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# Assessment of Fermented Kiwifruit on Morpho-Physiological and Productive Performances of *Fragaria* spp Plants, Grown Under Hydroponic Conditions

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## ABSTRACT

Climate change poses a significant threat to global agriculture by altering weather patterns, increasing the frequency of extreme events, and reducing the availability of arable land. Hydroponic systems offer a sustainable solution allowing efficient resource use, including water and nutrients, and enabling cultivation in areas with poor soil quality or limited space. The incorporation of biostimulants derived from plant byproducts further enhances sustainability by improving plant growth and resilience, reducing the use of synthetic fertilizers and the environmental footprint of agriculture, promoting, at the same time, healtier crop production. This study investigates the effect of biostimulants, derived from fermented kiwifruit byproducts, on the morpho-physiological and productive performances of *Fragaria vesca* (L.), cv. Malga, and of *Fragaria x ananassa* (Duch.), cv. Annabelle, grown in a column hydroponic system. Plants of both species, when treated with the biostimulant, demonstrated significant improvements for all the parameters evaluated, with healthier plants and improved quality features in fruits. These findings suggest that fermented kiwi byproduct could be an effective, sustainable integration to synthetic fertilizers, promoting better growth and fruit quality in strawberry cultivation under hydroponic systems.

## 1 | Introduction

Modern agriculture must face several accusations of being one of the main causes of climate change, for its hard impact on the environment, due to water consumption and synthetic fertilizer over-utilization (Malhi, Kaur, and Kaushik 2021).

To mitigate these problems, a possible solution is to resort to innovative soilless agricultural systems, such as hydroponics, which optimizes growing space utilization and resource efficiency (Fussy and Papenbrock 2022), since nutrients are distributed to plants via a water-based nutrient solution (Ahmed et al. 2001; Antón et al. 2004). The hydroponic system provides better pest and disease control than conventional farming. In addition, this type of cultivation system significantly reduces the cost of pesticides and produces higher quality and higher yielding plants (Lee and Lee 2015).

Column hydroponic system (CHS) represents a specific type of vertical farming and it is ideal for controlled environments. This system utilizes vertically stacked columns for plants suspended in a nutrient-rich water solution (Singh and Hati 2024). This method eliminates the need for soil, minimizes water usage, and allows for precise nutrient

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delivery, potentially leading to increased plant yields (Voutsinos et al. 2021).

The way to improve the sustainability of the agricultural sector is through the use of biostimulants (Alneyadi et al. 2024; Asif et al. 2023); they are products of natural origin proven to improve several aspects of plant growth, productivity and nutrient use, and to increase plant resistance to biotic and abiotic stresses. Moreover, biostimulants can be used to replace at least in part, synthetic agrochemicals like pesticides and fertilizers (Colla and Rouphael 2015; Rouphael and Colla 2020). Biostimulants have generally been applied to high-value crops, fruits and vegetables, and when used in soilless systems they can help to reduce fertilizers application and, at the same time, increase the sustainability of the entire production process (Ertani et al. 2021).

FAO (2020) reports that all over the world, 4 million tons of kiwi are produced, half of which is harvested in China (Chamorro et al. 2022). The extremely high production of kiwifruits generates, along the supply chain, an even higher amount of waste, constituted by seeds, skin and kiwifruit discards, but also by undersized fruits that do not comply the strict grading and sorting processes requested, mainly, by GDO (Chamorro et al. 2022; Dias et al. 2020).

A relatively new trend is the production of biostimulants from agri-food waste, with the beneficial side effect of finding a new life for products destined to be discarded (Zhang et al. 2024).

Fermentation is a technique widely used for a variety of purposes in food and biotechnology, and, in recent years, it has been used as a strategy for the valorization of agroindustrial wastes to obtain high-value molecules such as flavourings, antimicrobials, or new ingredients (Hadj Saadoun et al. 2021, 2022; Ricci et al. 2019). The use of this technique to produce biostimulants from waste has not yet been reported in the literature.

The strawberry (*Fragaria* spp.) is a fruit cultivated worldwide, well known for its vibrant colour, sweetness and adaptability to different climate conditions (Machado and Dol 2021). However, within a wide range of strawberry species, there is a clear difference between the ubiquitous *Fragaria* × *ananassa* and *Fragaria vesca* (Hardigan et al. 2019; Rugienius et al. 2021). *Fragaria* × *ananassa* is an annual plant, characterized by a larger yield, and more uniform fruits with a wider range of colours (red, white and orange). *F. vesca*, known as wild strawberry, is a perennial plant that produces small fruits with a very distinctive and strong aroma (Qarni et al. 2022; Tenea and Reyes 2024), a compact growth habit, and mostly does not produce runners (Ricci et al. 2023). It has been reported that in *F. vesca*, the application of biostimulants (Seaweed extract) can improve root development and nutrient uptake, resulting in increased plant growth and yield (Rana, Lingwal, et al. 2022).

Moreover, they can stimulate hormone production, leading to enhanced fruit quality in terms of size, shape, aroma and sweetness (Rana, Sharma, et al. 2022). The need to address the simultaneous challenges of increasing crop productivity and optimizing the use of synthetic resources results in the continuous production and evaluation of biostimulants from different sources (Rouphael and Colla 2020). With this premise, in this study, a biostimulant obtained from the fermentation of kiwifruit by-product was used as additive in the nutrient solution of a CHS for strawberry cultivation and its influence on plant vegeto-productive performances and microbial community in substrate was investigated. Fermenting the unsold kiwifruits and use it as biostimulant is an innovative way of valorizing a fresh produce by-product; in fact, in the concept of a circular economy, by-products are not only given a new lease on life but also contribute to improving agricultural sustainability by reducing the reliance on synthetic inputs. Moreover, for the best of authors' knowledge, this is the first paper in which fermented agri-food waste is used as biostimulant; in the literature, in fact, fermentation primarily serves to enhance the value of agricultural waste and by-products by transforming them into natural sources of biologically active compounds, mainly used in the development of pharmaceuticals and functional foods (Lu et al. 2019; Sabater et al. 2020).

