

# Foliar application of plant-based biostimulants improve yield and upgrade qualitative characteristics of processing tomato

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## Highlights

- The effects of three plant-based biostimulants on yield and quality of processing tomato was explored.
- Application of protein hydrolysates and seaweed extract improve marketable yield.
- The biostimulants had different effect on nutritional and functional quality of tomato.
- Hydrophilic antioxidant activity and ascorbic acid content increased under protein hydrolysate application.

## Abstract

Tomato (*Solanum lycopersicum* L.) is a diffused worldwide vegetable. Great amounts of fertilizers are often applied for increasing yield and quality, without considering the negative effect on the environment. A possible perspective for reducing this risk is to raise the nitrogen use efficiency (NUE) through the use of plant biostimulants, which also improve yield and quality concomitantly. The aim of the current study was to verify the potential beneficial effect of three vegetal-based biostimulants on agronomical, qualitative and nitrogen use efficiency of a processing tomato crop. The experiment provided three biostimulants (an extract of brown seaweed [SwE], a legume-derived protein hydrolysate [LDPH] and a tropical plant extract). The following assessments were carried out: marketable and unmarketable yields, mean fruits weight, firmness, pH, total soluble solids (TSS), colour parameters (a/b), hydrophilic antioxidant activity (HAA), lipophilic antioxidant activity (LAA), total ascorbic acid content (AsA), total phenols, nitrate and total nitrogen content, nitrogen use effi-

ciency, N-uptake efficiency, and N-utilization. The foliar application of biostimulants especially protein hydrolysates and seaweed extract significantly affected the marketable yield with an average increase of 18.3% over the control and 41.3% average decrease in unmarketable yield. The N-use and N-uptake efficiency followed a similar trend, with biostimulants boosting it higher than control, +18.4% and +59.3%, respectively; the nitrogen content was also higher in fruits of sprayed plants: +21.3% over control. This finding also reflects on higher dry matter accumulation and firmness in fruits of treated plants (+10.9% and +14.1% over control, respectively). The biostimulants application, in particular SwE and LDPH, also boosted TSS (+12.8%), the a/b colour ratio (+7.5%), HAA and AsA (9.8% and 114.6%, respectively). Therefore, the legume-derived protein hydrolysates and extract of brown seaweed *Ecklonia maxima* seem a good sustainable approach to improve yield and quality of tomato for canning industries.

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## Introduction

Tomato (*Solanum lycopersicum* L.) is among the most diffused vegetables in the world. Improving commercial yield, size, shape, firmness, colour, taste, and solid content of fruit represent the main goals to increase commercial values of this vegetable (Del Giudice *et al.*, 2016). These quality attributes as well as the yield are affected by climatic conditions and the agronomic management (irrigation, fertilisation, weed control, *etc.*) (Kalt, 2005; Flores *et al.*, 2009). For improving tomato production, the intensive application of chemical fertilizers has thrived as an ordinary practice among the farmers, notwithstanding its collateral damage to soil ecology and agricultural systems (Villarreal-Sánchez *et al.*, 2003). A viable perspective for reducing the risks linked to excessive or unbalanced use of chemical fertilizer is to raise the nitrogen use efficiency (NUE) that depends on the capacity of plants to uptake nutrients, as well as on their systems of transport, storage, and the mobilization, other than the N loss into the environment (Hawkesford *et al.*, 2014). In the last two decades, the use of plant biostimulants have been coupled to biotechnology and plant breeding strategies to improve NUE (Calvo *et al.*, 2014; Di Mola

