collect cells, which were washed twice with Ringer solution (VWR, UK). Finally, the cells were resuspended in bidistilled sterile water, and each strain was inoculated into 10 g of tomato to reach a final microbial density of 7 log CFU/mL.

2.2. Fermentation experimental design

The experiments were conducted following a Design of Experiment built with MODDE Pro v13.00 software (MKS Umetrics, Umea, Sweden). A D-optimal design was selected for the screening, and a model was built around one qualitative factor and two quantitative factors. The microbial strains employed for the fermentation of the tomato waste were the qualitative factor, while fermentation temperature (°C) and time (hours) represented the quantitative ones. For each of those, a maximum and a minimum value were set, along with centre points characterized by the average values of the factors. In particular, the selected values were 30 and 37 °C for temperature, and 24 and 48 h for time. The selected response was the final microbial load (log CFU/mL). Combining the factors, an experimental design was created, with 20 experimental conditions performed in duplicate, reaching a total of 40 experiments (Table S1). The worksheet obtained by the software was followed to perform the fermentations, and the selected response was evaluated by plate count on MRS agar medium. The plates were incubated at the optimal growth temperature of the microorganisms for 48 h. The best fermentation condition suggested by the software was tested in duplicate to validate the prediction made by the model. Finally, the process was scaled-up to produce a total of 7 kg of FTB. Until its use, FTB was stored at −20°C.

2.3. Fermented tomato based formulation

The experimental biostimulant was characterized prior to application. The pH was measured using a calibrated (pH 4.0 and 7.0) pH meter (METTER-TOLEDO LE409). The Total Soluble Solid content (TSS) and the Electrical conductivity (EC) of the experimental biostimulant were measured in triplicate with respectively a digital refractometer (Hanna Instruments HI96814) and a conductivity meter (Hanna Instruments, HI98331). The FTB sugar content was determined through extraction and chromatographic analysis. FTB (40 g) was centrifuged at 15,000 × g for 20 min at 4 °C, and the resulting supernatant was filtered through folded filter paper to a final volume of 25 mL. An aliquot of 5 mL was subjected to solid-phase extraction (SPE) using preconditioned Sep-Pak C18 cartridges, and a colorless eluate was collected and stored at −20 °C. Qualitative and quantitative analyses of glucose, fructose, and sucrose were carried out using ultra-high performance liquid chromatography (UHPLC) equipped with UV–Vis and refractive index detectors. Chromatographic separation was performed on a Luna NH_2 column (5 µm, 4.6 × 250 mm, 100 Å) under isocratic conditions with an acetonitrile/water mobile phase (80:20, v/v), at a flow rate of 1 mL/min and a

column temperature of 30 °C. Sugar identification was based on comparison with analytical standards, while quantification was obtained through replicate injections and data processing using the Chromeleon v.7 software. Titratable acidity (TA) was determined by titration method with standardized 0.1 N NaOH and expressed as % citric acid equivalents. Total polyphenol content (TPC) was quantified using the Folin–Ciocalteu method. For this, 5 g of the tomato mixture were extracted with 10 mL of a MeOH:Acetone solution (70:30), and the results were expressed as gallic acid equivalents (GAE). The same extract was used to evaluate antioxidant activity via the DPPH radical scavenging assay, calculating the percentage of inhibition relative to a Trolox calibration curve. Finally, nutrient composition was assessed in terms of potassium, sodium, and nitrate ion concentrations to establish appropriate application rates for the biostimulant. Specifically, the LAQUAtwin 4 M Kit was used to measure NO_3^-, Na^+, and K^+ concentrations in the fermented tomato-based biostimulant. Following the manufacturer's instructions, a few drops of the product were applied to the sensor, and the results were expressed in ppm.

2.4. Site description and plant material

The experiment was conducted at Soc. Agricola Anzola Achille and Stefania in Boretto, Reggio Emilia, Italy (44.8986, 10.5274), in controlled greenhouse conditions.

The tomato variety used in the experiment was "Solarino" (Rijk Zwaan-RZ), a red mini-plum fruit type with an average fruit weight of 8–12 g. The plants are generative, characterized by a long, open growth habit and reliable fruit set. "Solarino" produces uniform, oval-shaped fruits with a dark red, glossy appearance. The fruits are particularly well suited for the snack market. This cultivar can be successfully cultivated in soilless conditions. Seeds were sown in a local nursery in January 2024; after six weeks of growth, the seedlings were moved to the experimental farm site and transplanted in growth bags (Ageon Srl, Borgo San Dalmazzo, CN, Italy), designed for soilless agriculture, filled with a coconut fiber substrate. Each growth bag contained six tomato plants. The greenhouse experiment was conducted from February to June 2024, spanning 16 weeks. In the supplementary material, Figure S1 is available to provide a visual representation of the experimental setup and plant development.

2.5. Experimental design

FTB at three concentrations - 100 mL/L (FTB1), 200 mL/L (FTB2), and 300 mL/L (FTB3) - was applied to each plant, at collar level, using a syringe (Figure S2); per each plant, 50 mL of FTB was distributed. The plants in the control growth bags were treated weekly with 50 mL of water, reproducing the same method reported for FTB. For each treatment, 18 plants, distributed in three growth bags (6 plants per bag) were considered. To minimize positional effects, the growth bags subjected to different treatments were randomly relocated within the greenhouse. Fig. 1 graphically summarizes the spatial arrangement of the growth bags within the greenhouse and the overall experimental design.

2.6. Fertigation

Tomato plants were fertigated according to the farm's standard practices. This involved the application of a controlled nutrient solution according to the plants' developmental stage and the environmental conditions (light and temperature). The nutrient solution composition was the following: $Ca(NO_3)_2$ 137 g L^{-1}, KNO_3 12.5 g L^{-1}, Fe-EDDHA

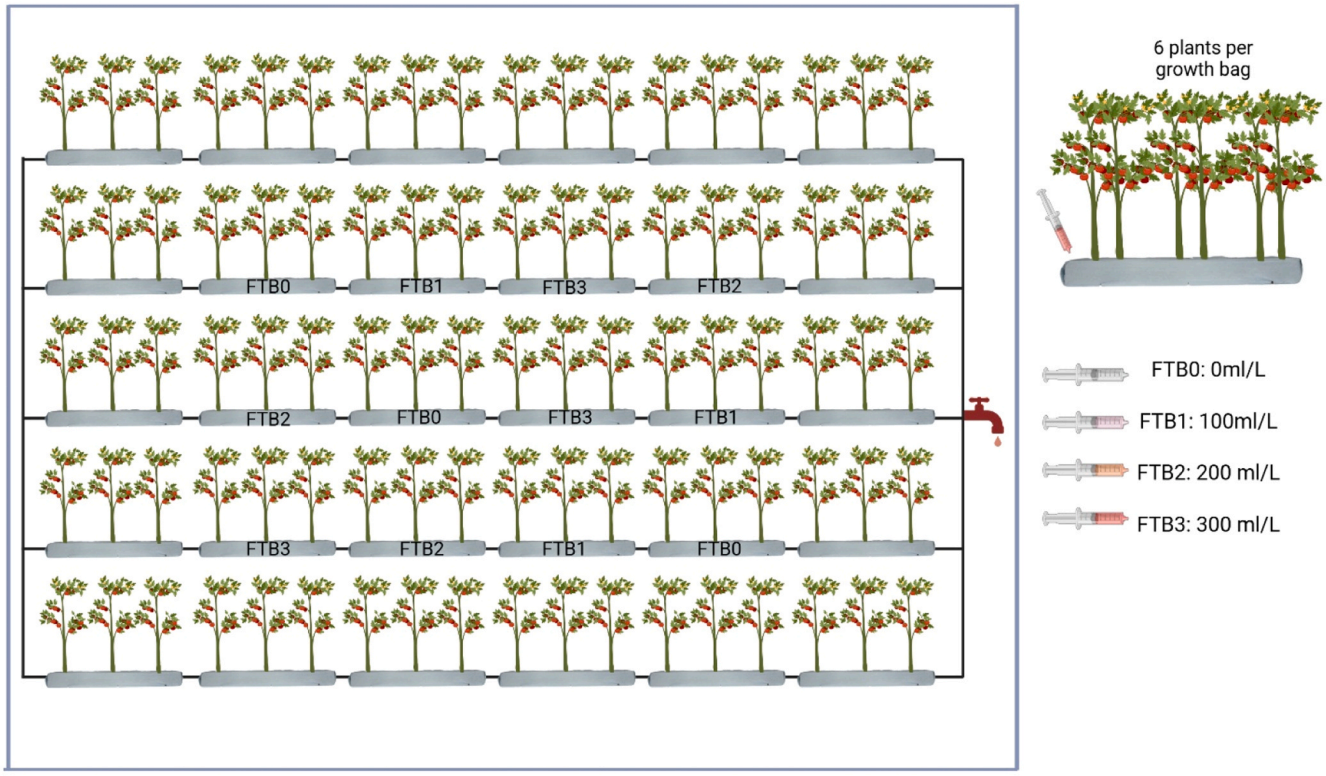


Fig. 1. Schematic representation of the experimental setup inside the greenhouse. Growth bags, each containing six plants, were arranged in horizontal rows and assigned to four treatments (FTB0: 0 mL/L, FTB1: 100 mL/L, FTB2: 200 mL/L, FTB3: 300 mL/L). The alternating distribution of the bags across different rows avoided edge placement, ensuring uniform exposure to environmental conditions and minimizing the influence of air currents typically present along the lateral sides of the greenhouse. The figure also illustrates the centralized fertigation system, which uniformly supplied all bags with the nutrient solution. Treatments were applied directly at the collar level of each plant, ensuring targeted and consistent delivery of the biostimulant. Created in BioRender. Agosti, A. (2025) https://BioRender.com/zbimfsf.