## 2 | Materials and Methods

### 2.1 | Plant Material

Plants of the wild strawberry Malga (CV1) and the strawberry Annabelle (CV2) were purchased for the study. Malga plants were selected based on their unique characteristics including the production of large fruit with an attractive orange tint. It highlights the moderately dense and juicy flesh, with a balanced flavour profile combining sweetness and a delicate tartness (Nour 2021). Additionally, Malga is known for its persistent aroma and smell (Nour 2021). Annabelle is a perennial herbaceous plant that grows naturally, produces edible fruits and can be easily cultivated (Del Bubba et al. 2016; Doumett et al. 2011). The fruits are soft and strongly flavoured and they are an important source of bioactive compounds, citric acid, sugar and phenolics (Del Bubba et al. 2016; Doumett et al. 2011).

The experiment started in July and ended at the end of October in 2023. Plants were cultivated resorting to a CHS, in which nutrient solution was contained in a 100 L tank. Four columns were used for the experiment; per each column six plants were cultivated. Two nutrient solutions were used, (i) Control-C consisting in the Hoagland solution and (ii) FKB consisting in the Hoagland solution added with the fermented kiwifruit by-product (FKB). FKB was added to the nutrient solution every week, at the concentration of 50 mL/L, as a biostimulant.

The composition of the Hoagland solution is the following: agricultural calcium nitrate 0.59 kg, ammonium nitrate 0.09 kg, chelated phenol EDDHA 37.23 g, magnesium nitrate 0.21 kg, monopotassium phosphate 0.23 kg, copper sulphate 0.25 g, zinc sulphate 1.44 g, potassium nitrate 0.27 kg + 0.19 kg, potassium sulphate 0.20 kg, boric acid 1.85 g, manganese sulphate 0.85 g and sodium molybdate 0.24 g.

### 2.2 | Biostimulant Production

Kiwifruit (*Actinidia deliciosa* 'Hayward') byproducts were collected from a local farm in Emilia Romagna (Italy). The fruits

were skinned and then ground with a laboratory blender (Knife Mill Grindomix GM 200, Retsch GmbH, Haan, Germany) to obtain a uniform pulp. The pulp was autoclaved (121°C—21 min) before inoculation. A strain of *Lactiplantibacillus plantarum* 4193 (isolated from Pecorino cheese) belonging to the University of Parma Culture Collection (UPCC) was used for fermentation. The microbial stock cultures were maintained as frozen at −80°C in De Man Rogosa and Sharpe (MRS) broth (Oxoid, Basingstoke, UK). Before fermentation, microbial strain was transferred twice in the medium (3% v/v) and incubated for 24 h at the optimal growth temperature. It was then inoculated in fresh MRS broth (3% v/v) and incubated for 15 h at 30°C to obtain a microbial density bacterial concentration of 9 Log CFU/mL. After centrifugation (Eppendorf centrifuge 5810 R, Eppendorf, Hamburg, Germany) (12,857 × g for 10 min at 4°C), cells were collected, washed twice in Ringer solution (VWR, UK), and suspended in sterile bidistilled water. The culture was inoculated into pulp to reach 7 Log cfu/g and incubated for 28 h at 25°C, to reach 8 Log cfu/g. Microbial load was assessed at the beginning and at the end of the incubation time by plate counts on optimal agar medium (MRS). The properties of FKB used in the experiment are shown in Table 1.

## 2.3 | Data Collection

During the whole experiment, every week, the following morpho-phisiological parameters were monitored: plant height (from the crown base to the longest leaf), number of leaves, leaf area (LA = ELA × 0.74; ELA: estimated leaf area based on the length and width of a trifoliate leaf with the formula (Abbott 1968), crown diameter (digital vernier caliper) (Zheng, Chen, and Yan 2019), leaf flavonol content (FLV), chlorophyll content (CCLa), soil plant analysis (SPAD) (Multi Pigment Meter, MPM-100S, Bio Scientific Ltd, UK). Fruits were harvested when fully ripen and the following data were registered: total number of fruits, fruit fresh weight (KERN EMB 1000-2), fruit length and width, petiole length, fruit skin colour, considering the CIE $L^*$ (for lightness), $a^*$ (for redness or blueness) and $b^*$ (yellowness) (CM 2600 d, Minolta Co.; Osaka, Japan), fruit firmness (Geotester Pocket Penetrometer with 3 mm plunger size) (Simkova et al. 2024; Zuñiga et al. 2020).

Fruits of both cultivars and from both treatments were blended to obtain a juice that was used to measure pH (pH510 Meter) (El-Mogy et al. 2019), total soluble solids (TSS) with a digital refractometer (Hanna instruments) using 50 μL of strawberry juice (Pontesegger, Rühmer, and Siegmund 2023), titratable acidity (TA), following the titration method (Teka 2013). TSS/TA was calculated to assess fruit quality, as mentioned by Tigist, Workneh, and Woldetsadik (2013). Electric conductivity (EC) of the strawberry juice was measured by using The Lovibond Senso Direct Con 110 Digital EC meter (Khalid et al. 2020).

On fruit juice and leaves, total phenolic contents (TPC) using a modified Folin-Ciocalteau method (Martelli et al. 2020) and the antioxidant activity, by applying the 2,2 diphenyl 1 picrylhydrazyl (DPPH) were calculated (Abram et al. 2015). Extracts were mixed with reagents incubated in the dark, and absorbance was measured at 760 nm. A calibration curve with gallic acid was used to quantify polyphenols. For the DPPH assay, aliquots of the sample extract or standard solution were mixed with a DPPH solution and incubated in the dark. The absorbance at 517 nm was measured. Trolox was used as a reference for calculating antioxidant capacity results expressed as mg TEAC/mL. TPC and DPPH analyses were duplicated with three consecutive measurements per sample (Martelli et al. 2020).