*et al.*, 2020a). Plant biostimulants are considered an important and sustainable approach to enhance the nutritional and functional quality of vegetable products and to maintain soil fertility (du Jardin, 2015). As emphasized by du Jardin (2015), biostimulants are used to improve nutrient uptake and increase yield and crop quality, stimulating natural processes in different conditions. Most researchers reported that the application of plant biostimulants can increase plant growth and development, productivity, and nutritional quality (Parađiković *et al.*, 2011; Koukounararas *et al.*, 2013; Ertani *et al.*, 2014; Bulgari *et al.*, 2015); moreover, it enhances soil-conditions influencing its microflora, modifying the root system architecture, and boosting their development (Caruso *et al.*, 2019a; Di Mola *et al.*, 2019). Nowadays, among the marketed biostimulants, seaweed extracts, particularly the brown algae (*Phaeophyceae*), protein hydrolysates, and plant extracts are the most representative plant-based biostimulant categories.

Biostimulants can be applied through foliar application or soil/substrate drenching. When the biostimulant is sprayed on the leaves, it is absorbed through the cuticle, epidermal cells and stomata (Fernández and Eichert, 2009); instead with drenching technique, it is absorbed through root epidermal cells and gets redistributed through the xylem (Subbarao *et al.*, 2015). Biostimulants can modify the primary and secondary metabolism of plants, improving productivity and decreasing the impact of abiotic stress on crops (Calvo *et al.*, 2014; Roupheal *et al.*, 2017a). Some biostimulants, as extracts rich in amino acids, improve tolerance to heat stresses (Colla *et al.*, 2014; Nardi *et al.*, 2016; Lucini *et al.*, 2015) and to saline stress (Di Mola *et al.*, 2021). Van Oosten *et al.* (2017) reported that lettuce plants treated with a hydrolysed protein based biostimulant, under cold stress showed higher fresh weight and better stomatal conductance compared to non-treated plants. More researches reported that the application of vegetal-based biostimulants on lettuce and tomato determined a significant increase of the nutritional and functional quality of edible tissues (Caruso *et al.*, 2019b; Cozzolino *et al.*, 2020). Also, Di Mola *et al.* (2020b) reported that the foliar application of biostimulants, especially seaweed extract and protein hydrolysates, improved the quality of baby leaf lettuce grown under different nitrogen levels, including non-fertilized ones. The effect of plant-derived biostimulants and seaweed extracts on yield and fruit quality of tomato are reported in a few articles. Colla *et al.* (2017a) reported a better yield and quality in greenhouse tomato treated with these biostimulants; these improvements were more evident with the foliar application of the commercial tropical plant extract 'Auxym'. Furthermore, Roupheal *et al.* (2017b) observed that tomatoes treated with legume-derived protein hydrolysate 'Trainer' accumulated more total solids soluble, lycopene, K, and Mg contents compared to untreated plants. Although the use of biostimulants increases production costs, Colla *et al.* (2017a) demonstrated that the yield increase of treated plants brings to higher gross returns and consequently also higher net returns, as compared to untreated plants cultivation. Based on the above mentioned and starting from the findings of Colla *et al.* (2017a), the aim of the present study was to verify the potential beneficial effect of the same three vegetal-based biostimulants on a processing tomato, a different variety in respect to Colla *et al.* (2017a), evaluating not only yield, and physical, chemical, and nutritional traits of fruits but also the biostimulants effect on nitrogen use, utilization, and uptake efficiency.

## Materials and methods

### Experimental site and design, crop management and biostimulant applications

The experiment was carried out in open field during the spring-summer growing season 2019, at the Department of Agricultural Sciences (Portici, NA, Southern Italy). The soil was sandy loam (70% sand, 12% silt, 18% clay), with a pH of 8.0, electrical conductivity (EC) of 0.25 dS m<sup>-1</sup>, 2.0% organic matter, 0.13% total nitrogen, N-NO<sub>3</sub> and N-NH<sub>4</sub> 15.0 and 6.4 mg kg<sup>-1</sup>, respectively, P<sub>2</sub>O<sub>5</sub> 75 mg kg<sup>-1</sup>, and exchangeable K<sub>2</sub>O 758 mg kg<sup>-1</sup>.

