Elwaziri E *et al*. (2023)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 51, Issue 2, Article number 13122
DOI:10.15835/nbha51213122
Research Article

# Biostimulant application of whey protein hydrolysates and potassium fertilization enhances the productivity and tuber quality of sweet potato

Emad ELWAZIRI[1], Hany ISMAIL[1], El-Sayed ABOU EL-KHAIRl[2], Salem M. AL-QAHTANI[3], Nadi A. AL-HARBI[3], Hany G. ABD EL-GAWAD[4], Wael A. OMAR[5], Khaled ABDELAAL[6]*, Ali OSMAN[7]

[1]*Zagazig University, Faculty of Agriculture, Horticulture Department, Zagazig, 44511, Egypt; Alwzrymad@gmail.com; hanyeldewah@gmail.com*
[2]*Horticulture Research Institute, Agriculture Research Center, Potato and Vegetatively Propagated Vegetables Research Department, Giza, Egypt; Sayedaboelkhair@yahoo.com*
[3]*University of Tabuk, University College of Tayma, Biology Department, P.O. Box 741, Tabuk, Saudi Arabia; salhtani@ut.edu.sa; naharbi@ut.edu.sa*
[4]*Ain Shams University, Faculty of Agriculture, Department of Horticulture, Cairo 11566, Egypt; hany_gamal2005@agr.asu.edu.eg*
[5]*Taif University, College of Science, Department of Biology, P.O. Box 11099, Taif 21944, Saudi Arabia; w.omar@tu.edu.sa*
[6]*Kafrelsheikh University, EPCRS Excellence Center, Faculty of Agriculture, Agricultural Botany Department, Plant Pathology and Biotechnology Laboratory, 33516, Egypt; khaled.elhaies@gmail.com (*corresponding author)*
[7]*Zagazig University, Faculty of Agriculture, Biochemistry Department, Zagazig, 44511, Egypt; aokhalil@zu.edu.eg*

## Abstract

Utilizing biostimulants like protein hydrolysates is one of the most creative and promising approaches to improving nutritional efficiency, abiotic stress tolerance, or crop quality traits. In the present study, whey protein was hydrolysed with trypsin for 4 h at an enzyme/substrate ratio (1/300, w/w). The obtained whey protein hydrolysates (WPH) were chemically characterized, and their antioxidant activity was estimated. WPH, which was produced using trypsin for 4 hours and presented the highest antioxidant activity. Therefore, it was selected as a bio stimulant with potassium fertilization for enhancing the productivity and tuber quality of sweet potatoes. A field experiment was carried out during the two successive summer seasons, at a private vegetable farm in Faques City, Sharkia Governorate, Egypt, to study the effect of different potassium rates (50, 75, and 100 kg $K_2O$/fad) and WPH at 0.10 and 0.20% as a foliar application compared to unsprayed plants (control). The interaction between $K_2O$ at 100 kg /fad and spraying with WPH at 0.15% increased shoot dry weight/ plant, N, P, and K uptake by shoots, yield/plant, marketable yield, and total yield/fad, as well as average tuber root weight. It was concluded that the most efficient bio-stimulating foliar spray treatment for increasing sweet potato productivity was WPH (0.20%).

***Keywords:*** biofertilizers; horticultural production; protein hydrolysates; sweet potato; potassium fertilization; tuber quality

*Received: 14 Mar 2023. Received in revised form: 10 Apr 2023. Accepted: 18 May 2023. Published online: 07 Jun 2023.*
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