Finally, on the uprooted plants the following parameters were registered: plant fresh weight, plant dry matter content (plant DMC, %), root fresh weight and root dry matter content.

## 2.4 | Community-Level Physiological Profiling (CLPP)

Microbial metabolism in samples treated with fermented kiwi byproduct (FKB), and substrate control (without any treatment) at the end of the harvest, was tested using the Biolog system with Biolog EcoPlate. This system is specifically designed for microbial

**TABLE 1** | Properties of fermented kiwi byproduct used as a biostimulant in the experiment.

| Parameters | Method | Fermented kiwi byproduct |
|---|---|---|
| Moisture (%) | Metodo III.1 MPAAF | 83.56 |
| Dry matter (%) | Metodo III.1 MPAAF | 16.44 |
| Ashes (% stq) | Combustion at 550°C | 0.73 |
| Orgnaic matter (%) | Calculation by difference | 15.71 |
| $C_{org}$ (%) | calculation from the s.o. | 9.13 |
| Boron (mg/kg) | — | 1.8 |
| Copper (mg/kg) | UNI EN ISO 6869-2001 | 2.0 |
| Iron (mg/kg) | — | 1.5 |
| Maganese (mg/kg) | UNI EN ISO 6869-2001 | < 0,3 (LQ) |
| Zinc (mg/kg) | UNI EN ISO 6869-2001 | 4.7 |
| Total Nitrogen (N%) | Modified Kjeldahl | 0.30 |
| Total Phospohorus ($P_20_5$%) | G.U. CEE n. L279/15 del 02/12/1971 | 0.06 |
| Total Pottasium (KO%) | EN ISO 6869:2001 (Flame photometry) | 0.37 |

community analysis and microbial ecology research. Each plate contains 31 of the most useful carbon sources in triplicate. Five grams of samples were decimal diluted with 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. One hundred microliters of solution per well were inoculated into EcoPlates in triplicates and incubated at 30°C. The plates were analysed by the Microplate Reader (dual-wavelength data: OD590) at T0 and after 24, 48 and 72 h to observe the dynamic utilization of different carbon sources from microbes for different carbon sources. For kinetic comparative analysis, we take the lecture measurement performed after 48 h (T48) as we observe the maximum change signal development at this point. The analysis of data was performed using AWCD (Average Well Colour Development) as a parameter that enables to capture of an integral fingerprinting of carbon sources used (Tian et al. 2017). The value of AWCD was calculated according to the below equation,

$$\mathrm{AWCD} = \frac{(C - R)}{n},$$

where $C$ is the OD value of each well with a carbon source, $R$ is the OD value of the control well (water), $n$ is the number of wells with carbon sources, and the value of $n$ is 31.

### 2.5 | Statistical Data Analysis

Two-way Analysis of Variance and Repeated Measure procedures were used for the analysis of data, for the factors 'Cultivar' and 'Treatment'; mean separation was carried out resorting to Tukey's test ($p \leq 0.05$) (IBM SPSS Statistics 29.0.1.0 software, SPSS Inc., Chicago, IL).

Phenotypic raw data from Biolog analysis were elaborated with Rstudio (R version 4.3.1, Package 'pheatmap' version 1.0.12) for statistical analysis and data visualization of assays, using the default algorithms for clustering. To reduce the noise levels, all absorbance values of carbon sources utilisation were referred against the negative control well (A1) and subsequently, all divided by the respective AWCD. Negative values were set to 0. Normalized data were used for statistical analysis.

## 3 | Results

### 3.1 | Vegeto-Productive Plant Performances

Repeated measures did not evidence any significant influence of the monitoring time on the response of plants in terms of height, while a significant interaction was recorded between the factors studied; in fact, the treatment with FKB determined an increase in the plant height for both cultivars (on the average $15.13 \pm 0.50$ cm for control and $17.27 \pm 0.71$ cm). Without any treatment, the two genotypes showed plants significantly different in their height, with plants of cv. Annabelle longer than those of cv. Malga (respectively, $15.51 \pm 0.27$ cm vs. $14.74 \pm 0.33$ cm); instead, when the FKB treatment was used, plants of both cvs. were of the same height ($17.08 \pm 0.36$ cm vs. $17.47 \pm 0.91$ cm) (Table 2).

**TABLE 2** | Effect of fermented kiwi byproduct on vegeto-productive characteristics of strawberry plants, grown in a hydroponic system.

| Cultivars | Treatments | PH (cm) | NL (n°) | LA ($cm^2$) | CD (cm) | Plant FW (g) | Plant DMC (%) | Root FW (g) | Root DMC (%) |
|---|---|---|---|---|---|---|---|---|---|
| Malga | Control | 14.74 ± 0.14 | 14.98 ± 0.91 | 4.69 ± 0.76 | 2.27 ± 0.08 | 13.15 ± 2.06 | 23.25 ± 0.86 | 18.71 ± 4.17 | 15.14 ± 0.64 |
| | FKB | 17.08 ± 0.15 | 8.81 ± 0.14 | 3.62 ± 0.32 | 2.10 ± 0.26 | 24.92 ± 2.25 | 38.81 ± 2.69 | 23.56 ± 0.61 | 13.50 ± 2.52 |
| Annabelle | Control | 15.51 ± 0.11 | 10.25 ± 0.35 | 5.00 ± 0.67 | 1.80 ± 0.27 | 25.10 ± 0.65 | 35.08 ± 0.66 | 16.98 ± 1.27 | 30.79 ± 2.20 |
| | FKB | 17.47 ± 0.39 | 15.04 ± 0.36 | 9.58 ± 0.94 | 2.98 ± 0.45 | 38.98 ± 3.52 | 43.87 ± 0.38 | 44.21 ± 6.40 | 22.30 ± 2.54 |
| Statistical analysis | | | | | | | | | |
| Cultivars (CV) | | 0.0194 | 0.1675 | 0.0003 | 0.4902 | 0.0006 | 0.0004 | 0.0411 | 0.0004 |
| Treatments (T) | | 0.0000 | 0.2041 | 0.0229 | 0.1018 | 0.0006 | 0.0000 | 0.0033 | 0.0445 |
| CV x T | | 0.4105 | 0.0000 | 0.0008 | 0.0339 | 0.6640 | 0.0500 | 0.0206 | 0.1488 |

*Note:* Two-way ANOVA, repetition measurements, Tukey's test ($p \leq 0.05$) (IBM SPSS Statistics 29.0.1.0 software, SPSS Inc.; Chicago, IL), at $p \leq 0.05$. Abbreviations: CD, crown diameter; LA, leaf area; NL, number of leaves; PH, plant height; plant DMC, plant dry matter content; plant FW, plant fresh weight; root DMC, root dry matter content; root FW, root fresh weight.