The tested crop was tomato (*Solanum lycopersicum* L.) 'Coronel' (ISI Sementi, Parma, Italy) F1 hybrid showing very consistent oval fruit, was adopted. The experimental plan consisted of a randomized complete-block design with three replicates per each treatment. The three treatments/biostimulants based on: an extract of brown seaweed *Ecklonia maxima* (Osbeck) - SwE; a legume-derived protein hydrolysate - LDPH; and a tropical plant extract - TPE, were compared with an untreated control.

The SwE is made by Kelpak Products (Ltd., Cape Town, South Africa), and marketed with the trade name Kelpak®; the LDPH, known with the trade name Trainer®, and the TPE, marketed with the trade name Auxym®, are produced by Italtollina S.p.A. (Rivoli Veronese, Italy). The elemental composition of the three biostimulants were reported by in Colla *et al.* (2017a).

Each experimental plot was 17.2 m<sup>2</sup> (5.60×3.08 m) and 55 plants per plots were transplanted on June 10 in single rows, corresponding to a plant density of 32,467 per ha. Before the transplant, a biodegradable black mulching film (15 µm thick MaterBi®, Novamont, Novara, Italy) was hand-placed on each row. Based on the Campania Region Fertilization Guide, the nutrient needs were calculated: potassium (K) was not needed, while phosphorus (P) was applied as mineral superphosphate at the rate of 20 kg ha<sup>-1</sup> before the transplant and nitrogen (N) was applied as ammonium nitrate and calcium nitrate at the rate of 130 kg ha<sup>-1</sup> of nitrogen by fertigation. Irrigations were made on a weekly basis, and they were stopped 10 days before harvest; the water lost by evapotranspiration was calculated by the Hargreaves formula and was completely restored. As for the biostimulants, the application started on June 25 on a bi-weekly basis, for a total of four applications; SwE and TPE were sprayed at a concentration of 2 mL L<sup>-1</sup>, and LDPH at 3 mL L<sup>-1</sup>, according to the manufacturers' recommendations, while control plants were sprayed with tap water. The harvest of tomato plants was on September 6.

### Yield assessments

At harvest, on 20 plants taken from the two central rows of each experimental plot, the fresh weight of marketable and unmarketable yield were weighted, and mean fruit weight were calculated, by dividing the fruit fresh weight by the number of fruits. The yield was expressed as tons ha<sup>-1</sup>. Then, a sample of plant biomass (stems and leaves) and fruits per each replicate (experimental plot) was oven-dried at 70°C, until reaching a constant weight in order to determine dry matter.

### Physical, chemical and nutritional traits of tomato fruits

The following physical, chemical and nutritional traits of tomato fruits were investigated: firmness, juice pH, total soluble solids (TSS), colour parameters (a/b), hydrophilic/lipophilic antioxidant activities (HAA and LAA, respectively), total ascorbic acid content (AsA), and total phenols. Firmness was performed on the two sides of the equa-

torial zone of five fruits per replicate, using a digital penetrometer (T.R. Turoni s.r.l., Forlì, Italy) with an 8 mm tip. The applied force for 4 mm penetration was expressed in  $\text{kg m}^{-2}$ . On fresh fruit juice, pH and TSS were assessed, using a digital pH-meter (METTLER TOLEDO MP 220) and a portable digital refractometer (Sinergica Soluzioni s.r.l., Pescara, Italy, model DBR 35), respectively. The TSS were expressed as °Brix. On ten-fruit samples per each treatment the colour space parameters ( $a^*$  and  $b^*$ ) were measured by a portable Hunter Lab Colorimeter (3NH model 310). The data were reported as red/yellow ratio ( $a/b$ ). The hydrophilic and lipophilic antioxidant activity and total phenols were determined on fresh vegetable samples, after freezing and lyophilizing, where AsA was determined on freeze fresh material. HAA, LAA and AsA were assessed spectrophotometrically, according to methods of Fogliano *et al.* (1999), Re *et al.* (1999), and Kampfinkel *et al.* (1995), respectively. The absorbance of solutions was measured at 505, 734, and 525 nm, for HAA, LAA and AsA, respectively. Finally, total phenols content was determined by Folin-Ciocalteu procedure (Singleton *et al.*, 1999).