## Introduction

Sweet potatoes (*Ipomoea batatas* [L.] Lam.) are a common food that people eat daily. Due to its nutritional properties, adaptability to various edaphoclimatic conditions, and quick yield, this crop is important to society and the economy (Amaro *et al*., 2017). Poor fertility, particularly low levels of potassium (K), phosphorus (P), nitrogen (N), sulfur (S), and micronutrients, limit sweet potato productivity (Uwah *et al*., 2013). Potassium influences the quality and growth of vegetables by increasing biomass production and leaf area and reducing sugar concentration (Mukhongo *et al*., 2017). The dry weight of biomass is significantly increased by fertilizing sweet potato with potassium (Taffouo *et al*., 2017), boosting production and its components (Hasanah *et al*., 2021), and gave the best tuber root quality (Nedunchezhiyan *et al*., 2010). The development of systems that are both environmentally friendly and sustainable in order to meet the need to feed the world's growing population is one of agriculture's greatest obstacles (Anitha, 2020). Utilizing bio stimulants like protein hydrolysates from various sources is one of the most creative and promising approaches to addressing these significant issues (Sitohy *et al*., 2020; El-Sanatawy *et al*., 2021; Osman *et al*., 2021a; Osman *et al*., 2021b). Regardless of its nutrient content, any substance or microorganism that is applied to plants with the intention of improving nutritional efficiency, abiotic stress tolerance, or crop quality traits is a bio stimulant, it is regulate or improve the physiological processes of plants (Du Jardin, 2015). Bio stimulants include peptides, vitamins, enzymes, substances with hormone-like effects (derived from algal extracts), antioxidants, humic and fulvic acids, silicon, and other minerals, as well as some strains of microorganisms (Almadi *et al*., 2020). Various studies have been carried out on the effect of protein hydrolysates as a bio stimulant on plant growth and production (Colla *et al.*, 2015; Colla *et al*., 2017; Almadi *et al*., 2020; Shahrajabian *et al.*, 2022). Some crops' yields have improved when bio stimulants are used in agriculture. However, there aren't many studies on how to use these products on crops like sweet potatoes, whose commercialized parts are the roots (Rós *et al*., 2015). In the present study, whey protein was hydrolysed with trypsin for 4 h at an enzyme/substrate ratio (1/300, w/w). The obtained whey protein hydrolysates (WPH) were chemically characterized, and their antioxidant activity was estimated. WPH, which was produced using trypsin for 4 hours and presented the highest antioxidant activity. Therefore, it was selected as a bio stimulant with potassium fertilization for enhancing the productivity and tuber quality of sweet potatoes.

## Materials and Methods

### *Whey protein hydrolysates preparation and characterization*

Trypsin was utilized for the enzymatic hydrolysis of WPH (buffer, 0.1 M phosphate buffer; pH, 8.8; temperature of 37 °C), and their optimal conditions are a ratio of enzyme to substrate of 1:300 w/w. Enzyme and substrate were thoroughly mixed. At the optimal temperature and with constant stirring, the mixture was incubated for four hours. To inactivate the enzyme, the mixture was then heated for ten minutes in a boiling water bath at 100 °C. Hydrolysate was clarified by centrifugation at 5000 xg for 15 min at 5 °C to remove insoluble substrate fragments, and the supernatant was lyophilized and frozen at -20 °C until further used (Abdel-Hamid *et al*., 2017). The percent of trichloroacetic acid (TCA) ratio was used to determine the degree of hydrolysis as described by (Hoyle and Merrltt, 1994). Degree of hydrolysis (DH) was calculated using the formula below:

$$DH (\%) = (\text{Soluble nitrogen in TCA } 10\% / \text{ Total nitrogen in the sample}) \times 100$$

Using the DPPH radical scavenging activity assay, the antioxidant activity of WPH (500 µg/mL) produced with trypsin at various times (0, 1, 2, 3 and 4 h) was estimated to determine the optimal time for producing antioxidant peptides (Göçer *et al*., 2011). The radical scavenging capacity of the samples was measured as a decrease in the absorbance of DPPH radicals, and it was calculated using the following equation:

Radical scavenging activity (%) = [(A control–A sample)/A control] × 100

A= absorbance at 517 nm.

Electrospray ionization mass spectrometry (ESI-MS) with positive and negative ions was used to analyse the protein hydrolysate with the highest antioxidant activity that had been obtained after 4 hours (Al-Mohammadi *et al.,* 2020).