The parameter 'number of leaves' did not show significant changes in response to treatment or between cultivars, but the interaction was statistically significant The two cultivars differently responded to the FKB treatment; when the biostimulant was present, cv. Annabelle plants produced a higher number of leaves ($10.25 \pm 0.81$ vs. $15.04 \pm 0.85$), while for cv. Malga, plants presented a lower number of leaves ($14.98 \pm 2.12$ vs. $15.04 \pm 0.32$) (Table 2).

The parameter Leaf Area was influenced positively by the FKB treatment, with a 47.75% of increase, only for the cv. Annabelle, while for cv. Malga no differences were observed. Comparing the two cultivars treated with FKB, the cv. Annabelle had leaves that were 60% larger than those of cv. Malga (Table 2).

A significant interaction between factors has been registered for the parameter crown diameter; specifically, only for the cv. Annabelle, the FKB treatment significantly increased the plant crown diameter ($1.80 \pm 0.63$ vs. $2.98 \pm 1.05$) (Table 2).

Analysing plant fresh weight and plant dry matter content of the epigeal part of the plant, both factors influenced the parameter independently (Table 2). It is clear that the two cultivars differently responded to the hydroponic culture system, with cv. Annabelle presenting values of all these parameters higher than cv. Malga; moreover, independently of the cultivar considered, concerning control, the FKB treatment determined an increase of 40% for FW (Figure 1) and of 30% for DMC.

For the parameter root FW, both factors, independently, influenced the plant response (Table 2). Specifically, the root fresh weight of plants grown with FKB was 47.34% higher than that of the control plants (Figure 1). Also, regarding the root dry matter content, the factors singularly influenced this parameter; cv. Annabelle plants better responded to hydroponic culture system than those of cv. Malga. A different trend was observed for this parameter; in fact, control plants presented a dry matter content higher than those treated with the biostimulant ($23.00\% \pm 8.51$ for the control and $17.90\% \pm 5.92$) (Table 2).

## 3.2 | Characterization of Strawberry Fruits

The plants of both cultivars produced a statistically comparable total number of fruits at the end of the experiment ($4.3 \pm 1.34$ for Malga and $4.8 \pm 1.95$ for Annabelle), independently of the nutrient solution composition (Table 3).

It was observed that cv. Malga started to produce earlier than Annabelle (data not shown). Moreover, in the last monitoring time, at the 6th week after the beginning of fruit ripening, a significant influence of FKB on the number of fruits produced was detected. Specifically, for Annabelle plants, the addition of the biostimulants to the nutrient solution determined the production of a significantly higher number of fruits. For Malga plants, this effect was not recorded, and comparing the response of the two cultivars to FKB, Malga plants produced a number of fruits significantly lower than Annabelle (Table 3).

The two tested cultivars produced fruits with a significantly different fresh weight. Also for this parameter, it seems that cv. Annabelle does not respond well to the hydroponic system; in fact, it produced fruits with a weight statistically lower than Malga (Table 3). The trend was the opposite when FKB was added to the nutrient solution, not only the Annabelle fruits fresh weight was statistically higher than those of Malga, but also, the addition of FKB determined a 60% increase of Annabelle fruit fresh weight (Table 3).

Plants of both cultivars produced fruits with the same length and width, when they were grown with the control nutrient solution; while, with the FKB treatment, plants of cv. Annabelle produced fruit with length and width higher than those of cv. Malga. Moreover, for cv. Malga, fruits had higher length and width in the control treatment, opposite trend was observed for fruits of cv. Annabelle that have a higher length when plants were grown with FKB biostimulant, no influence of FKB was observed for the width (Table 3).

As for other parameters analysed, Malga and Annabelle responded differently to the treatment studied, having the first

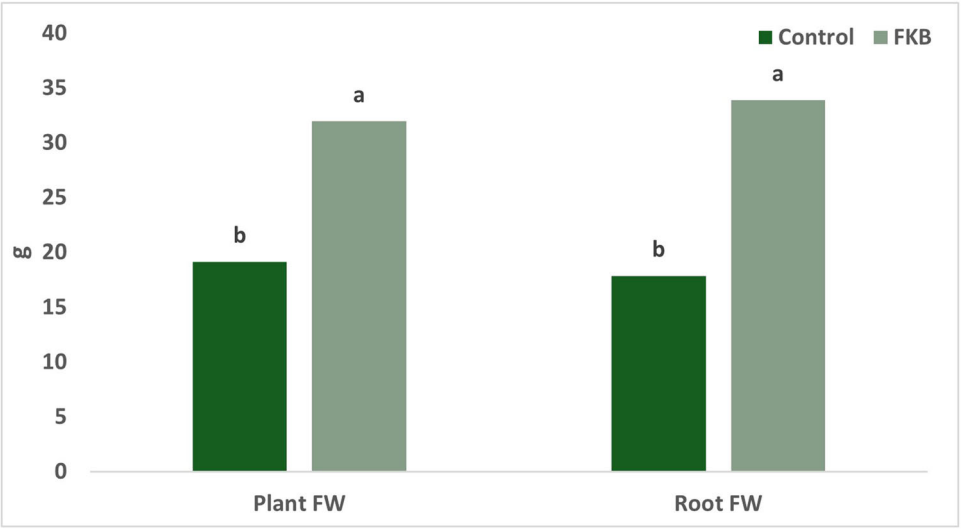



**FIGURE 1** | Influence of Fermented Kiwi Biostimolant (FKB) on fresh weight of epigeal (plant FW) and rooting (root FW) of strawberry plants. Two-way ANOVA, Tukey's test, $p < 0.05$.

