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Article

Yield, Quality, Antioxidants, and Mineral Composition of Traditional Italian Storage Onion Cultivars in Response to Protein Hydrolysate and Microalgae Biostimulation

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Abstract: Increasing interest is being devoted to environmentally friendly strategies, such as the use of plant biostimulants, to enhance crop performance and concurrently ensure food security under the perspective of sustainable management. The effects of two biostimulant formulations (protein hydrolysate and spirulina) on four Italian traditional storage onion cultivars (Ramata di Montoro, Rossa di Tropea, Rocca Bruna, Dorata di Parma) were investigated in Naples province (southern Italy), in terms of yield, quality, shelf-life, bioactive compounds, and mineral composition. Ramata di Montoro showed the highest levels of yield (66.4 t ha^{-1}) and vitamin C (31.5 mg g^{-1} d.w.) and the longest shelf-life (228 days). Significant increases in marketable yield were recorded under the applications of both protein hydrolysate (+15.5%) and spirulina (+12.4%) compared to the untreated control. The two biostimulant formulations significantly increased bulb shelf-life and the contents of polyphenols (201.4 mg gallic acid eq. 100 g^{-1} d.w. on average vs. 158.6 of the untreated control), vitamin C (26.8 mg g^{-1} d.w. on average vs. 22), and both lipophilic and hydrophilic antioxidant activities. These findings demonstrate the effectiveness of both protein hydrolysate and spirulina as sustainable tools for enhancing both yield and quality parameters within the frame of environmentally friendly farming management.

Keywords: *Allium cepa* L.; variety; biostimulants; spirulina; bulb shelf-life; polyphenols; vitamin C; macroelements; microelements

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1. Introduction

Onion (*Allium cepa* L.) is among the most important horticultural crops worldwide, with a global yield of 96.8 million tons in 2020 including about 419,000 tons in Italy [1,2]. The beneficial properties of its bulbs for human health mainly relate to the high content of bioactive substances, such as polyphenols and sulphur compounds, exerting antioxidant, anticancer, and anti-inflammatory activities [3–5].

Onion varieties show remarkable diversity in morphological, physiological, and biochemical traits, particularly in bulb characteristics such as size, shape, colour, and chemical composition [6–8]. Particularly, traditional genotypes have evolved distinct features adapted to local growing conditions and consumer preferences and also show appreciable accumulation of bioactive compounds, though significant variations in polyphenol content and antioxidant activity among varieties have been reported in previous research [4,8,9].

The growing demand for premium quality products and the need for sustainable agricultural practices have led to the development of alternative approaches in crop management and, in this respect, biostimulants play an important role based on their ability to stimulate plant nutrition processes regardless of their nutrient content, improving nutrient use efficiency, stress tolerance, and global crop quality.

Among biostimulants, protein hydrolysates and microalgae extracts have shown promising results in horticultural crops. Indeed, protein hydrolysates, obtained through chemical or enzymatic hydrolysis of proteins from plant or animal sources, enhanced nutrient uptake and plant metabolism, based on the reports of González-de-Peredo et al. [10] and Aguiar et al. [5]. According to the latter authors, spirulina (*Arthrospira platensis*), a blue-green microalgae rich in bioactive compounds, amino acids, and plant growth promoters, showed beneficial effects on crop growth and quality in previous research. In fact, their findings revealed substantial enhancements in plant growth, particularly in *Bayamred* spinach, where the mentioned treatment resulted in increases of 58.3%, 110.1%, and 155.8% in plant height, fresh weight, and dry weight, respectively, compared to the untreated control. Moreover, González-de-Peredo et al. and Aguiar et al.'s [4] studies revealed augmentations in germination rate (82% vs. 56% in control) and shoot development (25.1 cm vs. 15.3 cm in control) in *Trigonella foenum-graecum*, in addition to the improved photosynthetic capacity as evidenced by the high chlorophyll content (0.53 mg g^{-1} vs. 0.38 mg g^{-1} fresh weight control) [11,12].

The genotype-specific responses to biostimulant applications have been recorded by other authors regarding both yield and quality parameters in onion [13] and other horticultural crops [14]. Indeed, in the study by Giordano et al. [13], two different biostimulants (a protein hydrolysate and a tropical plant extract) led to different results in terms of yield and quality in two diverse lettuce cultivars (*Lactuca sativa* L. var. capitata, red and green Salanova). Moreover, in other research [14], the application of three different biostimulants (a humic/fulvic acids-based formulation, a *Trichoderma atroviride* fungal culture, and a seaweed extract derived from *Ecklonia radiata* and *Laminaria* spp.), elicited a greater root development of the onion cultivar Don Victoro, with a 29.7% root-to-shoot ratio, while the cultivar Carta Blanca showed enhanced shoot growth with up to a 34% increase in leaf area under combined treatments. The cultivar Sofire demonstrated intermediate responses, with a 28% increase in root dry weight when treated with microbial-seaweed combinations, underlying the strong genotype-dependence of biostimulant efficacy and related treatment optimisation strategies.