1.9 g L^{-1}, KH_2PO_4 20 g L^{-1}, $MgSO_4$ 750 g L^{-1}, K_2SO_4 0.07 g L^{-1}, $MnSO_4$ 1.19 g L^{-1}, $ZnSO_4$ 0.5 g L^{-1}, $Na_2B_4O_7$ 0.14 g L^{-1}, $CuSO_4$ 0.03 g L^{-1}, Na_2MoO_4 0.06 g L^{-1}. The pH and the EC of the solution were respectively 5.5 and 4.5 mS cm^{-1}. Moreover, the substrate was maintained within controlled ranges of pH (5.5–6.5) and EC (2.5–3.0 mS cm^{-1}) throughout the entire experiment.

2.7. Non-destructive in vivo estimation of leaf pigments and Fv/Fm

Leaf pigment indices flavonols (FLV), chlorophyll (Chl), and SPAD (SPADa) were non-destructively measured, using a portable multi-pigment meter (MPM-100, ADC BioScientific Ltd., Hoddesdon, UK), on five fully expanded leaves per plant, at the following phenological stages: vegetative stage (at week 3), flowering (at week 6), and harvesting (at week 9), according to Ali et al. (20). MPM-100 utilizes ratio fluorescence to determine flavonol content (F660nm/F325nm) and leaf transmittance in the near and far infrared to measure chlorophyll content (T850nm/T720nm) and SPAD. The maximum quantum efficiency of PSII (Fv/Fm) was measured on the same leaves, at the same phenological stages, using a FluorPen (FP-100, Photon Systems Instruments, Drásov, Czech Republic), according to Khalid et al. (2024). Specifically, for each plant within each treatment, five fully expanded leaves were used to assess Fv/Fm during the daytime. For Fv/Fm measurements, the leaves were dark-adapted for 20 min using light-retaining clips before taking the readings.

2.8. Vegetative and reproductive data collection

Plants were monitored weekly for a total of 16 weeks, until their uprooting. The following vegetative parameters were recorded for each plant and treatment: plant height, measured weekly from the stem base to the apex using a tape measure, was detected from the day of transplanting until topping, which was performed 11 weeks post-transplant to halt vegetative growth and enhance fruit quality; number of leaves was recorded weekly for 6 weeks, until defoliation, a technique used to promote fruit ripening. The stem diameter, measured 5 cm above the ground using a digital caliper, was recorded every other week for 12 weeks, for a total of 6 measurements, until topping.

Fruits were harvested at the mature color stage, following standard practices, from April to June. For growth-related parameters (plant height, stem diameter, and number of leaves), all plants within each treatment were individually measured to provide a more reliable estimate of the treatment effect on plant response.

At the harvest date, for each plant within each treatment, the number of fruits and their weight were recorded; this data was used to calculate the yield (g/plant).

Fruit fresh weight was recorded in grams (g) using an electronic scale (KERN® EMB 1000–2, Vicenza, Italy). Moreover, during the harvesting season, fruits were collected three times, thus fruits were analysed considering, other than the treatment, also the harvest date considering the truss position: lower trusses (1st and 2nd trusses, harvested at week 10), medial trusses (3rd 4th and 5th trusses, harvested at week 13) and upper trusses (6th, 8th and 9th trusses, harvested at week 16).

Harvested fruits collected from all plants within each treatment and harvest date were pooled to create a composite sample of fifty uniform fruits.

Fruit size was determined by measuring both equatorial (fruit width-FL) and longitudinal diameters (fruit length-FL) using a digital caliper, with results expressed in millimeters (mm).

2.9. Physico-chemical characterization of tomato fruits

To measure color, texture, Total Soluble Solids (TSS), a representative subsample of 50 fruits per treatment and harvest date was considered, and each fruit was measured.

Finally, a batch of 50 fruits per treatment was used to obtain juice by squeezing the fruits and removing the seeds with a sieve. The juice was then used to measure the Titratable Acidity (TA), Electrical Conductivity (EC) and pH. All analyses were performed in triplicate.

2.9.1. Colorimetric analysis

The color properties of the tomato skin were measured using a colorimeter (CM-36dG, Minolta Co., Osaka, Japan) with a standard D65 illuminant (Commission Internationale de l'Eclairage, https://cie.co.at/datatable/cie-standard-illuminant-d65). CIE-Lab color parameters including L* (lightness), a* (redness-greenness), and b* (yellowness-blueness), were recorded using reflectance at an 8 MAV lighting area aperture and a 10° standard observer position. Measurements were taken directly on the fruit surface in two different areas under consistent positioning and controlled lighting for accuracy. The instrument was calibrated before each analysis with appropriate reference standards for "zero" and blank. Each measurement was performed in triplicate, and the mean values were calculated for further analysis.

2.9.2. Texture analysis

Texture profile analysis was conducted using a TA.XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK) equipped with a 30 kg load cell. A P/3 flat-end cylindrical stainless-steel probe was used to perform a penetration test at a velocity of 1 mm/s, with a total penetration distance of 10 mm into the tomato pericarp at ambient temperature (25 ± 1°C). Firmness was measured as the maximum force required for penetration, expressed in Newtons (N). To ensure accuracy and reliability, each sample was tested with a total of eight replicates.

2.9.3. Total soluble solids (TSS)

TSS content was determined using the juice extracted from 50 fruits per treatment. A few drops of juice were analyzed with an optical portable refractometer (Hanna Instruments, Woonsocket, RI, USA). The results were reported in °Brix.

2.9.4. Titratable acidity (TA)

Titratable acidity was measured using a titration method reported by Teka, 2013. A 5 g aliquot of tomato juice was diluted with 100 mL of distilled water, and phenolphthalein was used as an indicator. The sample was then titrated with 0.1 N NaOH until the endpoint was reached, and TA was calculated as citric acid equivalents using the following equation:

$$\frac{(\textbf{Vol NaOH(ml)} \quad \textbf{x} \quad \textbf{0.1} \quad (\textbf{normality NaOH}) \quad \textbf{x} \quad \textbf{0.064})}{\textbf{gJuice}} \text{x100}$$

where 0.064 is the milliequivalent factor for citric acid. The sugar/acid ratio (TSS/TA) was subsequently calculated.

2.9.5. Electrical conductivity (EC)

The electrical conductivity (EC) of the tomato juice was measured using an EC meter (Knick Elektronische Messgeräte GmbH & Co. KG Beuckestraße, Germany). Electrodes were fully immersed in the juice, and measurements were recorded once stabilized. Each sample was analyzed in triplicate at 25°C.

2.9.6. pH determination

The pH of the juice was determined using a portable pH meter (LLG-pH meter 5, Hyde Manchester, UK). Measurements were performed in triplicate, and the mean values were used for subsequent analyses.

2.9.7. Total polyphenol content (TPC) and antioxidant activity (DPPH Assay)

A subsample of 50 representative fruits selected among fruits harvested from all the plants within each treatment and harvest date was homogenized using a home blender (Pimmy 200 W, Ariete, Florence, Italy). From the resulting tomato pulp, three indipendent extractions

were performed, each starting from 1 g of the sample, to provide analytica replicates for subsequent determinations. Total polyphenol content was determined using Folin–Ciocalteu's phenol reagent (Martelli et al. 2020). The pulp was then mixed with 20 mL of methanol:acetone (70:30 v/v) and thoroughly homogenized. The mixture was subsequently centrifuged at 3500 rpm for 10 mins to separate the solid and liquid phase. After centrifugation, a 250 μL aliquot of the extract was mixed with 1 mL of Folin–Ciocalteu reagent (1:10 v/v) and 2 mL of 20 % sodium carbonate solution. The mixture was incubated in the dark for 30 min at room temperature, and absorbance was measured at 760 nm using a spectrophotometer (JASCO V-530, Easton, MD, USA). Polyphenol content was quantified using a gallic acid calibration curve (10–100 mg/kg) and expressed as mg GAE/Kg FW.

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay described by Abram et al. (2015), with slight modifications. A 100 μL sample extract or standard solution was mixed with 2.9 mL of 0.05 mM DPPH in ethanol and incubated in the dark for 30 min at room temperature. The absorbance of the reaction mixture was recorded at 517 nm using a spectrophotometer (JASCO V-530, Easton, MD, USA). Trolox (Sigma-Aldrich, St. Louis, MO, USA) was used as a reference standard to generate a calibration curve from 0.1 to 1 m (5 points), and results were expressed as mM (Trolox Equivalent Antioxidant Capacity: TEAC). Radical inhibition percentage (I%) was calculated as follows: I% = [(AbsB—AbsS)/AbsB] × 100 where AbsB is the blank absorbance, and AbsS is the sample/standard absorbance. Each extract was analyzed in duplicate, with three consecutive measurements per replicate to ensure accuracy.

2.10. Lycopene content

Lycopene was extracted from 1 g of tomato pulp using 10 mL of hexane: acetone: ethanol (2:1:1 v/v) according to Tambunan et al. (2018). The mixture was stirred in the dark for 2 h and centrifuged at 3500 rpm for 10 min at room temperature. The absorbance of the hexane layer was measured at 473 nm and 502 nm using a spectrophotometer (JASCO V-530, Easton, MD, USA) and results were expressed as mg/Kg. Lycopene concentration was calculated using the following formula:

$$c = \frac{A}{\varepsilon \times d}$$

where A is absorbance, ε (1280 $L \cdot mol^{-1} \cdot cm^{-1}$) is the molar extinction coefficient, and d is the path length of the cuvette.

2.11. Microbial community analysis

To understand the effect of different treatments on microbial soil community, analysis was performed using BIOLOG system with BiologEcoplate. For each treatment, a sample of soil was collected during different stages of plant life cycle: vegetative, flowering, and harvest. The analysis was performed following the protocol by Agosti et al. 2024. Briefly, 5 g of samples were added to 50 mL Ringer solution (VWR, UK) and shaken at room temperature (22°C) for 30 min at 200 rpm. After 20 min settling, 1 mL supernatant was diluted in 9 mL of Ringer. 100 μL of solution per well were inoculated into EcoPlates in triplicate and incubated at 30°C. The plates were analyzed by the Microplate Reader (dual-wavelength data: OD590) after 24, 48, and 72 h to observe the dynamic utilization of different carbon sources from microbes. In this case, data were recorded at 48 h as, at this point, the maximum change signal development was observed.