### Nitrogen determination, and N-use, -uptake, and -utilization efficiency

The nitrate content and total nitrogen content of fruits were determined on dried samples by Foss FIAstar 5000 continuous flow Analyzer (FOSS analytical Denmark), based on colorimetric and Kjeldhal method (Bremner, 1965), respectively. Nitrogen use efficiency was calculated by dividing fresh yield by N applied (Aujla *et al.*, 2007); moreover, N-uptake efficiency was determined as the ratio between the nitrogen content of fruits and N applied; N-utilization was instead calculated as the ratio between fresh yield and nitrogen content of fruit. All three efficiency parameters were expressed as  $\text{kg kg}^{-1}$ .

### Statistical analysis

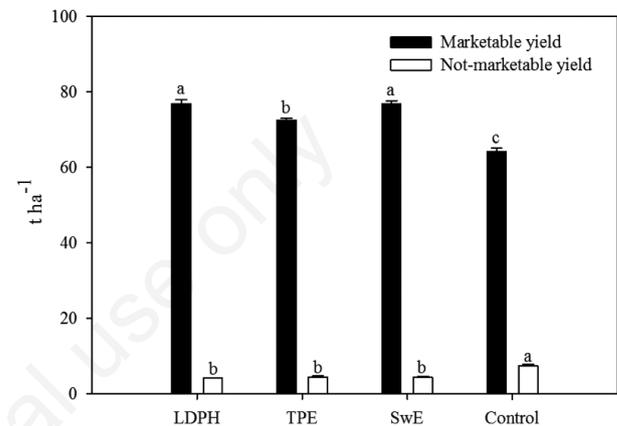
All data were subjected to the analysis of variance (one way-ANOVA), using a general linear model by the SPSS software package. Means were separated according to the Duncan's multiple range test at  $P \leq 0.05$ .

## Results and discussion

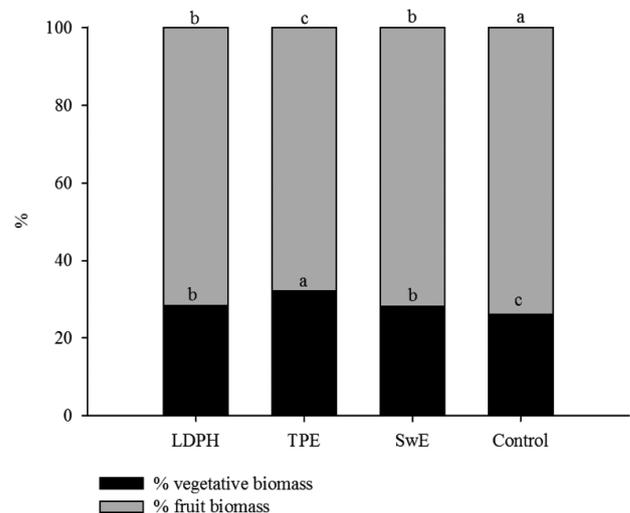
### Effects of biostimulants on yield and its parameters