Makhaye et al. [15] investigated the interaction between biostimulants (*Eklonia maxima* and PGPR) and *Abelmoschus esculentus* L. genotypes, revealing up to an 82% increase in germination rates for specific genotypes treated with the marine algae. Carvalho et al. [16] reported the significant variety-dependent effect of a protein hydrolysate (Triplus®) on the yield of six common bean cultivars, with the best results obtained with cultivars BRS Estilo and BRS Notàvel. The beneficial effects of microalgal biostimulants were described in previous studies: Faheed and Abd-El Fattah [17] recorded enhanced germination and seedling growth in lettuce treated with *Chlorella vulgaris* extracts, with an augmentation by about 12% of growth parameters (fresh and dry weight), in comparison with the untreated control but a decrease in soluble solids, proteins, and amino acids by 8.2%, 11.4%, and 7.2%, respectively.

The mentioned genetic diversity influences the overall production performance in onion crop systems, making the variety choice a crucial factor in combination with innovative agronomic strategies such as the biostimulant application, which requires further investigation.

Extensive research has documented different responses to biostimulants in several vegetable species, but scant investigations have been carried out in *Allium cepa* L. and, therefore, deepened knowledge about biostimulant applications on onion crops is required.

The present study investigated the comparative effects of two constitutionally different biostimulants, a protein hydrolysate and a microalgae extract, on four traditional Italian storage onion cultivars grown in the open field. It was hypothesised that the diverse chemical composition of the two mentioned formulations triggers cultivar-dependent physiological pathways in onion plants. The related consequences were assessed in terms of yield, quality, shelf-life, antioxidant properties, and mineral composition of bulbs, with the perspective to gain useful insights for optimising sustainable cultivation practices in *Allium cepa* L. crop systems.

2. Materials and Methods

2.1. Experimental Protocol and Growing Conditions

Research was conducted in Portici (Naples, Italy, 40°49′11′′ N, 14°20′28′′ E, 29 m above sea level), in a Mediterranean environment, on the storage onion (*Allium cepa* L.) cultivated in the open field in 2021 and 2022. Onion was grown after common bean (*Phaseolus vulgaris* L.) in a soil with the following characteristics at 0–35 cm depth: 1.45 g cm^{-3} bulk density, 63.6% sand, 21.4% silt, 15% clay, 2% organic matter, 0.13% total nitrogen, 58.6 ppm P_2O_5, 1670 ppm K_2O, pH 7.3, and 0.41 dS m^{-1} electrical conductivity (EC).

The mean monthly values of minimum and maximum temperature and rainfall in the two experiment years were the following: 4 °C and 12 °C, and 102 mm in January; 4 °C and 13 °C, and 99 mm in February; 6 °C and 15 °C, and 88 mm in March; 8 °C and 18 °C, and 74 mm in April; 12 °C and 23 °C, and 50 mm in May; 16 °C and 26 °C, and 34 mm in June; 18 °C and 30 °C, and 24 mm in July; 18 °C and 31 °C, and 42 mm in August; 14 °C and 27 °C, and 82 mm in September; 12 °C and 22 °C, and 130 mm in October; 8 °C and 17 °C, and 162 mm in November; 5 °C and 14 °C, and 111 mm in December.

The experimental protocol was based on the factorial combination of 4 cultivars (Ramata di Montoro, Rossa di Tropea, Rocca Bruna, Dorata di Parma) and 2 biostimulant treatments (protein hydrolysate Dynamic and spirulina microalgae powder), plus an untreated control. A split-plot design with three replications was used for the treatment distribution in the field, with the onion cultivars assigned to the main plots and the biostimulant treatments to the subplots; the experimental unit had a 4 m^2 surface area (2 m × 2 m). Regarding the two biostimulants applied, the protein hydrolysate Dynamic® (by Hydro Fert, Barletta, Italy) contains 5% N, 10% C, and 30% and 4.8% of total and free amino acids, respectively; the spirulina microalgae powder (by Micoperi Blue Growth, Ortona, Chieti, Italy) has 7% N, 40% C, and 60% and 55% of total and free amino acids, respectively.