2.12. Statistical analysis

Statistical analyses were performed using JASP software (version 0.19.3). Initially, the normality of the data was assessed. When data did not meet the assumption of normality, the non-parametric Kruskal-Wallis test was applied. Mean separation was conducted using Tukey's test for parametric analyses and Dunn's test for non-parametric cases, both at a significance level of $p \leq 0.05$.

A one-way Analysis of Variance (ANOVA) was carried out for all parameters, considering the factor "Treatment" per each phenological stage (e.g., FLV, SPAD, Chl, Fv/Fm) or at each truss position on the plant (FL, FW, L*, a*, b*, a*/b*, Firmness, TSS, TA, TSS/TA, EC, pH, TPC, AO, LC). The truss position was not included as a factor, in the statistical analysis, in order to evaluate the overall impact of the biostimulant on tomato production and quality, since it is well known that, in indeterminate tomato cultivars, fruit ripen progressively throughout the season.

For plant height, leaf number, and stem diameter, a repeated measures analysis was performed, considering the effects of "Time" and "Treatment" over the experimental period (12 weeks for height and diameter, until topping; 6 weeks for leaf number, until defoliation). Mean comparisons have been performed resorting to Tukey's test ($p \leq 0.05$).

Regarding total yield parameters (number of fruits, yield per plant, and individual fruit weight), a one-way ANOVA was conducted considering the "Treatment" factor. The plant responses to the application of FTB at different concentrations were summarized using a heatmap generated with the MetaboAnalyst 6.0 web tool. Pearson's distance was applied to assess similarity, and a heatmap was created considering the 4 different treatments performed. Because the variables were expressed in different units and showed large differences in scale, the data were transformed using a $\log_{10}$ function. This transformation reduces the impact of extreme values and allows for a more consistent comparison across measurements. The results were then visualized with a color scale (red = increased values, blue = decreased values).

Statistical analysis of data derived from Biolog analysis, were conducted with Rstudio (version 4.3.2) for principal component analysis (PCA).

3. Results

3.1. Modelling fermentation of tomato By-product

Fermentation of unsold mature green and red ripe tomatoes was monitored by plate counts on optimal agar medium, performed immediately after inoculation and at the end of the fermentation period. The experiments were conducted using the worksheet generated by MODDE Pro v13.00 software, and the plate count results obtained at the end of the fermentations are reported in Table S1. In general, all tested strains were able to grow in the tomato mixture and were not negatively affected by the fermentation conditions. The average final microbial load was 8.64 log CFU/mL, corresponding to an average increase of 1.63 log CFU/mL. Plate count results obtained at the end of the fermentation process were analysed with MODDE Pro v13.00 software to verify the effects of the factors considered on microbial growth and to find the best condition for the fermentation process. The accuracy of the model built to describe the growth of the microorganisms was described using four statistical parameters: R2, Q2, Model validity, and Reproducibility (R2 = 0.90, Q2 = 0.75, Model validity = 0.43, Reproducibility = 0.87) (Fig. 2a). Considering the good values obtained for the four statistical parameters, the model was considered suitable for predicting microbial growth. In particular, the optimization tool of the software was employed to identify the optimal fermentation conditions, and a response surface contour was built to summarize the results (Fig. 2b). According to the model prediction, the most suitable strain for fermenting the tomato mixture was *Weissella cibaria* 4253, with optimal fermentation conditions of 24 h at 30°C. Based on these model indications, the corresponding fermentation parameters were selected and employed for additional fermentations, made to validate the

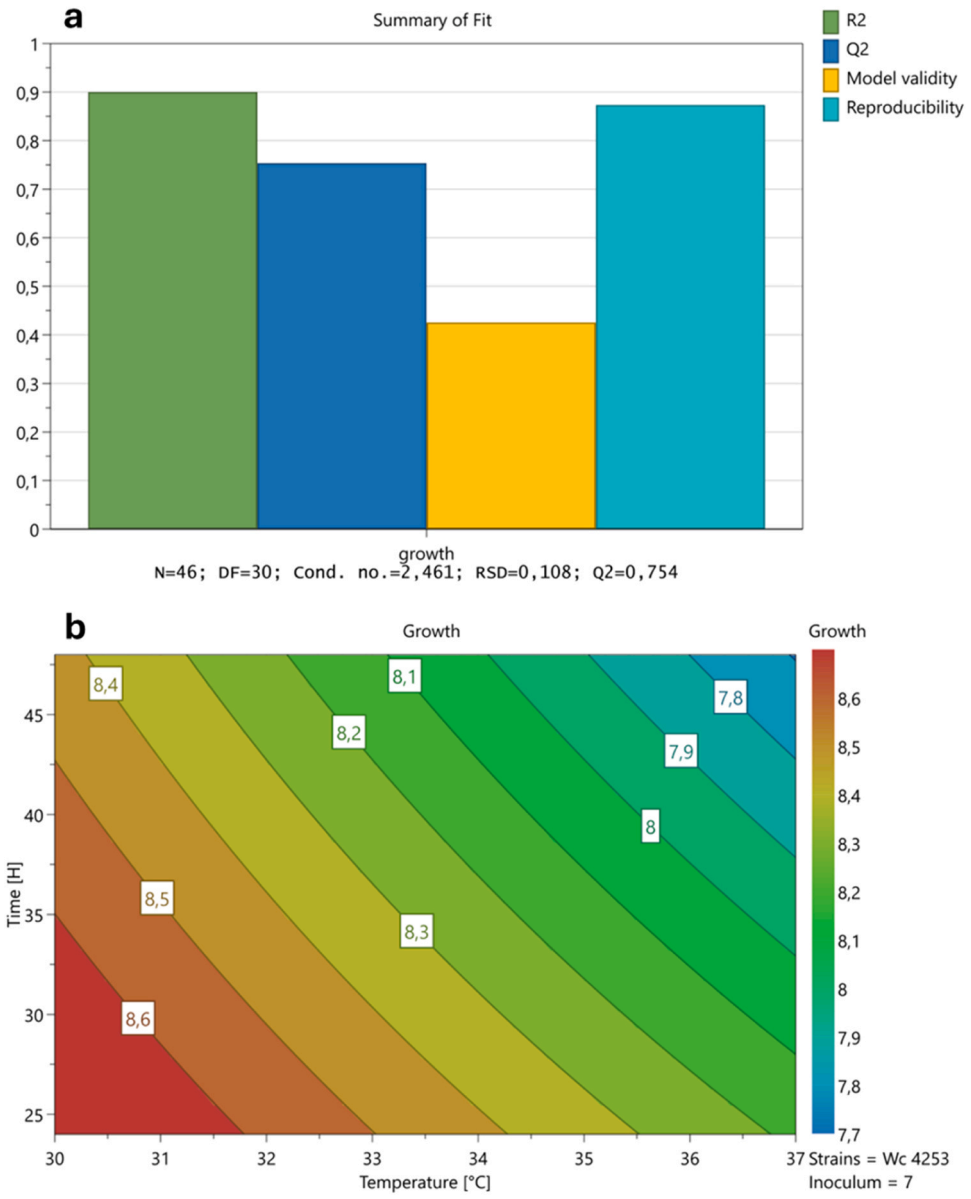


Fig. 2. a) Summary of Fit plot representing the values of R2 (green bar), Q2 (blue bar), model validity (yellow bar), and reproducibility (light blue bar); b) Response surface plot as a function of time and temperature for Weissella cibaria 4253 (Wc 4253) growth (log UFC/mL). The colours represent the minimum (blue) and maximum (red) growth predictions.

predictions. As shown in the contour surface plot (Fig. 2b), these conditions were expected to lead to an estimated increase in microbial load of 1 log CFU/mL. The prediction was validated and confirmed by the additional fermentations with *Weissella cibaria* 4253. The FTB was, then, prepared inoculating the strain at a concentration of 6 log CFU/g, with a final concentration of 7.34 log CFU/g.(Table 1)

3.2. Non-destructive in vivo estimation of leaf pigments and Fv/Fm

The statistical analysis performed using one-way ANOVA for each phenological stage (vegetative, flowering, and harvesting), considering only the "Treatment" factor of the data regarding flavonol (FLV) content, chlorophyll (Chl) content, SPAD values, and Fv/Fm is reported in Table 2. No statistically significant differences attributable to the treatment were observed.

Table 1
Chemical-physical characterization of the fermented tomato-based biostimulant (FTB) used on tomato plants. Values are reported as mean ± standard deviation.

Parameter	Measuring Unit	FTB
pH		4.14 ± 0.07
TSS	°Brix	6.66 ± 0.03
Glucose	mg/mL	19.37 ± 0.23
Fructose	mg/mL	10.72 ± 0.20
EC	mS/cm	0.92 ± 0.07
Na^+	ppm	200.00 ± 5.77
NO_3^-	ppm	220.00 ± 10.32
K^+	ppm	1720.00 ± 80.00
TPC	mg GAE/Kg	378.80 ± 46.57
AO	mM TEAC	0.031 ± 0.002

Abbreviations: GAE: Gallic Acid Equivalent: TEAC: Trolox Equivalent Antioxidant Capacity

Table 2
Influence of Fermented Tomato Biostimulant (FTB) and Plant Phenological Stage on leaf pigments and photosynthetic activity.