The results regarding marketable and unmarketable yield of tomato are reported in Figure 1. The foliar application of biostimulants, significantly increased marketable yield respect to the untreated control (+18.3%, on average). This mean increase was about double than the improvement for greenhouse tomato reported by Colla *et al.* (2017a), who also reported Auxym (TPE) as the best treatment in improving marketable yield. On the contrary, we found that LDPH and SwE reached higher similar values (76.8 and 78.8  $\text{t ha}^{-1}$ , respectively) than TPE treatment (72.6  $\text{t ha}^{-1}$ ). The positive effect of SwE on marketable yield was also observed by Ali *et al.* (2016), who applied *Ascophyllum nodosum* seaweed extract on the foliage of tomato plants. The ascribed beneficial effect of this biostimulant is due to the present polysaccharides, which improved plant productivity by enhancing endogenous hormone homeostasis (Rolland *et al.*, 2002). Regarding the effect of LDPH 'Trainer', Colla *et al.* (2014) highlighted that the used biostimulant triggers an auxin-like and to a lesser extent a gibberellin-like activities; moreover, de Jong *et al.* (2009) reported that the gibberellins

also play an important role in the onset of tomato fruit development by controlling both the expression of the genes regulating cell division and cell expansion in fruits. Additionally, Caruso *et al.* (2019b) observed similar results in cherry-like tomato landrace 'Piennolo del Vesuvio' treated with LDPH and TPE, which increased marketable yield around +14.9% over control. In addition, in our research all three biostimulants significantly affected the unmarketable yield, by reducing its incidence about 41.3% (on average) compared to control. The findings of our research highlight a similar overall response of tomato to the three plant-based biostimulants, but within the specie each variety seems to respond differently to the single biostimulant.



**Figure 1.** Marketable and unmarketable fruits of tomato as affected by biostimulants application. LDPH, legume-derived protein hydrolysates; TPE, tropical plant extract; SwE, seaweed *Ecklonia maxima*; Control, untreated. Different letters indicate significant differences according to Duncan's multiple range test ( $P \leq 0.05$ ).



**Figure 2.** Percentage repartition of dry matter between vegetative and fruit biomass of tomato as affected by biostimulants application. LDPH, legume-derived protein hydrolysates; TPE, tropical plant extract; SwE, seaweed *Ecklonia maxima*; Control, untreated. Different letters indicate significant differences according to Duncan's multiple range test ( $P \leq 0.05$ ).

The biostimulants treatment had a significant effect as well on the percentage repartition of dry matter between plant biomass and tomato fruits (Figure 2). All three biostimulants stimulated the plant biomass development, as highlighted by a higher incidence of vegetable biomass on total dry matter in sprayed plants respect to untreated plants (+13.1%). In particular, this increment was significantly higher for TPE treated plants compared to the other two biostimulants. A possible explanation of the TPE effect, could be both the stimulation of root growth of treated plants and the presence of phytohormones (e.g., auxins) and signalling compounds (e.g., amino acids, vitamins, phytochelatins), with an overall development of plants (Colla *et al.*, 2017a).

### Nitrogen use, uptake, and utilization efficiency

The used biostimulants significantly affected nitrogen use, uptake and utilization efficiency, as well as total nitrogen content of tomato fruits. The N-use and N-uptake efficiency followed a similar trend, highlighting increased values than the control (+18.4% and +59.3%, respectively). Anyway, the seaweed extract and legume derived-PH promoted the best effect (Table 1). Instead, the N-Utilization efficiency showed an opposite trend, with untreated control showing higher values than the three biostimulants (+32.9%). Finally, it was notable that total nitrogen content was higher in fruits of treated plants (+21.3% over the control). In a previous research (Di Mola *et al.*, 2020a), LDPH application proved to enhance the N-Use and N-Uptake efficiency in baby spinach and lamb's lettuce.

The nitrogen efficiency parameters represent very important indicators of the N metabolism in plants; in fact, a high supply of nitrogen does not always correspond to high yield, because this element can accumulate in other organs (stems and roots) or leached in the soil like nitrate. Furthermore, Du *et al.* (2017) and Ronga *et al.* (2019) observed low values of NUE in tomato plants fertilized with higher doses of nitrogen than the N0-control; Djidonou *et al.* (2013) also obtained the same results, regardless of