Prior to transplanting, the soil was ploughed at 40 cm and hoed at 15 cm soil depth. The transplant was conducted on 22 February, in both years, on raised beds with 7.5 cm spacing along the rows which were 65 cm apart (20.5 plants per m^2). Fertilisation was carried out with 167 kg ha^{-1} N (calcium nitrate and urea), 60 kg ha^{-1} P_2O_5 (simple superphosphate), and 200 kg ha^{-1} K_2O (potassium sulphate); phosphorus was completely supplied prior to transplanting, along with 50% N and K whose remainder moieties were supplied during the growing season; micronutrients were supplied through foliar application of B (1.5 kg ha^{-1}), Zn (1.5 kg ha^{-1}), Fe (2.5 kg ha^{-1}), Mn (1.5 kg ha^{-1}), Cu (1.0 kg ha^{-1}), and Mo (0.1 kg ha^{-1}) 3 weeks after transplanting. A post-emergence treatment for weed control was performed using ioxynil (0.3 L ha^{-1} of active principle); fungal disease control was performed with azoxystrobin + difenoconazole (1 L ha^{-1} of active principle) against Botrytis and Peronospora. Drip irrigation was activated when the available soil water capacity dropped to 80%, with irrigation water having pH 7.2, EC 0.81 dS m^{-1}, 170 mg L^{-1} bicarbonates, 80 mg L^{-1} chlorides, 65 mg L^{-1} sodium, 80 mg L^{-1} calcium, and 25 mg L^{-1} magnesium.

The biostimulants were applied, through fertigation, three times: the first one at the 5 true leaf stage; the second treatment at the beginning of bulb formation, and the third supply two weeks later. The following biostimulant doses were used: 10 L ha^{-1} (3 mL L^{-1}) for the protein hydrolysate and 5 kg ha^{-1} (4 g L^{-1}) for the spirulina powder.

The onion bulbs were harvested from 27 June (Rossa di Tropea) to 4 July (Ramata di Montoro), when 50% of the plant pseudo-stems softened and the leaves flattened. After harvest, onion plants were left in windrows in the field for a two week 'cure', then, the onion bulbs were considered marketable when they did not show decay signs, compromised tunic integrity, and bifurcated morphology. In each plot, the following measurements were performed: total weight and number of bulbs; mean bulb weight and diameter (along equatorial axis) on 20-unit random samples, along with firmness using a digital penetrometer (Fruti Tester, Effegi, Milan, Italy). Next, both total plant and bulb random samples collected in each plot were dried in an air-forced oven until achieving a constant weight to calculate the dry matter percentage.

To determine the storability, 20 bulbs were randomly sampled in the central area of each plot (60 bulbs for each treatment) and placed in a ventilated chamber at 2 °C with 60% relative humidity.

2.2. Sample Preparation

Further 'cured' bulb samples were collected in each experimental plot and immediately transferred to the laboratory, where one hundred g of the mentioned material were homogenised in a mixer to perform the following analytical determinations.

2.3. Ascorbic Acid

Fifty g of bulbs (from 15 bulbs powder mixture) were homogenised with 200 mL of 3% citric acid solution using a 1.0 L Waring blender. Aliquots were transferred to volumetric flasks containing 0.2 M NaH_2PO_4, pH 2.14, bringing the total volume to 100 mL under continuous stirring. After 15 min, the samples were centrifuged at 10,000× g for 30 min at 6 °C, and the supernatant was filtered through a 0.45 µm Acrodisc filter before HPLC analysis. Ten-microliter samples were injected into a Polymer Laboratories reverse-phase column mod. PLRP-S (100 Å, 5 µm, 4.6 mm × 250 mm). The column temperature was maintained at 35 °C, 0.2 M NaH_2PO_4 was the eluent, with pH 2.14 and 0.6 mL min^{-1} flow rate in isocratic mode, and the UV detector was set at 525 nm [18].

2.4. Polyphenols

Ten medium-sized bulbs were collected, lyophilised, and finely powdered. One gram of powder underwent pre-extraction with 25 mL petroleum ether for 24 h under continuous stirring, followed by three extractions with 25 mL of 60% v/v methanol/water solution. Methanolic extracts were combined and concentrated by vacuum evaporation using a Rotovapor (Heidolph, Kelheim, Germany). The residue was redissolved in 4.0 mL extraction solution and filtered through Anotop 10, 0.2 µm filters (Whatman, Maidstone, UK). Ten-microliter samples were analysed in triplicate for total polyphenol content using Folin-Ciocalteau reagent [19].