Plant Phenological Stage	Treatment	FLV±SD	CCIa±SD	SPAD±SD	Fv/Fm±SD
Vegetative	FTB0	0.44 ± 0.06	12.70 ± 0.82	31.91 ± 0.81	0.75 ± 0.01
	FTB1	0.44 ± 0.06	14.25 ± 0.48	33.4 ± 0.44	0.77 ± 0.00
	FTB2	0.07 ± 0.00	22.61 ± 1.89	38.95 ± 1.05	0.77 ± 0.00
	FTB3	0.36 ± 0.07	15.23 ± 1.58	33.98 ± 1.23	0.74 ± 0.02
p-value		0.266	0.282	0.093	0.319*
Flowering	FTB0	0.30 ± 0.07	13.80 ± 0.59	33.00 ± 0.55	0.75 ± 0.02
	FTB1	0.06 ± 0.00	18.75 ± 1.27	36.71 ± 0.87	0.70 ± 0.01
	FTB2	0.50 ± 0.04	16.03 ± 1.09	34.78 ± 0.87	0.72 ± 0.01
	FTB3	0.22 ± 0.03	12.70 ± 0.98	31.83 ± 0.95	0.74 ± 0.02
p-value		0.114	0.386	0.361	0.304
Harvesting	FTB0	0.09 ± 0.01	25.93 ± 2.61	40.51 ± 1.28	0.82 ± 0.00
	FTB1	0.33 ± 0.06	14.16 ± 1.30	31.9 ± 0.75	0.83 ± 0.00
	FTB2	0.31 ± 0.05	12.76 ± 0.80	31.94 ± 0.84	0.81 ± 0.00
	FTB3	0.07 ± 0.00	20.26 ± 1.07	37.71 ± 0.65	0.82 ± 0.00
p-value		0.092*	0.057	0.072	0.488

Statistical analysis was performed using one-way ANOVA (Tukey's test, $p \leq 0.05$) for each truss position on normally distributed data. For variables not meeting the normality assumption (indicated by *), the Kruskal–Walli's test was applied, followed by Dunn's post-hoc test. Within each parameter and within each phenological stage, different letters indicate values statistically different. Results are expressed as mean ± standard deviation (SD). FTB0: 0 mL/L of FTB, FTB1: 100 mL/L of FTB, FTB2: 200 mL/L of FTB, and FTB3: 300 mL/L of FTB, FLV: Flavonols, CCIa: Chlorophyll content, Fv/Fm: value to describe variations in the photonic efficiency of Photosystem II.

3.3. Vegetative and productive parameter analysis

The effect of the treatments was evaluated on the vegetative parameters of the plants, specifically on plant height, leaf number, and stem diameter. The treatments showed no effect on plant growth (Figure S3), as the statistical analysis indicated that the increase in the measured parameters was solely related to time and, consequently, to normal plant vegetative development. No statistically significant influence of the tested treatments was detected on the number of fruits harvested; however, differences were observed in the total yield per plant and the fruit weight (Table 3). Specifically, FTB1 plants exhibited the highest yield and fruit weight, with an increase of 23 % in yield and 8 % in weight compared to FTB0 (Table 3).

The one-way ANOVA analysis performed on the morphological traits of tomato fruits (fruit length and width), separately for each truss position, is reported in Table 4. The effect of the treatment on fruit length varied according to truss position; if in the lower truss, no significant differences in fruit length were detected among treatments, for the medial ones, FTB1 fruits were significantly longer (+6 %) than those of FTB0, while, for the upper trusses, no statistical significant differences were detected among treatments. It is important to underline that the maximum fruit length was observed in fruits harvested from the medial trusses (Table 4).

Considering fruit width, the statistical analysis highlighted that there were no differences during harvest from the lower and upper trusses, while statistically significant differences were observed in the medial ones. It was noted that in the medial trusses, only fruits harvested from plants treated with FTB3 had a smaller width compared to the others. A similar trend to that observed for fruit length was found for fruit width, but only in FTB0 fruits, with the highest values recorded in fruits from the medial trusses (Table 4).

Table 3
Effect of Fermented Tomato Biostimulant (FTB) on number of fruits, Yield per plant, and individual fruit weight.

Treatment	n° of fruits±SD	Yield/plant^{-1}±SD (g)	Fruit weight±SD (g)
FTB0	96.5 ± 9.3^{a}	1170.3 ± 121.3^{b}	12.41 ± 0.44^{bc}
FTB1	118.3 ± 9.0^{a}	1443.4 ± 52.4^{a}	13.41 ± 0.49^{a}
FTB2	108.8 ± 14.3^{a}	1172.7 ± 145.5^{b}	11.89 ± 0.52^{c}
FTB3	93.7 ± 2.2^{a}	1062.6 ± 82.7^{b}	12.96 ± 0.54^{b}

One-way ANOVA (Tukey's test, $p \leq 0.05$) Within each parameter, different letters indicate values statistically different. Results are expressed as mean ± standard deviation (SD). FTB0: 0 mL/L of FTB, FTB1: 100 mL/L of FTB, FTB2: 200 mL/L of FTB, and FTB3: 300 mL/L of FTB.

Table 4
Effect of Fermented Tomato Biostimulant (FTB) and Truss Position on Fruit Morphological Parameters.

Truss Position	Treatment	FL±SD (mm)	FW ±SD (mm)
Lower	FTB0	34.07 ± 0.39^{ab}	24.74 ± 0.43
	FTB1	35.21 ± 0.39^{a}	25.33 ± 0.31
	FTB2	33.81 ± 0.35^{b}	25.46 ± 0.19
	FTB3	34.00 ± 0.23^{ab}	25.38 ± 0.33
p-value		0.033	0.404
Medial	FTB0	37.04 ± 0.45^{b}	26.00 ± 0.40^{a}
	FTB1	39.33 ± 0.71^{a}	26.17 ± 0.43^{a}
	FTB2	38.08 ± 0.57^{ab}	26.31 ± 0.36^{a}
	FTB3	37.59 ± 0.53^{ab}	24.23 ± 0.36^{b}
p-value		0.049	0.002
Upper	FTB0	34.12 ± 1.02	22.12 ± 0.36
	FTB1	33.48 ± 1.26	23.17 ± 0.53
	FTB2	31.51 ± 0.41	23.06 ± 0.42
	FTB3	35.08 ± 1.08	23.73 ± 0.37
p-value		0.096	0.084

Statistical analysis was performed using one-way ANOVA (Tukey's test, $p \leq 0.05$) for each truss position. Within each parameter, different letters indicate values statistically different. Results are expressed as mean ± standard deviation (SD). FTB0: 0 mL/L of FTB, FTB1: 100 mL/L of FTB, FTB2: 200 mL/L of FTB, and FTB3: 300 mL/L of FTB, FL: fruit length, FW: fruit width.

3.4. Physico-chemical characterization of tomato fruits

Table 5 presents the results of the statistical analysis carried out on fruit color parameters and fruit firmness, considering the influence of the "Treatment" factor separately for each truss position. In the lower trusses, no statistically significant differences in fruit brightness were observed between fruits harvested from treated and untreated plants (Table 5); while, in upper trusses, fruits from FTB3-treated plants exhibited a brightness significantly higher than control, with a 7 % increase with respect to the FTB0 fruits (Table 5). On the contrary, in medial trusses, FTB3 presented the lowest brightness value. An interesting finding is that, if in the FTB0 and FTB2 fruits, the highest brightness was observed in medial trusses, in FTB1 lower and medial trusses had the same levels of lightness, and, finally, in FTB3, as harvesting progressed and thus truss position changed, fruit brightness remained stable.

Considering the fruit redness (a*), treatments tested exerted a different influence depending on the truss position considered. In

Table 5
Influence of Fermented Tomato Biostimulant (FTB) and Truss Position on fruit color parameters.

Truss Position	Treatment	L*	a*	b*	a*/b*	Firmness (N)
Lower	FTB0	33.46 ± 0.14	17.26 ± 0.42^{a}	16.63 ± 0.21	1.03 ± 0.02^{a}	6.99 ± 0.24^{a}
	FTB1	33.70 ± 0.17	15.28 ± 0.59^{b}	16.19 ± 0.25	0.94 ± 0.03^{ab}	6.55 ± 0.23^{ab}
	FTB2	33.72 ± 0.17	16.97 ± 0.46^{ab}	16.93 ± 0.24	1.00 ± 0.02^{ab}	6.58 ± 0.23^{ab}
	FTB3	33.66 ± 0.11	15.43 ± 0.52^{ab}	16.53 ± 0.21	0.93 ± 0.02^{b}	5.89 ± 0.26^{b}
p-value		0.636	0.011	0.174	0.036	0.026
Medial	FTB0	33.86 ± 0.16^{ab}	20.55 ± 0.35^{a}	17.28 ± 0.21^{a}	1.19 ± 0.01^{a}	5.85 ± 0.23^{b}
	FTB1	34.12 ± 0.12^{a}	19.83 ± 0.45^{a}	17.32 ± 0.18^{a}	1.14 ± 0.02^{ab}	7.57 ± 0.32^{a}
	FTB2	34.25 ± 0.14^{a}	20.14 ± 0.36^{a}	17.78 ± 0.22^{a}	1.13 ± 0.01^{ab}	6.54 ± 0.23^{ab}
	FTB3	33.42 ± 0.13^{b}	17.88 ± 0.39^{b}	16.22 ± 0.23^{b}	1.10 ± 0.02^{b}	6.91 ± 0.29^{ab}
p-value		< 0.001	< 0.001	< 0.001	0.028	0.002
Upper	FTB0	32.69 ± 0.25^{b}	17.78 ± 0.73	15.74 ± 0.24	1.12 ± 0.04	6.60 ± 0.32^{ab}
	FTB1	32.53 ± 0.21^{b}	17.2 ± 0.71	15.97 ± 0.2	1.07 ± 0.04	7.66 ± 0.25^{a}
	FTB2	32.96 ± 0.20^{ab}	18.86 ± 0.48	16.42 ± 0.32	1.15 ± 0.03	6.55 ± 0.23^{b}
	FTB3	33.24 ± 0.12^{a}	18.25 ± 0.73	16.05 ± 0.22	1.13 ± 0.03	6.91 ± 0.29^{ab}
p-value		0.025*	0.395*	0.307	0.645*	0.029

Statistical analysis was performed using one-way ANOVA (Tukey's test, $p \leq 0.05$) for each truss position on normally distributed data. For variables not meeting the normality assumption (indicated by *), the Kruskal–Walli's test was applied, followed by Dunn's post-hoc test. Within each parameter, different letters indicate values statistically different. Results are expressed as mean ± standard deviation (SD). FTB0 = 0 mL/L, FTB1 = 100 mL/L, FTB2 = 200 mL/L, FTB3 = 300 mL/L of FTB.

particular, the statistical analysis revealed lower redness in fruits harvested from FTB1-treated plants in the lower trusses. In the medial trusses, fruits from FTB3-treated plants showed the lowest redness compared to other treatments. In the upper trusses, redness levels were comparable across all treatments, with no statistically significant differences observed.