*Plant growing conditions, (plant materials) treatments, and experimental design*

A field experiment was carried out at a private vegetable farm in Faques City, Sharkia Governorate, Egypt to investigate the effects of various potassium rates (120, 180, and 240 kg $K_2O$/ha and whey protein hydrolysates (WPH) at 0.10 and 0.20% as foliar applications alongside unsprayed plants (as a control) on growth, yield, and tuber root quality of sweet potato cv. 'Buregard'. These treatments were arranged in a split-plot design in a randomized complete block design with three replications. Potassium rates were randomly distributed in the main plots, while the concentrations of WPH were randomly arranged in the sub-plots.

The physical and chemical properties of experimental soil in the two seasons showed that it was clay in texture and had 1.99 and 1.92% organic matter, 8.05 and 8.04 pH, 2.08 and 2.05 mmhos/cm EC, 8.41 and 8.92 ppm are available N, 0.048 and 0.044% available P and 0.93 and 0.89% available K, respectively.

Stem cuttings of sweet potato (about 20 cm length) were transplanted at 25 cm apart on 15 and 17$^{th}$ April in the 1$^{st}$ and 2$^{nd}$ seasons, respectively. The experimental unit area was 12.6 $m^2$. It contains three lines with 6m length each and 70 cm distance between each two lines. One line was used for taking the samples to measure the growth and chemical traits and the other two lines were used for yield determinations. Potassium rates were applied as soil application into three equal portions beginning 45 days after transplanting every two weeks in the form of potassium sulphate (48.5% $K_2O$). Plants were sprayed with the different concentrations of WPH or water four times at 15 days intervals beginning 45 days after transplanting in both seasons. Each plot was received 2 L. solutions of each concentrations using spreading agent in all treatments to improve adherence of the spray to the plant foliage for increasing WPH absorption by the plants. The untreated plants were sprayed with water and spreading agent. One line was left between each two experimental plots without spraying as a guard row to avoid the overlapping of spraying salutation. All treatments received equal amounts of ammonium sulphate (20.5 % N) and calcium superphosphate (15.5% $P_2O_5$) at a rate of 480 and 360 kg/ha, respectively.

*Sampling and measurements*

One line was used for taking the samples to measure the growth and chemical traits in the two growing seasons to measure leaf area/plant and the dry weight of shoots (leaves and branches) and Nitrogen, phosphorus, and potassium in shoots were determined in both seasons according to the methods described by (Reynolds and toxicology, 1989) and N, P and K uptake by shoots were calculated (mg/shoot). At harvest time, (at 150 days after transplanting), all tuber roots of each treatment were classified into two grades (marketable and non-marketable roots), then weighed to determine the total yield/ha (ton). Marketable tuber roots have a weight of about 100 to 250 g, while non-marketable roots have a weight of less than 100 g or more than 250 g. In addition, the average tuber root weight was calculated. Potassium use efficiency (KUE) was estimated by dividing the yield/ha, by the potassium quantity/fad., and expressed as kg tuber roots/kg $K_2O$ according to Clark (CLARK and RB 1982). Quality characters of sweet potato (tuber yield, dry matter, nitrogen content, protein content, starch content, sugar content, potassium content, and carotene content) were recorded at the time of harvest.

*Statistical analysis*

The recorded data were subjected to the statistical analysis of variance according to Hoshmand (2017), and means separation was done according to Duncan (1955) test.

## Results

*Whey protein hydrolysates characterization*

Figure 1 depicts the percentage of whey protein hydrolysed after being treated for four hours with trypsin (E/S ratio of 1:300). Over time, the degree of enzymatic degradation gradually rises. Variations in DH are typically brought about by the enzymatic reaction time, which influences the breaking of peptide bonds. For hydrolysates obtained from whey protein by enzymatic hydrolysis using trypsin, antioxidant activity using the DPPH assay was assessed. The results are shown in Figure 1. The results recorded an increase in antioxidant activity along with an increase in the degree of enzymatic hydrolysis. Whey protein hydrolysate prepared with trypsin increased its DPPH radical scavenging activity by up to 50% with increasing DH (4 h).