2.5. Antioxidant Activity

The lipophilic antioxidant activity (LAA) was measured using a radical cation assay, extracting 200 mg of lyophilised material by methanol, with the 2,20-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method described by Re et al. [20]. The hydrophilic antioxidant activity (HAA) was determined using the N,N-dimethyl-p-phenylenediamine (DMPD) method [21] by extracting 200 mg of lyophilised material in distilled water. AUV–Vis spectrophotometer was used to measure the absorbance reduction of the solutions at 734 and 505 nm wavelength for LAA and HA, respectively.

2.6. Mineral Elements

The desiccated bulb tissues from 'cured' onions were ground and used for the analysis of macro- and microelements [22] which were separated and quantified by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) coupled to a conductivity detector. An IonPac CG12A (4 × 250 mm, Dionex, Corporation) guard column and IonPac CS12A (4 × 250 mm, Dionex, Corporation) analytical column were used for cation analysis, whereas an IonPac AG11-HC guard (4 × 50 mm) column and IonPac AS11-HC analytical column (4 × 250 mm) were used for anion determination. The element contents were expressed in g kg^{-1} d.w.

2.7. Statistical Analysis

The obtained data were processed by two-way analysis of variance (ANOVA), with cultivar and biostimulant treatment as main factors, and mean separations were performed through Duncan's multiple range test at 0.05 probability levels, using SPSS version 29 software (IBM, Armonk, New York, NY, USA).

3. Results

3.1. Yield Parameters and Growth Indices

Since no significant differences between the two research years and no significant interactions between cultivar and biostimulant treatment arose, only the main effects of the two experimental factors on the examined variables are presented.

Onion yield was significantly influenced both by cultivar and biostimulant treatment (Table 1). Ramata di Montoro showed the best yield performance with 66.4 t ha^{-1}, followed by Rossa di Tropea (−12%), and Rocca Bruna and Dorata di Parma which did not significantly differ from each other (−22% on average). The production trend was positively correlated with the mean bulb weight which attained the highest value of 221.4 g in Ramata di Montoro (Table 1), followed by Rossa di Tropea (−12.7%), and the cvs. Rocca Bruna and Dorata di Parma (−28%). Ramata di Montoro showed an 8.34 cm bulb diameter, 22.3%, 34.5%, and 40.6% higher than Rossa di Tropea, Dorata di Parma, and Rocca Bruna, respectively (Table 1).

Table 1. Effects of cultivar and biostimulant treatment on onion yield parameters and growth indices.

Experimental Treatment	Marketable Yield (t ha^{-1})	Mean Bulb Weight (g)	Bulb Diameter (cm)	Plant Dry Weight (g m^{-2})
Cultivar Ramata di Montoro	66.4 a	221.4 a	8.3 a	1185.6 a
Rossa di Tropea	58.9 b	196.4 b	6.8 b	842.2 b
Rocca Bruna	51.7 c	172.4 c	5.9 c	812.3 b
Dorata di Parma	51.9 c	173.1 c	6.2 c	804.4 b

Table 1. *Cont.*

Experimental Treatment	Marketable Yield (t ha^{-1})	Mean Bulb Weight (g)	Bulb Diameter (cm)	Plant Dry Weight (g m^{-2})
Biostimulant				
Control	51.0 b	169.9 b	6.1 b	788.2 b
Protein hydrolysate	61.5 a	204.9 a	7.3 a	996.0 a
Spirulina	59.3 a	197.7 a	7.1 a	949.2 a

Within each column and experimental factor, values followed by different letters are significantly different according to Duncan test at $p < 0.05$.

Regarding the biostimulant formulations applied, protein hydrolysate and spirulina led to an 18% average yield increase, compared to the untreated control (51 t ha^{-1}), due to the higher mean bulb weights (204.9 and 197.7 g, respectively) than the untreated control (−20.6% and −16.4% vs. protein hydrolysate and spirulina, respectively).

Protein hydrolysate and spirulina also showed higher bulb diameters compared to the untreated control, by 20.2% and 16.1% respectively, but not statistically different from each other (7.2 cm on average),

The cultivar Ramata di Montoro had a 1185.6 g m^{-2} plant dry matter content, 45.9% higher than the average of the other three cultivars examined. Regarding biostimulant treatments, the values obtained under protein hydrolysate and spirulina applications were 996 and 949.2 g m^{-2}, respectively, significantly higher by 19% compared to the untreated control.