**TABLE 3** | Effect of fermented kiwi byproduct on fruit quality attributes of strawberry fruits, grown in a hydroponic system.

| Cultivars | Treatments | TNF | FFW (g) | FL (cm) | FW (cm) | PL (cm) | Fir. (kg/cm$^3$) | $L^*$ | $a^*$ | $b^*$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Malga | Control | 4.50 + 0.13 | 9.51 ± 3.62 | 2.94 ± 0.47 | 2.64 ± 0.34 | 2.16 + 0.04 | 0.68 ± 0.03 | 42.73 ± 5.04 | 32.36 ± 4.84 | 32.42 ± 2.11 |
| | FKB | 4.16 + 0.18 | 3.95 ± 0.74 | 1.95 ± 0.22 | 1.76 ± 0.13 | 3.21 + 0.05 | 1.88 ± 0.03 | 33.01 ± 7.75 | 42.85 ± 4.92 | 25.81 ± 10.98 |
| Annabelle | Control | 3.83 + 0.16 | 4.82 ± 0.32 | 2.48 ± 0.09 | 2.08 ± 0.11 | 1.42 + 0.09 | 0.83 ± 0.03 | 41.94 ± 4.14 | 32.88 ± 7.96 | 24.10 ± 9.12 |
| | FKB | 5.83 + 0.06 | 12.00 ± 0.33 | 3.16 ± 0.10 | 2.44 ± 0.10 | 4.36 + 0.07 | 1.78 ± 0.29 | 35.15 ± 11.43 | 44.84 ± 7.30 | 26.91 ± 15.47 |
| Statistical analysis | | | | | | | | | | |
| Cultivars (CV) | | 0.7082 | 0.1137 | 0.0477 | 0.7177 | 0.0294 | 0.8541 | 0.4226 | 0.0801 | 0.0036 |
| Treatments | | 0.3309 | 0.4378 | 0.3923 | 0.0947 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.1102 |
| CV x T | | 1.0000 | 0.0000 | 0.0010 | 0.0002 | 0.0000 | 0.3661 | 0.0867 | 0.3040 | 0.0002 |

*Note:* Two-way ANOVA, repetition measurements, Tukey's test ($p \leq 0.05$) (IBM SPSS Statistics 29.0.1.0 software, SPSS Inc.; Chicago, IL), at $p \leq 0.05$. Abbreviations: $a^*$, redness; $b^*$, yellowness; FFW, fruit fresh weight; Fir, firmness; FL, fruit length; FW, fruit width; $L^*$, lightness; PL, petiole length; TNF, total number of fruits.

statistically longer petioles in the control treatment and the second in the FKB; but, when the treatments were compared within the cultivars, the addition of FKB to the nutrient solution, it induced the production of longer petioles (Table 3).

Statistical analysis evidenced a strong influence of the factor 'Treatment' on the strawberry fruit firmness; in fact, as a result of the FKB treatment, fruits were characterized by a significantly higher firmness than those from the control ($1.82 \pm 0.34$ kg/cm$^2$ vs. $0.75 \pm 0.09$ kg/cm$^2$) (Table 3).

FKB treatment does not improve fruit lightness, but their redness; in fact, values of $a^*$ significantly higher were recorded in fruits from plants treated with the biostimulants ($43.85 \pm 2.05$ vs. $32.62 \pm 2.12$), with an increase of 25% in redness (Figure 2). For the $b^*$ parameter, instead, a significant interaction was detected (Table 3); in fact, only for cultivar Malga, fruits from treated plants showed a yellowness significant lower than those from the control; moreover, Malga presented fruits showing more yellowness than those of Annabelle (Table 3).

Evaluating quality parameters of strawberry fruits, the same trend evidenced above was recorded.

At the control conditions, Malga fruits presented values of pH, TSS and TSS/TA ratio higher than Annabelle, confirming the weak adaptability of the second cultivar to the hydroponic conditions; when FKB was added to the nutrient solution, the trend was reversed, and Annabelle fruits presented higher values. For the cv. Annabelle, the addition of FKB determined a 50% increase in TSS/TA ratio. For the parameters EC and TA, when the control nutrient solution was used, values significantly higher were observed in Annabelle fruits. FKB determined an inversion of trend and treated fruits presented values higher than those nontreated (Table 4).

### 3.3 | Biochemical Analysis of Strawberry Fruit and Leaves

The biochemical analysis of fruits and leaves harvested from the plants of both cultivars evidenced that, the FKB biostimulants did not influence the accumulation of (poly)phenols in fruits and neither the antioxidant activity of extracts obtained from fruits (Table 5). A different response was obtained when the same parameters were analysed in leaves; in fact, if in the control plants, the TPC and antioxidant activity of leaves did not vary between the two cultivars tested, when FKB was used, for Annabelle, evidenced values significantly lower than Malga. The effect of FKB in reducing the TPC and antioxidant activity is particularly marked in the cv. Annabelle, rather than in Malga.