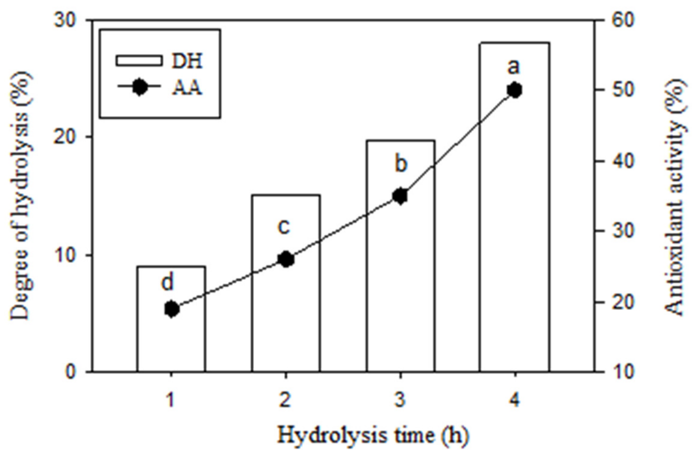


**Figure 1.** Degree of hydrolysis (DH %) whey protein treated with trypsin (E/S ratio, 1/300) for 4 h, and their antioxidant activity (AA %)

The protein hydrolysate obtained after 4h with the highest antioxidant activity (50%) was subjected to electrospray ionization mass spectrometry (ESI-MS) with positive and negative ions for molecular weight estimation and results are presented in Figure 2 and Table 1. The hydrolysate was composed on 35 peptides for positive ion with the molecular weight ranging from 141 Da to 859 Da, and 35 peptides for negative ion with the molecular weight ranging from 159 Da to 802 Da.

**Table 1.** Peptides retention time (RT, min), and molecular weight (MW, Da) of whey protein treated with trypsin (E/S ratio, 1/300) for 4 h including positive and negative ions by ESI-MS

| Peak No. | RT (min) | MW (Da) | Peak No. | RT (min) | MW (Da) |
|---|---|---|---|---|---|
| Positive ion | | | Negative ion | | |
| 1 | 0.13 | 141 | 2 | 0.71 | 180 |
| 3 | 0.75 | 203 | 3 | 0.78 | 215 |
| 4 | 2.16 | 305 | 5 | 5.85 | 247 |
| 8 | 6.74 | 245 | 6 | 6.02 | 173 |
| 9 | 7.08 | 171 | 7 | 6.25 | 175 |
| 16 | 8.90 | 195 | 8 | 6.75 | 261 |
| 21 | 10.16 | 455 | 9 | 7.07 | 187 |
| 22 | 10.95 | 959 | 10 | 7.19 | 243 |
| 23 | 11.73 | 285 | 11 | 7.34 | 648 |
| 24 | 11.85 | 274 | 12 | 7.69 | 317 |
| 25 | 11.99 | 318 | 13 | 7.83 | 159 |
| 31 | 14.71 | 277 | 14 | 8.00 | 201 |

| | | | | | |
|---|---|---|---|---|---|
| 32 | 15.65 | 279 | 15 | 8.30 | 193 |
| 33 | 16.19 | 496 | 17 | 8.48 | 417 |
| 36 | 16.93 | 383 | 18 | 9.65 | 329 |
| 37 | 17.30 | 319 | 19 | 9.88 | 169 |
| 40 | 18.65 | 425 | 20 | 9.98 | 431 |
| 41 | 18.88 | 280 | 21 | 10.17 | 487 |
| 42 | 19.69 | 279 | 22 | 10.95 | 452 |
| 43 | 20.29 | 256 | 23 | 11.72 | 283 |
| 44 | 20.68 | 282 | 26 | 12.26 | 311 |
| 45 | 21.26 | 313 | 27 | 12.48 | 587 |
| 46 | 23.25 | 284 | 28 | 13.07 | 601 |
| 47 | 23.79 | 523 | 29 | 14.22 | 315 |
| 49 | 25.75 | 693 | 30 | 14.63 | 265 |
| 50 | 27.73 | 442 | 31 | 14.72 | 293 |
| 51 | 27.89 | 482 | 32 | 15.66 | 295 |
| 52 | 27.99 | 859 | 34 | 16.27 | 540 |
| 53 | 28.13 | 441 | 35 | 16.63 | 298 |
| 54 | 28.31 | 366 | 37 | 17.27 | 485 |
| 55 | 28.44 | 419 | 38 | 17.50 | 363 |
| 56 | 28.6 | 455 | 39 | 17.60 | 527 |
| 57 | 29.35 | 394 | 47 | 23.80 | 499 |
| 58 | 31.10 | 758 | 48 | 24.08 | 525 |
| 59 | 31.26 | 187 | 58 | 31.07 | 802 |