the different experimental conditions. In addition, Cammarano *et al.* (2020) investigated the role of projected changes and increased CO<sub>2</sub> on the water and nutrient efficiency of tomato and they found that the impact of the CO<sub>2</sub> on the crop physiological efficiencies was positive for the NUE and WUE, but these efficiencies decreased when the irrigation and fertilization was optimized. Our findings reflected the yield increase occurring in plants treated with LDPH and SwE, but the increase in N-uptake efficiency also resulted in an accumulation of nitrogen in tomato fruits. On the other hand, LDPH in other researches already showed its ability to boost crops' resources use efficiency (in terms of water and nutrients) (Colla *et al.*, 2017b; Rouphael *et al.*, 2018b), especially N uptake and assimilation. The positive effect of foliar application of LDPH on the N efficiency parameters can be due to the improvement of root architecture, which is related to an increase in nutrient accessibility caused by its ability to boost the capacity of absorption, translocation and assimilation of macro and micro-minerals (Ertani *et al.*, 2009; Schiavon *et al.*, 2008). Finally, the highest value of N-Utilization (ratio between fresh yield and N removed by fruits) recorded for the control, suggested that untreated plants although producing less but did remove a quantity of nitrogen lower as well, that was totally used for sustaining yield (high N-Utilization efficiency), at the expense of N content in fruits.

### Physical, chemical and nutritional traits of tomato fruits

Total soluble solids content, pH, a/b colour, firmness, fruit weight and dry matter content were reported in Table 2; all parameters except pH were significantly affected by biostimulant treatments.

In our research, applied biostimulant increased FW compared to the untreated control (82.3 vs 69.8 g fruit<sup>-1</sup>, respectively) with LDPH showing significantly higher effect than TPE (Table 2). These results resembled the effect induced on yield, highlighting a good correlation (0.988) between the two parameters. The adopted

**Table 1. Effect of biostimulants application on nitrogen (N) use efficiency, nitrogen uptake efficiency, nitrogen utilization efficiency, and N total content of tomato fruits.**

| Treatments    | N-Use efficiency<br>kg kg <sup>-1</sup> | N-Uptake efficiency<br>kg kg <sup>-1</sup> | N-Utilization efficiency<br>kg kg <sup>-1</sup> | Total N<br>%       |
|---------------|---|--|---|--------------------|
| Biostimulants |   |  |   |                    |
| SwE           | 606.4 <sup>a</sup>                      | 0.92 <sup>ab</sup>                         | 668.1 <sup>b</sup>                              | 2.47 <sup>ab</sup> |
| TPE           | 558.4 <sup>b</sup>                      | 0.84 <sup>b</sup>                          | 678.8 <sup>b</sup>                              | 2.58 <sup>a</sup>  |
| LDPH          | 591.0 <sup>a</sup>                      | 1.06 <sup>a</sup>                          | 560.8 <sup>b</sup>                              | 2.82 <sup>a</sup>  |
| Control       | 494.5 <sup>c</sup>                      | 0.59 <sup>c</sup>                          | 845.3 <sup>a</sup>                              | 2.16 <sup>b</sup>  |
| Significance  | **                                      | **   | *   | *                  |

SwE, seaweed *Ecklonia maxima*; TPE, tropical plant extract; LDPH, legume-derived protein hydrolysates; Control, untreated. \*, \*\*Significant at P<0.05 or P<0.01, respectively. <sup>a-c</sup>Different letters within each column indicate significant differences according to Duncan's multiple range test P≤0.05.