### 3.4 | Analysis of Physiological Parameters of Strawberry Fruit and Leaves

Physiological parameter analysis evidenced that the two cultivars performed differently in terms of CCLa and SPAD index, with the cv. Malga presenting values statistically higher than cv. Annabelle. Instead, for cv. Annabelle, the flavonol content (FLV) was statistically higher than cv. Malga; moreover, for this

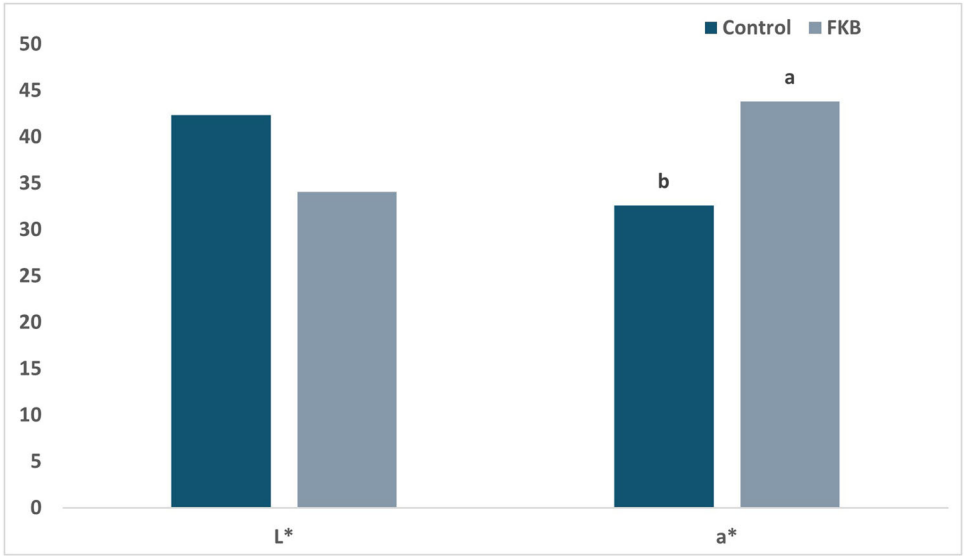



**FIGURE 2** | Influence of Fermented Kiwi Biostimolant (FKB) on lightness ($L^*$) and redness ($a^*$) of strawberry fruits. Two-way ANOVA, Tukey's test, $p < 0.05$.

**TABLE 4** | Effect of biostimulants on physico-chemical attributes of strawberry cultivars, grown in a hydroponic system.

| Cultivars | Treatments | pH | TSS (g/L) | TA (g/L) | TSS/TA (%) | EC (S/m) |
|---|---|---|---|---|---|---|
| Malga | Control | 4.64 ± 0.00 | 6.40 ± 0.06 | 1.15 ± 0.04 | 5.57 ± 0.22 | 1.07 + 0.01 |
| | FKB | 3.75 ± 0.01 | 5.37 ± 0.03 | 2.23 ± 0.02 | 2.41 ± 0.02 | 2.53 + 0.01 |
| Annabelle | Control | 4.06 ± 0.02 | 5.80 ± 0.06 | 1.99 ± 0.02 | 2.91 ± 0.01 | 1.34 + 0.01 |
| | FKB | 5.97 ± 0.01 | 7.33 ± 0.03 | 1.26 ± 0.03 | 5.84 ± 0.13 | 0.96 + 0.02 |
| Statistical analysis | | | | | | |
| Cultivars (CV) | | 0.0000 | 0.0000 | 0.0497 | 0.0153 | 0.0000 |
| Treatments (T) | | 0.0000 | 0.0007 | 0.0004 | 0.3857 | 0.0000 |
| CV x T | | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

*Note:* Two-way ANOVA, Tukey's test ($p \leq 0.05$) (IBM SPSS Statistics 29.0.1.0 software, SPSS Inc.; Chicago, IL), at $p \leq 0.05$.
Abbreviations: EC, electric conductivity; TA, titratable acidity; TSS, total soluble sugar (Brix); TSS/TA, ratio between sugar and acid.

parameter, plants treated with FKB presented higher values of FLV than those from the control plants (Table 5).

### 3.5 | Community-Level Physiological Profiling (CLPP)

Analysis of the substrate microbial community reported some significant differences in metabolic activity. Analysis of the substrate microbial community reported some significant differences in metabolic activity. Hierarchical clustering was used to analyse data related to substrate utilization from the substrate ecosystem, correlating the Ecoplates data from each soil sample to the normalized colour development values for each substrate. Data are visualized through a heatmap, shown in Figure 3, highlights which compounds present in the Ecoplates was metabolized at higher or lower levels by the microbial community present in the different samples. As can be seen, for most compounds, the substrate samples did not differ according to treatment. Substrates cluster in three groups: the first one is the largest, including the highly utilized substrates; cluster number two includes two compounds that show variable utilisation across the samples (glycogen and 2-hydroxy benzoic acid); the third cluster corresponds to substrates characterised by low utilization by the substrate microbial community. Looking within the same strawberry cultivar, remarkable differences were found for specific compounds, such as 2-hydroxy benzoic acid which is used by the community present in the substrate of cv. Malga sample treated with biostimulant and not in the control sample. Within the cv. Annabelle, differences are observed on the ability to metabolize polymers (alpha cyclodextrin and glycogen), difference also found for the cv Malga in which only after the treatment there is this substrate utilization.

## 4 | Discussion

Scientific literature in recent years has become enriched by an increasing number of research evaluating biostimulant of natural origin, with the aim of supporting the modern agriculture to guarantee food for the growing world population and, at the same time, to lower its environmental impact (Rouphael and Colla 2020). Mostly used biostimulants are humic substances (humic and fulvic acids), protein hydrolysates, seaweed-botanical extracts, but also, biopolymers, fungi and beneficial bacteria (du Jardin, 2015), but, for the best of authors

**TABLE 5** | Effect of biostimulant on photosynthetic activity and antioxidant capacity of strawberry cultivars, grown in a hydroponic system.