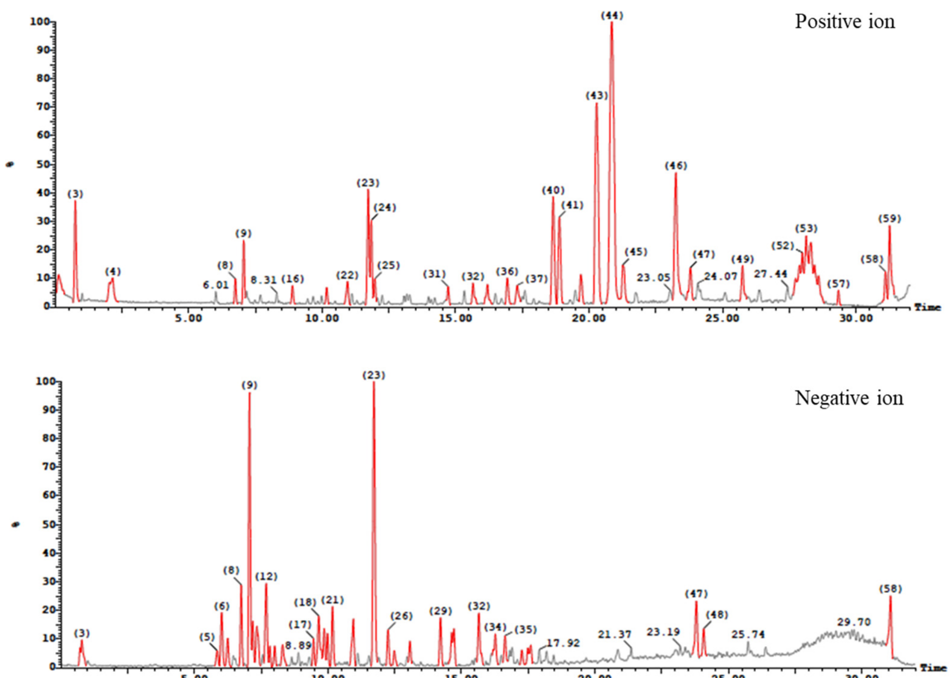


**Figure 2.** Chromatogram of peptides from whey protein whey protein treated with trypsin (E/S ratio, 1/300) for 4 h including positive and negative ions by ESI-MS

*Vegetative growth characteristics*

The effect of potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) and foliar whey protein hydrolysate at different concentrations (0, 0.1, and 0.2%) on vegetative growth characteristics during the 2020 and 2021 seasons are presented in Figure 3A, and B. Figure 3A depicts the positive effects that potassium fertilizer had on sweet potato plants. The treatment that received 240 kg $K_2O$/ha) had the highest values of vegetative growth characteristics (leaf area and dry weight of shoots). Figure 3B depicts the positive effects that WPH had on sweet potato plants. The treatment that received 0.2% had the highest values of vegetative growth characteristics (leaf area and dry weight of shoots). It is clear from Figure 3 that potassium played an important role in vegetative growth characteristics (leaf area and dry weight of shoots). The increasing rate of potassium fertilizers from 120 to 240 kg $K_2O$/ha resulted in a significant effect on the vegetative parameters. Also, the same results were observed with WPH foliar application. The effect of interaction between potassium fertilizer at different levels (120, 180 and 240 Kg $K_2O$/ha) and foliar application of WPH at different concentrations (0, 0.1, and 0.2%) on vegetative growth characteristics during 2020 and 2021 seasons are presented in Table 2. The highest value of interaction effect was recorded with treatment that received 180 Kg $K_2O$/ha with 0.2% WPH in both seasons.