**Table 2. Effect of biostimulants application on total soluble solids, pH, colour a/b, firmness, fruit average weight, fruit dry matter.**

| Treatments    | TSS<br>°Brix      | pH   | Colour a/b        | Firmness<br>kg cm <sup>-2</sup> | FAW<br>g fruit <sup>-1</sup> | DM fruit<br>%      |
|---------------|-------------------|------|-------------------|---------------------------------|------------------------------|--------------------|
| Biostimulants |                   |      |                   |                                 |                              |                    |
| SwE           | 5.87 <sup>a</sup> | 4.22 | 2.59 <sup>a</sup> | 0.82 <sup>a</sup>               | 86.1 <sup>a</sup>            | 6.10 <sup>ab</sup> |
| TPE           | 5.47 <sup>b</sup> | 4.23 | 2.48 <sup>b</sup> | 0.76 <sup>b</sup>               | 78.4 <sup>b</sup>            | 5.83 <sup>b</sup>  |
| LDPH          | 5.97 <sup>a</sup> | 4.18 | 2.64 <sup>a</sup> | 0.84 <sup>a</sup>               | 82.5 <sup>a</sup>            | 6.37 <sup>a</sup>  |
| Control       | 5.13 <sup>b</sup> | 4.22 | 2.39 <sup>c</sup> | 0.71 <sup>c</sup>               | 69.8 <sup>c</sup>            | 5.50 <sup>c</sup>  |
| Significance  | **                | NS   | **                | **                              | **                           | *                  |

TSS, total soluble solids; FAW, fruit average weight; DM, dry matter; SwE, seaweed *Ecklonia maxima*; TPE, tropical plant extract; LDPH, legume-derived hydrolysate proteins; Control, not treated; NS, non-significant. \*, \*\*Significant at P<0.05 or P<0.01, respectively. <sup>a-c</sup>Different letters within each column indicate significant differences according to Duncan's multiple range test P≤0.05.

**Table 3. Effect of biostimulants application on lipophilic antioxidant activity, hydrophilic antioxidant activity, phenols and ascorbic acid.**

| Treatments    | LAA<br>mmol Trolox 100g <sup>-1</sup> dw | HAA<br>mmol AA 100g <sup>-1</sup> dw | Phenols<br>mg gallic acid g <sup>-1</sup> dw | AsA<br>mg 100 g <sup>-1</sup> fw |
|---------------|--|--------------------------------------|--|----------------------------------|
| Biostimulants |  |                                      |  |                                  |
| SwE           | 8.86 <sup>ab</sup>                       | 11.19 <sup>a</sup>                   | 1.39   | 61.86 <sup>b</sup>               |
| TPE           | 9.02 <sup>a</sup>                        | 8.66 <sup>c</sup>                    | 1.38   | 40.15 <sup>c</sup>               |
| LDPH          | 8.47 <sup>c</sup>                        | 10.76 <sup>a</sup>                   | 1.49   | 83.37 <sup>a</sup>               |
| Control       | 8.57 <sup>bc</sup>                       | 10.00 <sup>b</sup>                   | 1.41   | 33.84 <sup>c</sup>               |
| Significance  | **                                       | **                                   | NS   | **                               |

LAA, lipophilic antioxidant activity; HAA, hydrophilic antioxidant activity; AsA, ascorbic acid; SwE, seaweed *Ecklonia maxima*; TPE, tropical plant extract; LDPH, legume-derived hydrolysate proteins; Control, not treated. \*\*Significant at P<0.01, respectively. <sup>a-c</sup>Different letters within each column indicate significant differences according to Duncan's multiple range test P≤0.05.

biostimulants did not seem to influence the number of fruits per plant, that was 28.5 (mean value of three biostimulants) vs 28.4 of the control (data not reported).

The biostimulants application also promoted dry matter accumulation in the fruits, whose increasing was about 10.9% than control (Table 2). This finding could be partially related to the improved N fruit content in the treated plots. Likewise, firmness showed a similar trend, with 14.1% increase respect to untreated plants (Table 2). Several authors reported that fruit firmness is related to membrane properties, cell size and wall structure (Chapman *et al.*, 2012; Huang *et al.*, 2018) and represent an important indicator of tomato fruit quality. Maach *et al.* (2020) found that the application of two biostimulants (Tecamin Flower and Tecamin Brix), alone or combined, improved firmness of tomato fruits, probably because the algal extract has a beneficial effect on turgidity and wall components (Hawkesford *et al.*, 2012). Our findings were in line with these results, highlighting a greater effect of Seaweed algal extract and LDPH on firmness, than TPE and untreated control.