| Cultivars | Treatments | DPPH (mg/mL) | TPC (mg/mL) | DPPH (mg/mL) | TPC (mg/mL) | FLV | CCLa | SPAD |
|---|---|---|---|---|---|---|---|---|
| | | Fruits | | Leaves | | | Leaves | |
| Malga | Control | 5.79 ± 0.37 | 11.79 ± 88.74 | 80.19 ± 0.75 | 54.73 ± 0.61 | 0.71 ± 0.01 | 20.83 ± 0.50 | 37.31 ± 0.43 |
| | FKB | 5.31 ± 0.96 | 11.34 ± 201.54 | 81.94 ± 0.31 | 57.09 ± 2.11 | 0.82 ± 0.02 | 19.67 ± 0.55 | 36.80 ± 0.38 |
| Annabelle | Control | 5.09 ± 0.21 | 11.35 ± 56.67 | 81.46 ± 2.14 | 59.65 ± 2.04 | 0.91 ± 0.02 | 18.11 ± 0.48 | 35.78 ± 0.37 |
| | FKB | 5.45 ± 0.04 | 11.06 ± 33.68 | 68.55 ± 0.35 | 45.22 ± 0.55 | 0.96 ± 0.02 | 17.71 ± 0.96 | 34.87 ± 0.37 |
| Statistical analysis | | | | | | | | |
| Cultivars (CV) | | 0.6135 | 0.7614 | 0.0000 | 0.0417 | 0.0000 | 0.0006 | 0.0000 |
| Treatments (T) | | 0.9169 | 0.7555 | 0.0000 | 0.0019 | 0.0000 | 0.2367 | 0.0716 |
| CV x T | | 0.4446 | 0.944 | 0.0000 | 0.0001 | 0.1056 | 0.5601 | 0.6126 |

*Note:* Two-way ANOVA, repetition measurements, Tukey's test ($p \leq 0.05$) (IBM SPSS Statistics 29.0.1.0 software, SPSS Inc., Chicago, IL), at $p \leq 0.05$. Abbreviations: CCLa, chlorophyll content; DPPH, 2,2-diphenyl-1-picrylhrdrazl (antioxidant); FLV, flavonoid content; SPAD, soil plant analysis development; TPC, total phenolic content.

knowledge, this is the first study reporting the use of a fermented fruit waste, such as the kiwifruit, as biostimulant. For this reason, results obtained in this study, will be discussed in comparison with the most used biostimulants present in literature.

The application of fermented kiwi biostimulant has demonstrated significant improvements in various vegetative and productive parameters for both cultivars tested. When compared to other well-known biostimulants, such as fulvic acid, seaweed and botanical extract, FKB shows promising results (Rouphael and Colla 2020; Wang et al. 2022).

As reported for Moringa extracts, in strawberry (Ismail and Ganzour 2021) or for biostimulants like protein hydrolysates, in hydroponically grown basil (Ciriello et al. 2022), also FKB improved several growing parameters such as plant height, number of leaves, leaf area and root fresh weight. The lower root dry matter registered in plants grown with FKB could be due to the optimal nutrient availability that might limit the need for extensive root systems, suggesting biostimulants might focus on other growth and fruit quality aspects (Drobek, Frąc, and Cybulska 2020; Ertani et al. 2021).

In this study, FKB improved number and weight of strawberry fruits, as reported in other research carried out on tomato plants, in which seaweed and humic substances were used as biostimulant (Aytaç et al. 2024; Murtic et al. 2018). Fruit firmness seems to be improved by the use of FKB; this outcome is of particular interest, since it is linked to lower fruit mechanical damages and prolonged shelf life; same result is reported, in strawberry, after the use of Moringa extract as foliar biostimulant (Ismail and Ganzour 2021). As reported by Popescu and Popescu (2018) that used humic acids as foliar biostimulants in grape, also in this study the use of FKB enhance fruit quality, specifically in terms of TSSs, and most of all, improved the balance between TSSs and TA, a crucial parameter for flavour profiles in fruits like strawberries (Aghaeifard et al. 2016).

The evaluation of TPC and AO activity of fruit and leaf extracts confers more insights to better understand the role of FKB in columnar hydroponic strawberry cultivation.

For the authors' knowledge, this is the first study in which the effect of the biostimulant application has been evaluated, analysing the TPC and AO of leaves, other than fruits. Measuring these parameters in leaves helps in valorizing the strawberry vegetative biomass that is normally discarded; instead, in the framework of the circular bioeconomy, this biomass can be destined to different productive sectors, representing a secondary income for strawberry producers (Tan and Lamers 2021; Villamil-Galindo, Van de Velde, and Piagentini 2021). FKB treatment determined a reduction in the (poly)phenols in fruits and neither the antioxidant activity of extracts obtained from fruits; previous studies report that the plant response in terms of TPC and AO is strictly affected by several factors, among which the genotype (Aaby et al. 2012) and the growth and environmental conditions (Crespo et al. 2010).

In this study, the FKB treatment did not affect the TPC and AO of fruits; this result can be due to the experimental conditions, as reported by Kapur et al. (2024) for strawberry fruits. On the