The statistically highest level of this parameter was reached, for all the treatments, for fruits from the medial trusses (Table 5). However, it is interesting to note that while for FTB0 and FTB1 fruits, the redness decreased from medial to upper trusses, for FTB2 and FTB3 fruits, it remained constant in the same interval (Table 5).

For the b* parameter, the statistical analysis evidenced a "Treatment" effect, only for fruits harvested from medial trusses. Specifically, it was found that fruits harvested from FTB3-treated plants were characterized by a lower b* compared to other treatments. It is interesting to note that fruits harvested from FTB0, FTB1 and FTB2 treated plants, a decrease in yellowness was observed from medial to upper trusses, while for FTB3 fruits the level of this parameter remained fairly stable across all the harvesting date (Table 5). The a*/b* ratio was subsequently analyzed, and the statistical analysis revealed a treatment effect in the lower and medial trusses, with fruits from FTB3-treated plants showing lower values compared to those from FTB0-treated plants in both positions. In the upper trusses, no statistically significant differences were detected.

Regarding fruit texture, the statistical analysis was performed separately for each truss position, considering the effect of the "Treatment" factor. The results are presented in Table 5. In the lower trusses, a significant decrease in the texture was observed in FTB3 fruits compared with those of FTB0 (Table 5). In medial and upper trusses FTB1 fruits showed the highest texture (Table 5).

The statistical analysis for the TSS parameter is presented in Table 6. In the lower trusses, fruits from FTB3-treated plants showed the highest °Brix values among treatments. Although no statistically significant differences were observed in the medial and upper trusses, FTB3 fruits consistently exhibited higher values compared to the control, particularly in fruits harvested from upper trusses.

Regarding TA, expressed as a percentage of citric acid, statistical analysis showed an influence of "Treatment" factor for each truss position (Table 6). Within lower trusses, FTB1 fruits were characterized by the highest acidity among all treatments performed. Within the medial trusses, the trend was different, with FTB3 fruits showing a lower TA compared to FTB1 and FTB2 fruits (Table 6). In the upper trusses the lowest TA was detected for FTB1 fruits, while the highest for FTB2. Considering the truss position, for all the treatments studied, TA decreased as the harvest season progressed; particularly interesting it is that, differently from the others, within FTB3 fruits, the TA remained stable from medial to upper trusses (Table 6).

The sugar-acid ratio (TSS/TA), also known as the maturity index, was analyzed, and the results are presented in Table 6. In the lower and medial trusses, the highest values were observed in fruits from FTB3-treated plants. In the upper trusses, the ratio appeared to be unaffected by the treatment. An increase in the TSS/TA ratio was detected as the harvesting season progressed in fact, fruits from upper trusses had a TSS/TA ratio significantly higher than both medial (on average 14 %) and lower (on average 21 %) (Table 6).

The EC parameter was not influenced by the factor "Treatment" in any of the truss position analyzed (Table 6), however it was noticed that fruits harvested from the upper trusses showed values significantly higher (on average 48 %) than those collected from the lower and medial trusses (Table 6).

The effect of the "Treatment" factor on the pH of tomato fruits was evaluated through separate one-way ANOVA analyses for each truss position and the results are reported in Table 6. In lower and medial trusses the highest pH was detected for fruits harvested from non-treated plants (FTB0), while in the upper trusses the trend was reversed, with the highest pH registered for fruits harvested from FTB-treated plants (Table 6).

The one-way ANOVA analysis results of fruit TPC for each truss position revealed a "Treatment" effect, specifically in the lower and medial ones (Table 6). At the lower truss harvest, FTB0 fruits exhibited the highest TPC levels; for the medial trusses, instead, the significantly highest TPC were observed in FTB1 fruits.

Antioxidant activity (AO) increased significantly throughout the harvest times, with a 63 % increase from the lower to the medial trusses on average, and a further 22 % increase from the medial to the upper trusses and, moreover, a significant "Treatment" effect was registered in medial and upper trusses (Table 6). In particular in these truss position, the highest AO was detected for FTB3 fruits. The parameter Lycopene Content (LC) was influenced by the factor "Treatment" in lower and medial trusses (Table 6). Within the lower trusses, fruits from the FTB0 treatment had a lycopene content significantly higher (+43 %) than those from treated plants. However, in the second harvest (fruits from the medial trusses), a significantly positive influence of the treatments was noted, with fruits from FTB3-treated plants exhibiting LC values higher than those from FTB0 treatment (+55 %). Finally, within the upper trusses, any of the treatments tested seemed to influence the LC of fruits.

Table 6
Influence of Fermented Tomato Biostimulant (FTB) and Truss Position on fruit quality parameters.

Truss Position	Treatment	TSS±SD (°Brix)	TA±SD (% citric acid)	TSS/TA±SD	EC±SD (mS)	pH±SD	TPC±SD (mg GAE/Kg)	AO±SD (mMTEAC)	LC±SD mg/Kg
Lower	FTB0	8.23 ± 0.08^{b}	0.83 ± 0.002^{b}	9.88 ± 0.15^{bc}	2.71 ± 0.05	4.46 ± 0.015^{a}	982.49 ± 123.82^{a}	1.01 ± 0.51	965.31 ± 102.4^{a}
	FTB1	8.20 ± 0.15^{b}	0.85 ± 0.001^{a}	9.59 ± 0.31^{c}	2.57 ± 0.06	4.17 ± 0.003^{c}	643.39 ± 48.09^{c}	0.68 ± 0.32	378.39 ± 54.82^{c}
	FTB2	8.43 ± 0.08^{ab}	0.79 ± 0.015^{c}	10.57 ± 0.03^{ab}	2.57 ± 0.13	4.32 ± 0.025^{b}	798.48 ± 48.60^{bc}	0.59 ± 0.27	507.98 ± 118.81^{bc}
	FTB3	8.94 ± 0.17^{a}	0.82 ± 0.003^{b}	10.83 ± 0.39^{a}	2.87 ± 0.18	4.11 ± 0.012^{c}	851.16 ± 92.63^{ab}	0.83 ± 0.27	752.55 ± 190.06^{ab}
p-value		0.015	< 0.001	0.001	0.329	< 0.001	< 0.001	0.397	< 0.001
Medial	FTB0	8.60 ± 0.10	0.72 ± 0.022^{ab}	11.84 ± 0.43^{ab}	2.63 ± 0.13	4.19 ± 0.005^{a}	734.91 ± 50.13^{b}	1.03 ± 0.06^{b}	651.15 ± 205.71^{b}
	FTB1	8.40 ± 0.11	0.74 ± 0.015^{a}	11.30 ± 0.25^{b}	2.81 ± 0.02	4.13 ± 0.005^{b}	952.14 ± 39.49^{a}	1.21 ± 0.08^{ab}	802.03 ± 195.25^{ab}
	FTB2	8.44 ± 0.13	0.76 ± 0.006^{a}	11.06 ± 0.22^{b}	2.67 ± 0.07	4.15 ± 0.005^{b}	650.26 ± 38.45^{b}	1.11 ± 0.20^{b}	666.89 ± 62.10^{ab}
	FTB3	8.73 ± 0.06	0.68 ± 0.031^{b}	12.77 ± 0.73^{a}	2.53 ± 0.11	4.12 ± 0.012^{b}	557.14 ± 47.02^{c}	1.75 ± 0.47^{a}	1015.48 ± 182.17^{a}
p-value		0.178	< 0.001	0.008	0.311	< 0.001	< 0.001	0.009	0.038
Upper	FTB0	8.97 ± 0.03	0.68 ± 0.007^{ab}	13.07 ± 0.16	3.94 ± 0.04	4.13 ± 0.016^{b}	490.53 ± 32.15	1.71 ± 0.17^{ab}	725.76 ± 158
	FTB1	9.64 ± 0.36	0.65 ± 0.014^{c}	14.7 ± 1.25	3.98 ± 0.01	4.27 ± 0.017^{a}	467.76 ± 74.34	1.17 ± 0.12^{b}	882.44 ± 65.84
	FTB2	9.64 ± 0.43	0.69 ± 0.01^{a}	13.94 ± 1.00	3.96 ± 0.03	4.34 ± 0.037^{a}	651.78 ± 178.73	1.35 ± 0.47^{b}	873.08 ± 119.9
	FTB3	10.03 ± 0.52	0.66 ± 0.006^{bc}	15.18 ± 1.35	3.95 ± 0.02	4.28 ± 0.012^{a}	558.12 ± 108.81	2.02 ± 0.28^{a}	874.53 ± 187.61
p-value		0.362*	0.007	0.075*	0.839*	< 0.001	0.298*	0.007	0.366

Statistical analysis was performed using one-way ANOVA (Tukey's test, $p \leq 0.05$) for each truss position followed by on normally distributed data. For variables not meeting the normality assumption (indicated by *), the Kruskal–Wallis test was applied, followed by Dunn's post-hoc test. Within each parameter, different letters indicate values statistically different. Results are expressed as mean ± standard deviation (SD). FTB0: 0 mL/L of FTB, FTB1: 100 mL/L of FTB, FTB2: 200 mL/L of FTB, and FTB3: 300 mL/L of FTB, TSS: Total Soluble Solids, TA: Titratable acidity, EC: Electrical Conductivity, TPC: Total Polyphenol Content, AO: Antioxidant Activity, LC: Lycopene Content.