Total soluble solids (measured as °Brix), are the soluble part (sugars, acids, mineral salts) of dry matter of tomato fruit, and represent one of the most important quality traits for tomato industries and for consumer (Kader, 2002). The biostimulants application increased TSS in biostimulant-treated plants compared to the control (+12.8% as reported in Table 2). Colla *et al.* (2017a) and Ertani *et al.* (2014) also found an increase in TSS, glucose and sucrose in tomato and chili pepper when plants were treated with LDPH and biostimulants based on alfalfa and red grape, respectively.

The colour is one of the most important physical parameters, both for fresh and processed tomato; in this crop it is mainly linked to lycopene biosynthesis, which is under genetic control and depends on the temperature (Brandt *et al.*, 2006). The colour is also affected by irrigation (Nangare *et al.*, 2016) and nitrogen fertilization (Ronga *et al.*, 2020). Values greater than 1.70 up to 2.60 are desirable for canned products. In our research, the a/b values ranged from 2.39 for the control up to 2.64 for LDPH-treated tomatoes; the all three biostimulant improved colour parameters (+7.5% over control), with TPE-treatment being less effective than SwE and LDPH (Table 2).

Low juice pH of processed tomato is crucial in controlling the proliferation of harmful microorganisms in processed products; values inferior to 4.3 require temperatures lower than 100°C for sterilization. In our study, biostimulant application did not affect the pH of fruit juice (pH=4.21 as means of all treatments), which was optimal for tomato canning. Our results were consistent with

those of Colla *et al.* (2017a) and Rouphael *et al.* (2017b) reporting no effects of biostimulants on tomato pH.

The lipophilic and hydrophilic antioxidant capacities represent good parameters to evaluate the tomato nutritional quality. These two measurements concern antioxidant molecules having a beneficial effect on human health, delaying or/inhibiting oxidative damage, hence evading a broad range of diseases (Khanam *et al.*, 2012; Kyriacou and Rouphael, 2018). In our research, the lipophilic and hydrophilic antioxidant activities, as well as the total ascorbic acid were affected by biostimulants application. Indeed, SwE and LDPH application increased HAA and AsA (+9.8%, and +114.6% in, respectively), LAA was instead improved by application of SwE and TPE which were statistically comparable (Table 3). Our findings are consistent with the results reported by Caruso *et al.* (2019b) showing a beneficial effect of LDPH on AsA and TPE on LAA of a cherry-like tomato landrace. The synthesis and built up of bioactive compounds like AsA (LAA) can be correlated to indirect or direct aftermath of the application of biostimulants on the formation of antioxidants in plant tissues (Rouphael *et al.*, 2017b). For instance, as mentioned by the same authors, plant-derived protein hydrolysates can promote the activity of certain enzymes tangled in antioxidant cells homeostasis, or an indirect effect such potassium accumulation can lead to the increase of fruit antioxidants.

## Conclusions

The findings of the current research indicate that foliar application of plant-based biostimulants improved tomato yield and mean fruit weight, especially when legume-derived protein hydrolysates and seaweed extract were applied. The effect of these biostimulants on yield is probably due to their beneficial effect on nitrogen use efficiency (N uptake and the N-use). Moreover, some quality attributes of tomato fruits (TSS, colour and firmness) were improved by the same two biostimulants, offering additional benefits to tomato industry both on the qualitative and the economic aspects.

Therefore, the use of the legume-derived protein hydrolysates and extract of brown seaweed *Ecklonia maxima* seem a good sustainable approach to improve yield and quality of processing tomato. However, future research seems necessary in order to verify the effect of these commercial products in different environmental and on varieties showing different fruit type and length of the crop cycle.

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