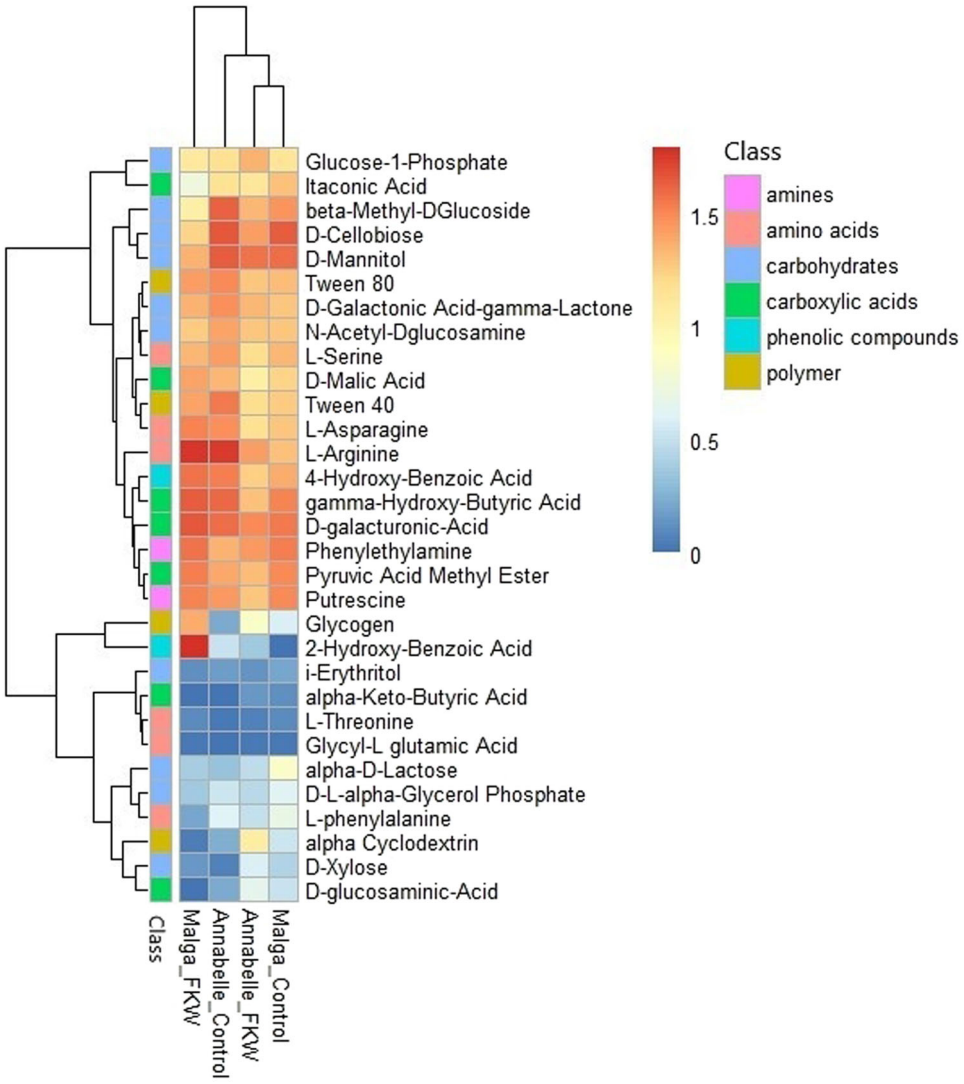



**FIGURE 3** | Heatmap displaying the metabolic profiling of microbial community in substrate samples of different strawberry cultivars exposed to the fermented kiwi product. The substrate utilisation patterns are clustered based on the Euclidean distance measure, calculated on the normalized Ecoplates data, and the colour intensity represents the efficiency of strains in metabolising the substrates supplied.

contrary, FKB, or nonetheless, it should be noted that the lack of statistically significant differences may be attributed to factors such as the varying experimental conditions, dosage or timing of the bio-stimulant, and genotype.

In this study, only the flavonol content was influenced by the FKB treatment; several are the studies reporting that enhanced flavonoid content is linked to healthier plants (Cardarelli et al. 2024; Dara 2019). Instead, FKB does not seem to improve chlorophyll efficiency and photosynthetic activity, in contrast with what was reported by Colla et al. (2014, 2021) and El-Nakhel et al. (2023) for several horticultural species, treated with different types of biostimulants. As reported for other types of biostimulants, such as humic acids and amino acids, improve boosting biomass production in horticultural plants.

Integrating biostimulants into agricultural practices aligns with sustainability goals by enhancing plant growth, yield and resilience while potentially reducing the need for chemical fertilizers and pesticides. This approach promotes healthier plants and more efficient resource use, contributing to sustainable agriculture.

The findings indicate that FKB favours plant growth, potentially due to the unique nutrient profile present in kiwifruit and metabolites released in the substrates after fermentation or by a specific microbial community present in substrate sample. As reported in previous sections, the differences in metabolic ability between control samples and the treated ones suggest that a different microbial community could be established, expressing different degradation pathways. The observed increase in strawberry plant growth and yield with FKB application suggests that these biostimulants could play a crucial role in enhancing agricultural productivity. Longer plants with robust plant vigour are likely to be more resilient to environmental stresses, which can lead to higher yields and improved plant quality.

## 5 | Conclusion

The study provides a valuable starting point for understanding the influence of fermented kiwi byproduct on plant growth and development in hydroponically grown strawberries. Results indicate that fermented kiwi byproduct biostimulant can

significantly enhance morphological and physiological parameters, as well as improve fruit quality attributes. To go deeper in investigating these results, further research should explore the specific mechanisms by which fermented kiwi byproduct affects plant height in the cv. Malga through hormone analysis. In addition, evaluating the interaction effects (cultivar × treatment) for other parameters could elucidate the physiological or genetic basis for the observed cultivar-dependent responses. The inclusion of a wider range of cultivars in future studies will help to determine the applicability of fermented kiwi byproduct, to better exploit its potential benefits in different agricultural settings.

The combination of hydroponics and biostimulants represents a promising environmentally friendly production strategy for greenhouse-grown vegetables. However, to fully leverage this approach, further research is needed to understand the mode of action of biostimulants, such as fermented kiwi byproduct, in plants and their effects on other vegetables.

## Author Contributions

**Samreen Nazeer:** formal analysis, investigation, data curation, writing–original draft preparation, writing–review and editing. **Anna Agosti:** formal analysis, investigation. **Lorenzo Del Vecchio:** formal analysis, investigation, data curation, writing–original draft preparation. **Leandra Leto:** formal analysis, investigation. **Andrea Di Fazio:** formal analysis, investigation. **Jasmine H. Saadoun:** conceptualization, methodology, validation, formal analysis, investigation, data curation, writing–original draft preparation, writing–review and editing, visualization, supervision. **Alessia Levante:** methodology, formal analysis, investigation, data curation, writing–original draft preparation, writing–review and editing. **Camilla Lazzi:** conceptualization, methodology, validation, resources, writing–review and editing, visualization, supervision, project administration, funding acquisition. **Martina Cirlini:** conceptualization, methodology, validation, formal analysis, resources, writing–review and editing, visualization, supervision, project administration, funding acquisition. **Benedetta Chiancone:** conceptualization, methodology, validation, formal analysis, resources, writing–review and editing, visualization, supervision, project administration, funding acquisition.


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## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

Data sharing is available on request.

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