3.2. Quality Parameters, Firmness, and Shelf-Life of Onion Bulbs

The values of dry matter, soluble solids, and titratable acidity (Table 2) were the lowest in the cultivar Rossa di Tropea bulbs (8.6%, 6.9 °Brix and 2.9 mg malic acid g^{-1} d.w., respectively, whereas the other three varieties did not statistically differ from each other referring to the two first parameters, and Rocca Bruna had the highest titratable acidity of 3.4 mg malic acid g^{-1} d.w. Firmness was not significantly affected by variety, whereas Ramata di Montoro bulbs showed a 25.8% longer shelf-life than the average value of the other three cultivars).

The biostimulant treatments with protein hydrolysate and spirulina led to significantly higher values of dry matter and soluble solids by 8.3% and 9.3% on average, compared to the control, but they did not differ from each other. On the contrary, a higher titratable acidity level was recorded for the untreated control, compared to the two biostimulant formulations. The latter positively affected both firmness and shelf-life which resulted in higher values (2.36 N and 197.9 days as an average of the two formulations) by 12.9% and 8%, respectively, than the untreated control.

Table 2. Effects of cultivar and biostimulant treatment on quality parameters, firmness, and shelf-life of onion bulbs.

Experimental Treatment	Dry Matter (%)	Soluble Solids (°Brix)	Titratable Acidity (mg Malic Acid g^{-1} d.w.)	Firmness (N)	Shelf-Life (Days)
Cultivar					
Ramata di Montoro	9.8 a	7.8 a	3.1 b	2.22	228.0 a
Rossa di Tropea	8.6 b	6.9 b	2.9 c	2.23	177.3 b
Rocca Bruna	9.7 a	7.6 a	3.4 a	2.34	184.7 b
Dorata di Parma	9.6 a	7.5 a	3.2 b	2.29	182.0 b
				n.s.	

Table 2. *Cont.*

Experimental Treatment	Dry Matter (%)	Soluble Solids (°Brix)	Titratable Acidity (mg Malic Acid g^{-1} d.w.)	Firmness (N)	Shelf-Life (Days)
Biostimulant					
Control	8.9 b	7.0 b	3.3 a	2.09 b	183.3 b
Protein hydrolysate	9.8 a	7.7 a	3.1 b	2.40 a	199.0 a
Spirulina	9.6 a	7.6 a	3.1 b	2.32 a	196.8 a

d.w.: dry weight; N: Newton; n.s.: not significant. Within each column and experimental factor, values followed by different letters are significantly different according to Duncan test at $p < 0.05$.

3.3. Compounds and Antioxidant Activity of Onion Bulbs

The polyphenol concentration (Table 3) was not significantly affected by cultivar. Ramata di Montoro bulbs showed 31.5 mg vitamin C g^{-1} f.w., 36.2% higher than the average of the other three varieties which did not statistically differ from each other. The lipophilic antioxidant activity in cv. Ramata di Montoro bulbs attained a 43.8% higher value (6.9 mmol trolox equivalents 100 g^{-1} d.w.), compared to the average value of the other three varieties. The hydrophilic antioxidant activity also reached the top values in Ramata di Montoro bulbs (7.72 mmol ascorbic acid equivalent 100 g^{-1} d.w.) but the lowest in Rossa di Tropea ones (6.36 mmol ascorbic acid equivalent 100 g^{-1} d.w.).

Table 3. Effect of cultivar and biostimulant treatment on antioxidant compounds and activities.

Experimental Treatment	Polyphenols (mg Gallic Acid eq. 100 g^{-1} d.w.)	Vitamin C (mg g^{-1} d.w.)	Lipophilic Antioxidant Activity (mmol Trolox eq. 100 g^{-1} d.w.)	Hydrophilic Antioxidant Activity (mmol Ascorbic Acid eq. 100 g^{-1} d.w.)
Cultivar				
Ramata di Montoro	182.3	31.5 a	6.9 a	7.7 a
Rossa di Tropea	189.8	22.2 b	5.0 b	6.4 c
Rocca Bruna	196.0	24.1 b	4.7 b	6.8 b
Dorata di Parma	180.3	22.9 b	4.8 b	6.7 bc
	n.s.			
Biostimulant				
Control	158.6 b	22.0 b	4.6 b	6.2 b
Protein hydrolysate	201.4 a	27.2 a	5.8 a	7.3 a
Spirulina	201.3 a	26.3 a	5.7 a	7.2 a

d.w.: dry weight; n.s.: not significant. Within each column and experimental factor, values followed by different letters are significantly different according to Duncan test at $p < 0.05$.