3.5. Heatmap analysis of biostimulant influence on tomato quality and nutritional properties

A heatmap analysis (Fig. 3) was conducted for all evaluated parameters to provide an overview of the effects of the FTB applied at different concentrations (FTB0, FTB1, FTB2, and FTB3). The results revealed distinct response patterns in fruit quality attributes depending on the FTB treatment. The heatmap (Fig. 3) illustrates the relative changes of fruit quality parameters across tomato plants treated with increasing concentrations of the experimental biostimulant (FTB0, FTB1, FTB2 and FTB3). Red cells indicate values above the mean, whereas blue cells indicate values below the mean. Pearson distance was applied as the similarity metric, but no clustering was performed, allowing a direct visual comparison of treatment-specific effects on individual quality attributes. FTB3 was associated with higher values of TSS/TA and TSS. FTB3 also showed higher levels of lycopene content and antioxidant activity, whereas total polyphenol content was higher in FTB0 fruits, and lower in FTB3 fruits. Lightness (L*) was relatively higher in FTB2 and lower in FTB0. Moreover, FTB3 was associated with higher electrical conductivity (EC) and lower titratable acidity (TA). Finally, FTB1 treatment was linked to an increase in Texture.

3.6. Monitoring soil microbial community

The results of substrate utilization patterns of soil microbial community are reported in Fig. 4. The ten most significant variables include polymers, amino acids, phenolic compounds and carboxylic acids. Depending on when the soils were sampled (Fig. 4a) and the treatment to which they were subjected (Fig. 4b), we can observe that the samples differ in their metabolic capacities. Looking at the ripening stage that corresponds to the last point of detection, FTB0 and FTB1 samples were both grouped in the metabolization of the amino acid L-asparagine, while sample FTB2 and FTB3 on the opposite side show different metabolic capacity, one for phenyletilamine and the other for alpha cyclodextrin. In addition, a separation between vegetative, flowering, and harvest stage was found.

4. Discussion

In this study, FTB was distributed, as an experimental biostimulant, to indeterminate tomato plants, cv. "Solarino", from February to June. This study aimed to assess the effects of FTB on the morpho-physiological traits of tomato plants, physico-chemical properties of the fruits, and overall fruit yield.

Before discussing the results in detail, it is important to note that, since no commercial product of this type exists and this represents one of the first attempts to valorize waste biomass through fermentation, the application rates of the biostimulant were tested experimentally. The selected doses (100, 200, and 300 mL L^{-1}) were based on those reported by Adekiya et al. (2022), who applied an organic biostimulant obtained by a spontaneous fermentation of fresh mix of leaves and stems of *Tithonia diversifolia* biomass at lower concentrations (5, 15, 25, 35, 45, and 55 mL L^{-1}). The higher doses in this study were chosen to explore potential effects beyond the previously tested range. In a previous study by Kisvarga et al. (2020), an alfalfa-based biostimulant, both fermented and non-fermented through lactic fermentation, was tested, and interesting results were observed for both types of products. However, the fermented product proved to be more stable at room temperature and exhibited improved nutritional characteristics, due to the conversion of sugars—present at high levels in the non-fermented product—into organic acids, which contributed to lowering the pH to around 4.

In this study, since, the main purpose was to assess the efficacy of an experimental biostimulant, rather than to analyze the impact of fermentation on tomato fruit waste or to contrast fermented and non-fermented substrates, a control treatment consisting of non-fermented tomato fruit waste was not included in the experimental design;

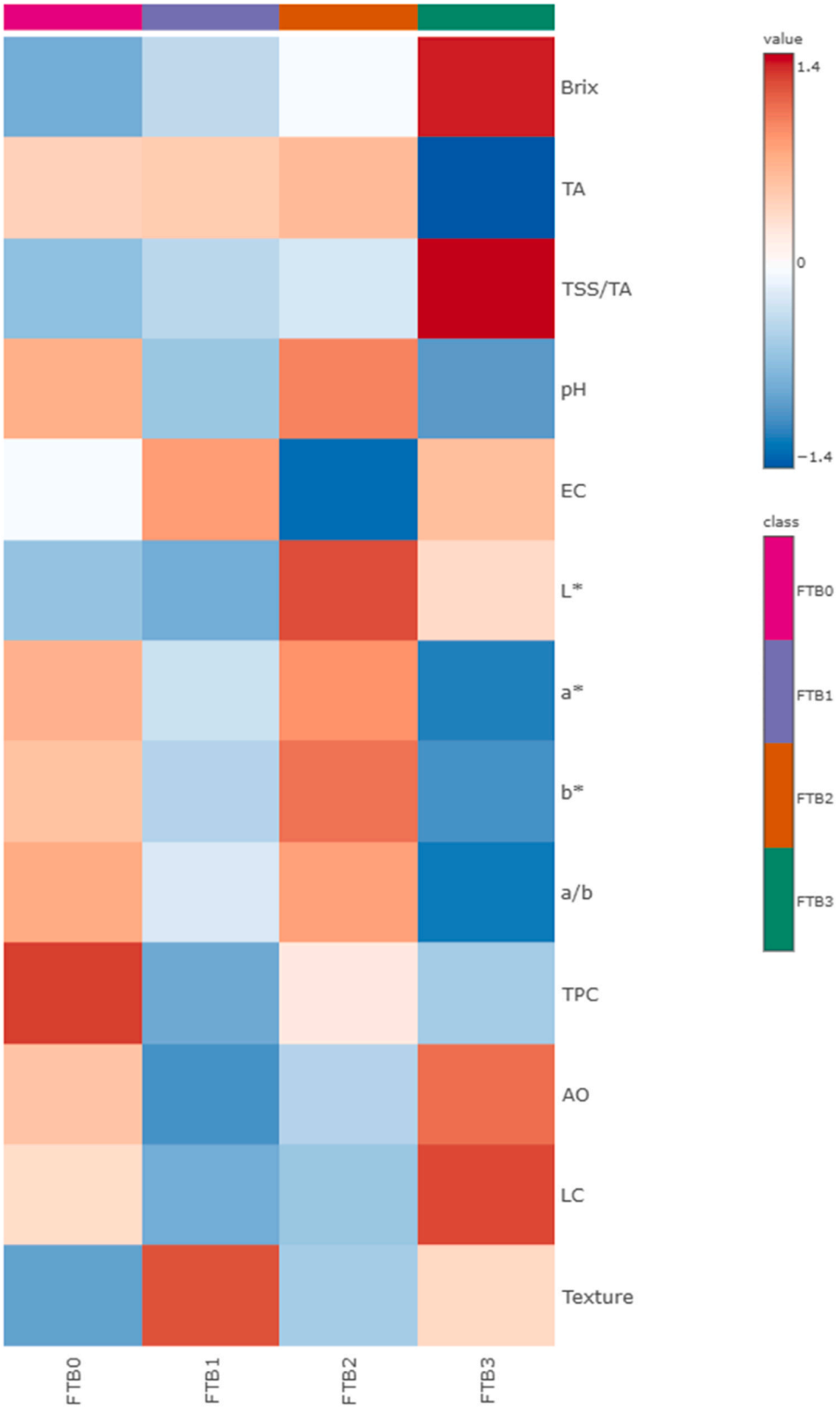


Fig. 3. Heatmap created with Pearson's distance based ondisplaying the relative distribution offruit quality parameters of tomato plants grown under different concentrations of an experimental tomato-based biostimulant (FTB0: 0 mL/L – control; FTB1: 100 mL/L; FTB2: 200 mL/L; FTB3: 300 mL/L) applied weekly to soilless-grown tomato plants (cv. Solarino). Colors indicate deviations from the mean value (red: higher; blue: lower).

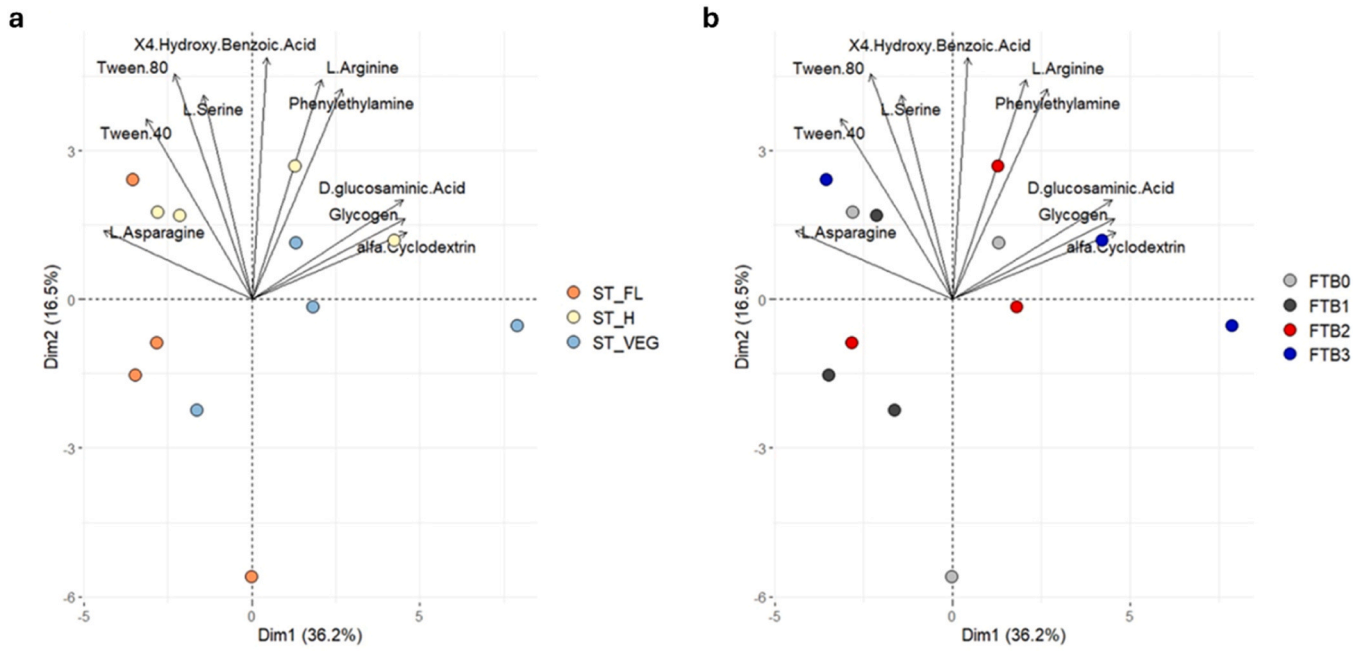


Fig. 4. Biplot relative to the principal component analysis carried out on the metabolic activity of soil microbial community and representing the ten most impacting variables, data are displayed for soil sample collected at different plant stage (a) and subjected to different treatment (b).

instead, different concentrations of the fermented product were evaluated.