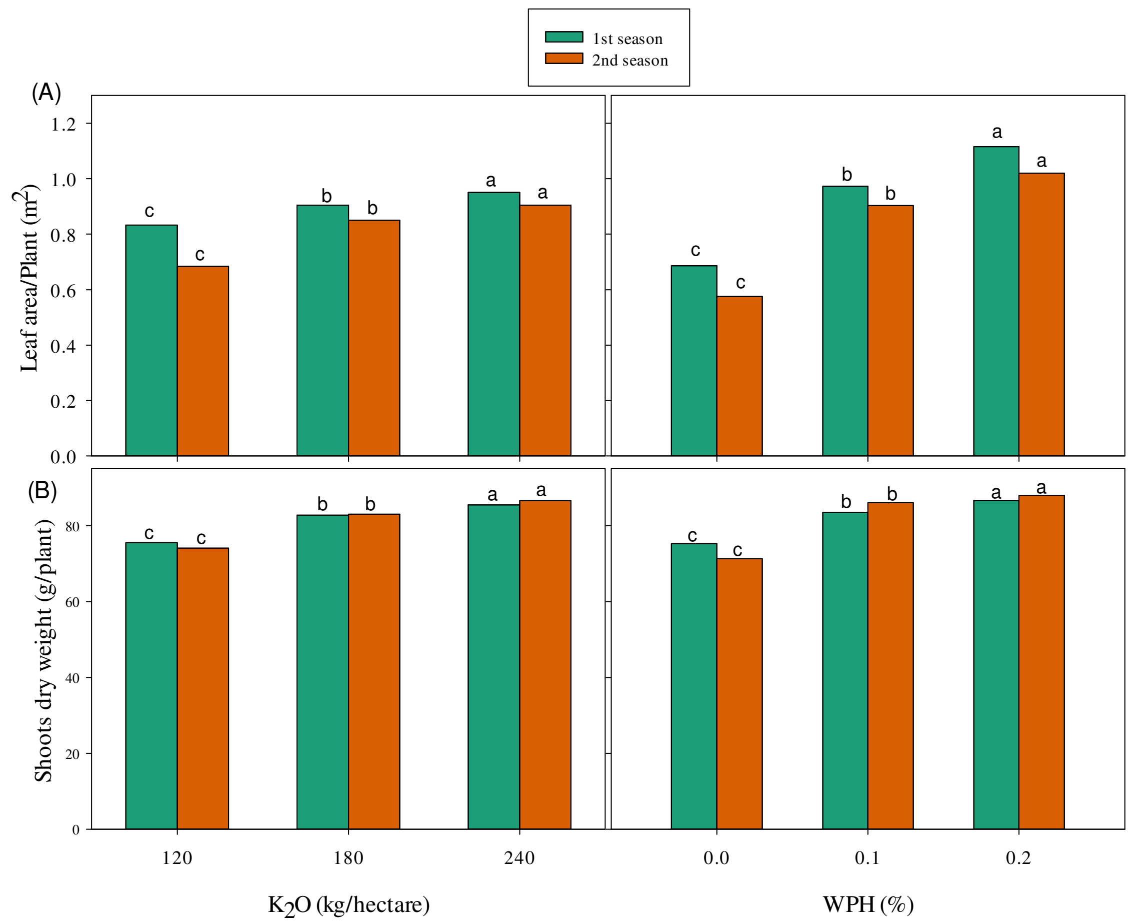


**Figure 3.** Effect of potassium fertilizer at different levels (120, 180, and 240 kg $K_2O$/hectare) and foliar whey protein hydrolysate at different concentrations (0, 0.1, and 0.2%) on vegetative growth characteristics during 2020 and 2021 seasons. Mean values in the same box for each trait followed by the same lower-case letter is not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