The biostimulant treatments resulted in higher values of the antioxidant compounds and activities measured, compared to the untreated control, with no significant differences between the two formulations applied. Particularly, the polyphenol content in the bulbs treated with the biostimulants (201.4 mg 100 g^{-1} d.w.) was 27% higher compared to those of the untreated control. Vitamin C attained the top level under biostimulation (26.8 mg g^{-1} on average), 22% higher compared to the untreated control. Both the lipophilic and hydrophilic antioxidant activities benefited from the biostimulant application (+25.1% and +16.9%, respectively), compared to the untreated control.

3.4. Mineral Elements

The contents of calcium, magnesium, sodium, and phosphorus were the highest in Rossa di Tropea bulbs which showed the lowest potassium level (Table 4). The latter element did not significantly differ between the other three cultivars, whereas Ramata di Montoro accumulated the most nitrates, though the values were always very low. Rocca Bruna and Dorata di Parma always had the lowest levels of all the macroelements examined, except K, not differing from Ramata di Montoro which only displayed higher nitrate concentration.

Table 4. Effects of cultivar and biostimulant treatment on macroelement content of onion bulbs.

Experimental Treatment	Ca^{2+} (g kg^{-1} d.w.)	Mg^{2+} (g kg^{-1} d.w.)	K^+ (g kg^{-1} d.w.)	Na^+ (g kg^{-1} d.w.)	PO_4^{3-} (g kg^{-1} d.w.)	Nitrate (NO_3^-) (g kg^{-1} d.w.)
Cultivar						
Ramata di Montoro	2417.3 b	949.3 ab	9414 a	143.0 b	2529.7 b	225.3 a
Rossa di Tropea	3007.7 a	1002.0 a	8438 b	161.3 a	2858.7 a	172.0 b
Rocca Bruna	2299.0 b	927.3 ab	9229 a	123.7 c	2544.7 b	187.3 b
Dorata di Parma	2341.3 b	915.7 b	9194 a	118.3 c	2446.0 b	171.7 b
Biostimulant						
Control	2310.0 b	866.5 b	8375 b	123.3 b	2353.0 b	169.3 b
Protein hydrolysate	2621.5 a	987.0 a	9419 a	143.0 a	2731.5 a	203.8 a
Spirulina	2617.5 a	992.3 a	9412 a	143.5 a	2699.8 a	194.3 a

Within each column and experimental factor, values followed by different letters are significantly different according to Duncan test at $p < 0.05$.

Regarding the biostimulant application, both protein hydrolysate and spirulina treatments elicited the highest contents of all the elements analysed in the bulbs, compared to the untreated control (Table 4).

Unlike the macroelements, the trends of microelement accumulation differed among the four minerals studied (Table 5). Indeed, iron showed the highest content in Ramata di Montoro bulbs, copper and zinc in Rossa di Tropea, and manganese in Rocca Bruna and Dorata di Parma.

Table 5. Effects of cultivar and biostimulant treatment on microelement content of onion bulbs.

Experimental Treatment	Fe^{2+} (mg kg^{-1} d.w.)	Cu^{2+} (mg kg^{-1} d.w.)	Mn^{2+} (mg kg^{-1} d.w.)	Zn^{2+} (mg kg^{-1} d.w.)
Cultivar				
Ramata di Montoro	30.9 a	5.8 b	9.8 b	17.2 b
Rossa di Tropea	27.7 b	6.5 a	10.0 b	19.0 a
Rocca Bruna	24.0 c	5.9 b	11.7 a	15.4 c
Dorata di Parma	25.8 bc	5.2 c	11.3 a	17.0 b
Biostimulant				
Control	24.9 b	5.4 c	9.8 b	15.6 b
Protein hydrolysate	28.0 a	6.2 a	11.1 a	17.9 a
Spirulina	28.4 a	5.9 b	11.2 a	18.0 a

Within each column and experimental factor, values followed by different letters are significantly different according to Duncan test at $p < 0.05$.

The two biostimulants applied resulted in higher contents of the four microelements analysed, compared to the untreated control, not significantly differing from each other except for Cu which was more concentrated under the protein hydrolysate treatment.

4. Discussion

In the present study, differences in yield and quality parameters among the four analysed onion cultivars presumably relate both to genetic potential and specific adaptation to the production area. Indeed, they are traditionally cultivated in distinct Italian regions, which contribute to morphological, physiological, and biochemical diversity, particularly referring to bulb features such as size, shape, colour, and chemical composition [22]. The best overall performance shown by the cultivar Ramata di Montoro is likely due to its optimal adaptation to the environmental and soil characteristics of the research location, similar to those of its original cultivation area [6].