The treatment was applied once a week from the beginning of the vegetative cycle until fruit harvest. Whereas some studies recommend a staggered application of biostimulants according to the plant's phenological stage—reflecting the varying demands during seedling, flowering, and fruiting phases of tomato (Mannino et al., 2020; Della Lucia et al., 2022), other authors have reported the use of a constant application at a uniform dose throughout the entire crop cycle (Manai et al., 2024; Vultaggio et al., 2025). The experimental biostimulant tested in the present study, was produced by fermenting unsold mature green and red ripe tomatoes with *Weissella cibaria* 4253. LABare known for their ability to grow in different agricultural wastes, including tomatoes, and produce several high-value compounds (Ricci et al. 2019; Pereira et al. 2023). Prior to discussing the main findings, it should be emphasized that this study represents a preliminary trial, with results based on data from a limited number of plants and fruits, considered as biological replicates in the statistical analysis. Although this limits the assessment of within-treatment variability, it was considered appropriate for the exploratory nature of the study and the homogeneous greenhouse conditions.

4.1. Assessment of model validity and reproducibility

A model was built to describe the growth of the five LAB strains tested in the DoE, and four statistical parameters described the accuracy of the model. The R2 value indicates how well the data fit the model, and an R2 value greater than 0.5 reflects a good significance (John et al. 2020). This is the case of the proposed model, which showed an R2 value of 0.90 (Fig. 2a). The Q2 value reflects the precision of the model in prediction, and a value of 0.1 indicates a significant model. The model had a value of 0.75 (Fig. 2a), and values above 0.5 are considered indicative of strong predictive power (Consonni et al. 2010). In addition, the Model validity value was 0.43 (Fig. 2a), and values higher than 0.25 indicate a valid model, appropriate for the problem. Reproducibility, the fourth parameter, was 0.87 (Fig. 2a), a good value considering that values higher than 0.5 signify reproducible results (Eriksson et al. 2008). *Weissella cibaria* 4253 resulted as the most suitable strain for fermenting the tomato mixture. According to Di Cagno et al. (2009), *Weissella* strains were previously isolated from tomatoes and have already demonstrated the ability to ferment and positively influence these matrices.

4.2. Physico-chemical characterization of the fermented tomato-based biostimulant (FTB)

The experimental biostimulant based on a mix of green and red tomatoes showed a pH of 4.14 ± 0.07, consistent with a product fermented by lactic acid bacteria (Barna et al. 2021; Simões et al. 2022). Notably, it contained a significant amount of K^+, an essential ion crucial for the production of marketable fruits, leaf and fruit composition, enzyme activation, protein and carbohydrate synthesis, and osmotic balance, directly influencing the content of bioactive compounds and the nutritional value of the fruits (Yumita et al. 2023; Johnson et al. 2022).

Numerous studies have demonstrated that fermentation can enrich specific valuable components in agrowastes, thereby varying their bioactivity (Erskine et al. 2023). The FTB used in this study exhibited low values of total phenolic content and antioxidant activity. This can be explained by the fact that the fermented tomato mix is composed of both green and red fruits, which naturally vary in phenolic content and associated antioxidant activity depending on their ripening stage. For instance, Hdider et al. (2013) reported that phenolic levels in tomato fruits vary according to cultivar and ripening stage, with some cultivars showing peaks at the orange-red or green-orange stages, while others may have lower levels at the same stages.

4.3. Chlorophyll content and photosynthetic efficiency in response to biostimulant treatments

In Cardarelli et al. (2020), the SPAD value, used as a non-destructive measurement of leaf chlorophyll content and an indicator of the photoinhibition of the Photosystem II (PSII), measured in tomato did not appear to be affected by the combined treatment with a microbial biostimulant based on mycorrhizal fungi and a non-microbial biostimulant. In this study, however, it is noteworthy that during the vegetative stage,

plants treated with FTB2 showed a 9 % higher SPAD value compared to untreated plants. Interestingly, the detection of statistically significant differences at a specific phenological stage is partially in contrast with previous findings by Dasgan et al. (2024) and Weisser et al. (2023), where the application of biostimulants did not result in treatment-dependent effects associated with the plant's developmental stage at which the measurements were performed.

4.4. FTB1 treatment increases yield and fruit weight

Tomato yield is a complex trait, influenced by multiple genetic, environmental, and physiological factors that collectively shape plant growth and development (Iacuzzi et al. 2024). To account for the natural variability, especially related to fruit position along the truss and maturation stage (Tsouvaltzis et al., 2023), each fruit was weighed individually. This approach allowed for a more robust estimation of average fruit weight within each treatment. Biostimulants are proved to improve yield and fruit weight; in this study, in fact, a 23 % higher yield and an 8 % higher fruit weight was observed in plants treated with FTB1 compared to the control. This result is in line with what is reported by Distefano et al. (2022), who observed an increment of 16 % in the weight of cherry tomato fruits, harvested from plants treated with a biostimulant, containing plant extracts. These preliminary results are encouraging, as they open the door to further, more detailed investigations, particularly regarding the dynamics of photoassimilate flow between source and sink organs as reported by Osorio et al. (2014). The FTB1 treatment, in fact, may have positively influenced the sink strength of the fruit, defined as the product of sink size and sink activity, by enhancing processes such as cell expansion, phloem unloading, and assimilate partitioning towards developing fruits. Galaverni et al. (2025) reported that a fermented kiwifruit-based biostimulant had a positive effect under water deficit conditions, where stressed tomato plants achieved yields comparable to well-watered plants in open-field conditions. This finding supports the hypothesis that fermentation-derived bioactive compounds can help sustain sink activity and fruit development even under suboptimal growing conditions. According to Smith et al. (2018), yield formation in many crops is increasingly recognized as being limited more by sink capacity, the ability of reproductive organs to receive, unload, and utilize assimilates, than by source photosynthetic potential itself. This concept aligns with the idea that yield realization largely depends on how efficiently assimilates are partitioned from source tissues to developing sinks (Reynolds and Langridge, 2016). A possible explanation for the yield increase observed with FTB1 could also be related to the fact that, as previously mentioned, the biostimulant contains a substantial amount of potassium. As reported in the literature, proper K application promotes fruit yield by enhancing the transport of photoassimilates from source to sink (Shah et al. 2024). The stimulatory effect of FTB on tomato production is probably mediated by multiple factors, including the concentration of minerals, bioactive compounds, the composition of plant growth-promoting microbes, and phytohormones, mechanisms that are not yet fully understood, as previously reported for fermented alfalfa-based biostimulants (Barna et al. 2021). The increase in fruit yield per plant could be related to an improvement in physiological parameters, particularly SPAD and chlorophyll content. However, in this study, no significant physiological differences were observed, despite the yield increase recorded when the biostimulant was applied at 100 mL/L. A similar pattern was reported by Cardarelli et al. (2020), where plants treated with the biostimulant showed no changes in SPAD values but still exhibited an increase in fruit yield, primarily due to higher fruit weight rather than an increased number of fruits per plant, as observed in our study. In a two-year study conducted by Patanè et al. (2025) on tomato plants, treatment with two commercial plant-derived biostimulants obtained through enzymatic hydrolysis of legume seeds significantly increased plant productivity, with an average yield improvement of + 57 % across the two biostimulants in the first year. In another study by Mauro et al. (2024), a different commercial plant-based biostimulant was applied to two indeterminate tomato genotypes, "Creativo" and "Caravaggio". An increase in yield of 15 % and 7 % was observed for the two genotypes, respectively, highlighting the genotype-dependent effect of the same biostimulant on different cultivars. Therefore, the yield increase per plant observed with FTB1 is highly noteworthy and consistent with the percentages previously reported in the literature by other authors. Since this study mainly addressed agronomic and fruit-quality responses, the specific physiological mechanisms underlying the observed yield improvement remain to be elucidated in future work. The proposed interpretation, should thus be considered hypothetical, as no direct measurements of assimilate flow, enzymatic activity, or hormonal regulation were performed.

Future research should include targeted physiological and biochemical analyses to validate these mechanisms, as no direct measurements of assimilate flow, enzymatic activity, or hormonal regulation were performed.

4.5. Temporal and treatment-dependent changes in tomato fruit characteristics

As Biais et al. (2014) and Bénard et al. (2009) demonstrated, fruit metabolite composition varies depending on the moment fruits are collected. This aspect is particularly relevant for indeterminate tomato varieties, as that used in this study, for which it is necessary to proceed with several manual harvesting, that lead to the collection of fruits ripened at different times under variable environmental and light conditions (Gautier et al. 2005; Rosales et al. 2011; Tsouvaltzis et al. 2023). Actually, Tijskens et al. (2020) and Cockshull, Ho, 1995), reported that tomato fruits change in size during the harvesting season; in this study, fruits bigger in size were collected from medial trusses, and, particularly, from FTB1 treated plants; an increase in the size of tomato fruits due to the application of biostimulants, such as fulvic acids, was reported by Suh et al. (2014). Li et al. (2015) demonstrated that fruit size potential during the fully fruiting stage is negatively correlated with the plant source/sink ratio under a standard fruit load, the larger fruits observed in the medial trusses, particularly in FTB1-treated plants, may reflect a more favorable source–sink balance that allowed greater assimilate allocation to individual fruits with the lowest concentration of biostimulant tested. Although the mechanism underlying the increase in fruit yield, size, and weight following biostimulant treatment remains poorly understood (Mannino et al. 2020), some authors have suggested that these effects may be related, for example, to auxin- and gibberellin-like activities (Khan et al. 2009).