In this experiment, no interaction between cultivar and biostimulant treatment arose, in contrast with the findings by Mikulewicz et al. [23]. The latter authors recorded significant cultivar-specific responses to amino acid-based biostimulant applications in *Allium cepa* L. cultivation: the cultivar White Wing F_1 exhibited optimal response to Maximus Amino Protect (L-α-amino acids-based biostimulant), with mean bulb weight increasing from 194.7 g in control specimens to 223.7 g in treated plants (+14.9%); Spirit F_1 displayed the best response to Calleaf Aminovital (Protein Hydrolysate) treatment, with mean bulb weight increasing from 127.3 g to 147.0 g (+15.5%); in contrast, the cultivar Red Baron demonstrated negative growth responses to both biostimulant applications, with control specimens showing higher bulb weight (221.6 g) compared to treatments with Calleaf Aminovital and Maximus Amino Protect (−17.9% and −29.4%, respectively).

Regarding the main effects of the biostimulant treatments, both protein hydrolysate and spirulina enhanced all the measured yield and quality parameters except for titratable acidity, but their effects were never significantly different from each other despite the distinct mechanisms of action. A study by Canellas et al. [24] conducted on *Allium cepa* L. revealed fundamental insights into the mechanisms of biostimulant action on root development. The latter investigation demonstrated that humic substance applications (Humic Total) significantly influenced root system architecture through multiple pathways, including enhanced root hair proliferation, secondary development, and initiation processes. The mentioned root modifications were directly associated with improved nutrient uptake efficiency and overall plant performance in onion, with consequent higher yield.

In addition, Kandil et al. [25] provided critical evidence regarding the cellular-level impacts of biostimulant applications in *Allium cepa* L., demonstrating that amino acid-based treatments elicited significant modifications in cellular ultrastructure, particularly affecting chloroplast organisation within mesophyll tissues. The latter structural development turned into improved photosynthetic efficiency, leading to optimised assimilate production and enhanced dry matter accumulation in onion bulbs.

Another study revealed that the biostimulant influence on plant root development was characterised by a well-defined molecular mechanism involving signalling molecules eliciting responses like the phytohormone auxin [26]. The latter auxin-like activity of biostimulants (humic substances) provided mechanistic evidence of the effects on root proliferation and architectural modification in plant systems.

Moreover, protein hydrolysates primarily act by stimulating the expression of nitrate transport genes (*NRT1.1* and *NRT2.1*) and activating plasma membrane H^+-ATPase proton pumps [27]. The latter mechanism is further enhanced by the activation of genes involved in secondary metabolite synthesis through the phenylpropanoid pathway, which may explain the increased phenolic compounds observed in our study.

Spirulina has a different action mechanism, based on the activation of plant defence responses by its polysaccharides and phycocyanins through the jasmonic acid pathway, encouraging synthesis of secondary metabolites and antioxidant compounds [28]. In our research, the increased yield and diameter of onion bulbs may be due to enhanced photosynthetic activity elicited by natural phytohormones present in spirulina, particularly cytokinins and auxins, which also promote cell division and tissue expansion. Moreover, the synergy of biostimulants with humic acid and glycine in soil reportedly promotes nutrient assimilation leading to the increase of vegetative growth and plant biomass, with a consequent energy accumulation devoted to bulb formation [29,30].

Consistently with the enhancement in yield, quality, and shelf-life recorded in our investigation, Abd Alla et al. [31] found that foliar applications of biostimulants significantly improved cabbage performance. Specifically, applications of phenolic compounds, micronutrient complexes, and seaweed extracts increased marketable head yield by 17.3–22.8% compared to untreated controls, as well as head firmness (+28.4%), vitamin C content (+31.2%), and total soluble solids (+15.7%).

In our research, bulb shelf-life was significantly extended under the application of the two biostimulant formulations and was the longest in the cultivar Ramata di Montoro. The latter outcome can be attributed to multiple factors, among which is the higher bulb firmness connected with the enhanced cell wall integrity, which Gómez-Merino and Trejo-Téllez [32] associated with improved calcium allocation elicited by biostimulants such as seaweed extracts, humic substances, and protein hydrolysate; concerning spirulina, the mentioned effect was probably due to the presence of phytosiderophores enhancing calcium assimilation and wall integrity [14]. In our study, both biostimulants presumably contributed to maintain optimal sugar levels without triggering premature sprouting; in this respect, the increased sugar content, reflected in the higher soluble solids, plays a key role in onion storage, as the sucrose concentration rise acts as a trigger for the transition from dormancy to the onset of sprouting [30,33].

The improved post-harvest preservation can also be attributed to increased antioxidant compounds (polyphenols and vitamin C) and enhanced antioxidant activity in treated bulbs, consistent with the findings of Petropoulos et al. [34], who demonstrated that higher antioxidant content correlates with reduced post-harvest deterioration and better resistance to storage pathogens.

Previous studies reported an 11.5–12.0% increase in onion bulb dry weight and dry matter content under protein hydrolysate, spirulina, and algae-extract application, both in white and red varieties [14,35,36]. In our research, the higher dry matter content in treated bulbs compared to the untreated control (9.7% vs. 8.94%, respectively) supports their improved storage potential, as also found by Sekara et al. [37].

Pobereżny et al. [38] demonstrated the protein hydrolysate efficacy in increasing Vitamin C content and promoting cellular division in carrots, while Vojnović et al. [39] reported significant increases in bulb polyphenol concentration under combined protein hydrolysate application in onions. Cristofano et al. [40] recorded an improved nutritional quality, including enhanced hydrophilic and lipophilic antioxidant activity, in fruit and leafy vegetables treated with various biostimulants, including natural brown seaweed extracts and protein hydrolysates.

As demonstrated by Golubkina et al. [22], mineral composition in onion bulbs significantly varies among cultivars, independent of the crop management system, indicating the strong genetic influence on mineral uptake and translocation mechanisms.

Protein hydrolysate application reportedly results in the overall enhancement in mineral uptake due to the improved root architecture of two basil cultivars [41]. Particularly, in a previous investigation [42] conducted on onion crops, the treatments with both protein hydrolysate and spirulina were effective in increasing macro- and micronutrient content, either under a conventional or organic system, confirming the outcome of our research.

Notably, minerals like nitrogen, phosphorus, potassium, calcium, and magnesium are pivotal for chlorophyll synthesis, enzyme activation, and cell wall development; their absorption may have been influenced by the amino acids contained in protein hydrolysate and spirulina extracts applied in our research, which promote the transcription for mineral element carriers increasing nutrient uptake and translocation, as shown in studies carried out on oilseed rape (*Brassica napus* L.) and *Zea mais* L. demonstrating that seaweed and black peat-derived biostimulants significantly enhanced chloroplast division and mineral nutrient dynamics in oilseed rape [43,44]. In addition, the latter investigations reported increased concentrations of Mg, Mn, Na, and Cu, along with improved root-to-shoot translocation of Fe and Zn. Moreover, their findings revealed that cultivar-specific characteristics, rather than environmental conditions, predominantly determined treatment efficacy, highlighting the critical role of genotypic factors in biostimulant response patterns.

Copper, iron, zinc, and magnesium are essential for chloroplast metabolism and enzymes, components of the electron transport chain and chlorophyll structure; the high levels of these elements following biostimulant treatments were likely associated with the overexpression of transporters, such as *COPT2* for copper and *NRAMP3* for iron and zinc translocation [43]. Interestingly, the amino acids derived from protein hydrolysates are useful for preserving the protein structure needed for cell division, facilitating cellular division, enlargement, differentiation, and the effective formation of polyamines aiding mineral absorption activities [27]. The amino acid profile of spirulina, including methionine, glycine, and cysteine, was demonstrated to optimise nutrient uptake, translocation, and metabolism, also enhancing the uptake of nitrogen, phosphorus, and potassium as well as root development [37]. Moreover, the presence of antioxidant compounds in spirulina protects cellular structures during nutrient uptake and assimilation processes [37].

Our findings are consistent with Shafeek et al.'s [45] reports, indicating that ready-to-use biostimulants, such as spirulina powder, in some cases did not differ from protein hydrolysates in promoting plant growth and yield and improving qualitative characteristics, under sustainable management, safety, and health perspective [46].

5. Conclusions

From research carried out comparing four traditional Italian storage onion cultivars treated with two biostimulant formulations (protein hydrolysate and spirulina), it arose that the variety Ramata di Montoro showed the best overall performance in terms of yield, shelf-life, vitamin C content, and both lipophilic and hydrophilic antioxidant activities of bulbs. Better productivity, storability, and nutritional outcomes were recorded under the biostimulant application compared to the untreated control.

The application of protein hydrolysate and spirulina on onion resulted in positive quantitative and qualitative outcomes. The benefits related to the improved yield, enhanced quality, nutritional value, and shelf-life of bulbs make the biostimulant utilisation a valuable strategy within the framework of sustainable onion management.

In this study, the small number of both cultivars and biostimulant types examined did not trigger significant interactions between the two experimental factors on the measured variables.

Future research should deepen the investigation of physiological mechanisms associated with the yield, quality, and antioxidant responses of different onion varieties to biostimulant applications, even in terms of potential synergistic effects of formulation mixtures.

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