4.6. Effects of biostimulant treatments on tomato fruit quality traits across the harvesting season

The skin color of tomato fruits, particularly the a* value that indicates the level of redness, is considered the most important parameter for the quality standards set by the European Union (Passam et al. 2007), nevertheless, is crucial for consumer perception and, thus, influences purchasing preferences. In this study, the treatment with FTB did not improve the tomato skin color; results in contrast with the findings of Nazeer et al. (2024) that reported a 25 % increase in strawberry fruit redness due to the treatment with a LAB fermented kiwi waste. Another study (Soppelsa et al. 2018) on apple trees treated with various biostimulants, including seaweed extract, reported a fruit color improved by 87 % in 2016 season and by 50 % in 2017 season. Moreover, Rivelli et al. (2024) found that during the harvesting season, the tomato skin color varies in terms of lightness, redness and yellowness; in this study, FTB3 treatment contributed to maintaining the color more stable from medial to upper truss fruits. This outcome could help the grower in guaranteeing a more stable and standardized quality to consumers.

Firmness is a key indicator of fruit maturity and quality, playing a crucial role preserving organoleptical features for fresh consumption

tomatoes and extending their shelf life (Chang et al. 2024). Cardarelli et al. (2024) reported that strawberry fruit firmness was significantly higher in plants treated with Trainer®, a vegetal-derived protein hydrolysate, compared to those treated with a standard synthetic auxin (SA) while the bacterial filtrate treatment and the control group showed intermediate firmness values, which were not significantly different from the SA or control treatments. Additionally, several studies in recent years have highlighted that biostimulants, such as chitosan, potassium silicate, and algae, can improve fruit firmness (El-Miniawy et al. 2013; Sajid et al. 2020). Similarly, in the present study, tomato fruit firmness increased in plants treated with the FTB. This effect was particularly evident for FTB1-treated plants from which collected fruits presented a firmness increased by 29 % (medial) and 16 % (upper) compared to those harvested from FTB0 plants.

TSS is one of the key parameters for tomato quality, influencing both sweetness and flavor intensity since it represents the sum of sugars, acids and other minor components in the tomato fruit (Kasim, Kasim, 2015). As reported by Rivelli et al. (2024), in tomato fruits, the TSS varies during the harvesting season; in this study, a 12 % increase of °Brix was registered in fruits from medial to upper trusses. This study confirms what is reported by Mzibra et al. (2021) who testing a biostimulant derived from seaweed, observed an increase in the °Brix of tomato fruits; in fact, the fruit TSS increased with the FTB concentration. This effect on tomato fruit quality represents an improvement, both for producers and consumers (Li et al. 2021). Regarding the fruit acidity, expressed in this study as the percentage of citric acid, the results align with those observed in other studies, showing a variation in acidity depending on fruit truss position. In accordance with Rosales et al. (2011), it was observed that there was a decrease in the acidity of tomatoes as the production period progressed, with a decrease of 12 % between the lower and medial trusses, and 7 % between the medial and upper trusses. Worth to be highlighted is the lower TA detected in fruits collected from plants treated with FTB3, throughout the harvesting season; in fact, since a low TSS/TA ratio is associated with bland-tasting (Mencarelli and Saltveit, 1988), to maintain high the quality of fruits, it is necessary that they have a lower TA, to a parity of TSS value.

The high TSS and the low TA in fruits harvested from FTB3-treated plants determined a high TSS/TA; the positive effect of the treatment with FTB3 on this parameter (7 % increase compared to FTB0 fruits) is in line with what is reported by Distefano et al. (2022), who obtained a 9 % increase in the TSS/TA, after a treatment with a plant-based biostimulant to cherry tomato plants, cv. “Eletta”. 7 % higher than FTB3 treatment in several studies, environmental conditions and fruit position on the plant are reported as relevant factors influencing the secondary metabolism (Rosales et al. 2011; Toor et al. 2006). In this study, the interaction between treatment and truss position influenced both total phenolic content (TPC) and lycopene levels in tomato fruits. While TPC generally decreased over successive harvests, in contrast with previous findings (Rosales et al. 2011; Toor et al. 2006), treated plants (FTB1 and FTB2) maintained higher TPC levels in medial and upper trusses. In line with previous research (Rosales et al. 2011; Toor et al. 2006), this study noted that the highest antioxidant activity value was reached by fruits harvested from the upper tiers, as the antioxidant content of the fruits increased in response to the rise in temperature, particularly observed in the harvest from the higher tiers, compared to the first harvest from the lower tiers in April. Temperature is known to influence antioxidant accumulation, with levels decreasing when it falls below 12°C or rises above 32°C (Rosales et al. 2011; Dumas et al. 2003). Interestingly, while control plants (FTB0) exhibited a progressive decline in lycopene content over the course of the harvest—particularly in the upper trusses during the hottest period—treated plants demonstrated the opposite trend, with lycopene levels increasing in the medial and upper trusses. This suggests that biostimulant application may mitigate temperature-induced declines, potentially enhancing fruit quality under varying environmental conditions.

4.7. *Evaluating microbial community responses to agricultural treatments via BIOLOG analysis*

The BIOLOG system is used in environmental microbiology to assess how different agricultural practices influence the functional diversity of soil microbial communities, as microorganisms respond rapidly to environmental disturbances or stress. Changes in their metabolic profiles can serve as early and effective indicators of ecosystem shifts (Ramut et al. 2025). Differences in bacterial carbon resource preferences were associated with both treatment and plant developmental stage, suggesting shifts in the quality of plant root exudates and in the bacterial populations’ ability to respond to competition (Houlden et al. 2008). Variations in the metabolic activity of the microbial community are presumed to have altered plant–substrate feedback mechanisms (Gfeller et al. 2023), thereby contributing to the enhanced plant performance observed in this study.

4.8. *Integrative overview of tomato quality parameters under biostimulant treatments*

To summarize the overall effects of the experimental biostimulant on fruit characteristics, a heatmap was generated following the approach previously adopted by Dasgan et al. (2024) and Campana et al. (2025) to provide a comprehensive overview of the results, independently of the harvest effect. Pearson’s distance analysis highlighted that FTB3 was associated with an increase in sweetness relative to acidity. Moreover, the highest biostimulant concentration tested in this study showed a positive correlation with both lycopene content and antioxidant capacity, suggesting that fruits from these plants tended to accumulate more lycopene, consequently resulting in higher antioxidant activity. Interestingly, contrary to expectations, TPC was negatively affected by FTB3 treatment, however, this parameter is positively influenced by the FTB0 conditions, indicating a higher accumulation of phenolic compounds in the control. However, this increase in phenolic content was not reflected in antioxidant activity measured by the DPPH assay employed in this study. It is also noteworthy that the L* value (lightness) of fruit color was positively influenced by the FTB2 treatment, and a negative effect was registered with FTB0 treatment. This indicates that fruits from untreated plants tended to be darker, whereas those from plants treated with the intermediate biostimulant concentration (FTB2) exhibited higher lightness. Furthermore, the positive effect of FTB3 was registered on electrical conductivity (EC), along with a decrease in titratable acidity (TA), suggesting that higher concentrations of the biostimulant may promote ion accumulation while reducing fruit acidity. These trends are consistent with the observed increase in both TSS and the TSS/TA ratio, supporting the hypothesis that FTB application enhances fruit sweetness while reducing acidity.

5. Conclusion

This study demonstrates the potential of an experimental fermented tomato-based biostimulant (FTB) derived from unsold green and red tomatoes to improve the quality of tomato fruit in soilless cropping systems. By integrating agronomic, biochemical and microbiological analyses, this research shows how the application of this biostimulant can influence key fruit quality traits, including sweetness, antioxidant capacity and lycopene content, depending on the harvest time. The use of a Design of Experiment (DoE) approach to optimise fermentation conditions in combination with BIOLOG-based profiling of microbial metabolic activity provides a comprehensive understanding of plant and substrate responses. Notably, the most pronounced effect of the FTB was observed on the medial trusses, corresponding to the May harvest. This harvest represented the peak production period and was also the most abundant. Improving fruit quality during this critical phase is particularly valuable from a commercial perspective, as farmers face high market competition. The positive impact of the biostimulant on key

quality parameters during this period not only underscores its practical applicability in tomato cultivation, but also opens avenues for future studies on the economic benefits of biostimulant use. Moreover, the results support the role of biostimulant in sustainable agriculture and emphasise the value of circular economy strategies in crop production. Although the molecular mechanisms were not directly investigated, the observed changes in fruit composition and microbial metabolic activity suggest a positive effect of FTB, underlying biochemical and possibly genetic modulations and paving the way for future studies at the molecular level. Moreover, the experimental design could be further expanded, for example, by including the non-fermented tomato matrix in the tested treatments and with deeper post-harvest quality analyses that would provide additional valuable insights. In addition, future work should incorporate physiological and source–sink assessments, including gas-exchange measurements, carbohydrate allocation profiling between leaves and fruits, nutrient-use efficiency assessments, and detailed quantification of fruit set, to gain deeper insight into the mechanism of action of FTB and to idenify the optimal biostimulant concentration.

CRediT authorship contribution statement

Leandra Leto: Investigation, Formal analysis. **Rohini Dhenge:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Luca Fontechiari:** Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Samreen Nazeer:** Investigation, Formal analysis. **Massimiliano Rinaldi:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition. **Angela Paterna:** Investigation, Formal analysis. **Alessia Levante:** Writing – review & editing. **CHIANCONE Benedetta:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Anna Agosti:** Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Jasmine Hadj Saadoun:** Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Camilla Lazzi:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Martina Cirlini:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.plantsci.2025.112865.

Data availability

Data will be made available on request.

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