**Table 2.** Effect of interaction between potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) and foliar application of whey protein hydrolysates (WPH) at different concentrations (0, 0.1, and 0.2%) on vegetative growth characteristics during 2020 and 2021 seasons

| Treatments | | Leaf area/ plant ($m^2$) | | Dry weight of shoots (g/plant) | |
|---|---|---|---|---|---|
| $K_2O$ (kg/ha) | WPH (%) | 1st season | 2nd season | 1st season | 2nd season |
| 120 | 0 | 0.580f | 0.450g | 67.33h | 64.11g |
| | 0.1 | 0.790d | 0.720e | 78.79g | 79.70 e |
| | 0.2 | 1.110a | 0.970c | 82.55e | 81.09d |
| 180 | 0 | 0.660e | 0.550f | 78.89f | 74.55f |
| | 0.1 | 1.090a | 1.050a | 83.33d | 85.39 c |
| | 0.2 | 1.120a | 1.070a | 86.95c | 89.59b |
| 240 | 0 | 0.820c | 0.730e | 79.55f | 75.10f |
| | 0.1 | 1.040b | 0.940d | 88.55b | 92.93a |
| | 0.2 | 1.120a | 1.020b | 90.28a | 93.36a |

Mean values in the same column for each trait followed by the same lower-case letter is not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

*Minerals uptake (N, P and K)*

The data in Figure 4 present the uptake of N, P, and K by sweet potato plants when treated with potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) and foliar whey protein hydrolysate at different concentrations (0, 0.1, and 0.2%). Increased rate of potassium caused significant increment in N, P and K uptake in sweet potato shoots in both seasons. Plants that received 240 kg $K_2O$/ha had the highest N, P and K contents in comparison with other treatments. Results illustrated in Figure 4 presented that WPH had a positive effect on N, P and K uptake in sweet potato shoots in both seasons. Increasing rat of WPH foliar application up to 0.2% resulted in a significant effect on minerals uptake.

The effect of interaction between potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) and foliar application of WPH at different concentrations (0, 0.1, and 0.2%) on N, P, and K uptake (mg/plant) during 2020 and 2021 seasons are presented in Table 3. The highest minerals (N, P, and K) uptake values of interaction effect were recorded with treatment that received 240 Kg $K_2O$/ha with 0.2% WPH in both seasons.

**Table 3.** Effect of interaction between potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) and foliar application of WPH at different concentrations (0, 0.1, and 0.2%) on N, P, and K uptake (mg/plant) during 2020 and 2021 seasons

| Treatments | | N uptake | | P uptake | | K uptake | |
|---|---|---|---|---|---|---|---|
| $K_2O$ (kg/ha) | WPH (%) | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season |
| 120 | 0 | 2161i | 2000k | 240h | 204i | 1340j | 1090g |
| | 0.1 | 2836fg | 2909fgh | 303g | 342ef | 1781i | 1841e |
| | 0.2 | 3129d | 3090de | 381de | 366d | 2097ef | 2295d |
| 180 | 0 | 2761gh | 2721hij | 318g | 317h | 1822gh | 1782ef |
| | 0.1 | 3167cd | 3288 cd | 403 c | 385c | 2267cd | 2562c |
| | 0.2 | 3548ab | 3602bc | 459b | 477b | 2530b | 2885b |
| 240 | 0 | 2904ef | 2779ghi | 368ef | 330gh | 2013f | 1810e |
| | 0.1 | 3480b | 3708ab | 458b | 480b | 2311c | 2806b |
| | 0.2 | 3692a | 3837a | 526a | 524a | 2862a | 3286a |

Mean values in the same column for each trait followed by the same lower-case letter is not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

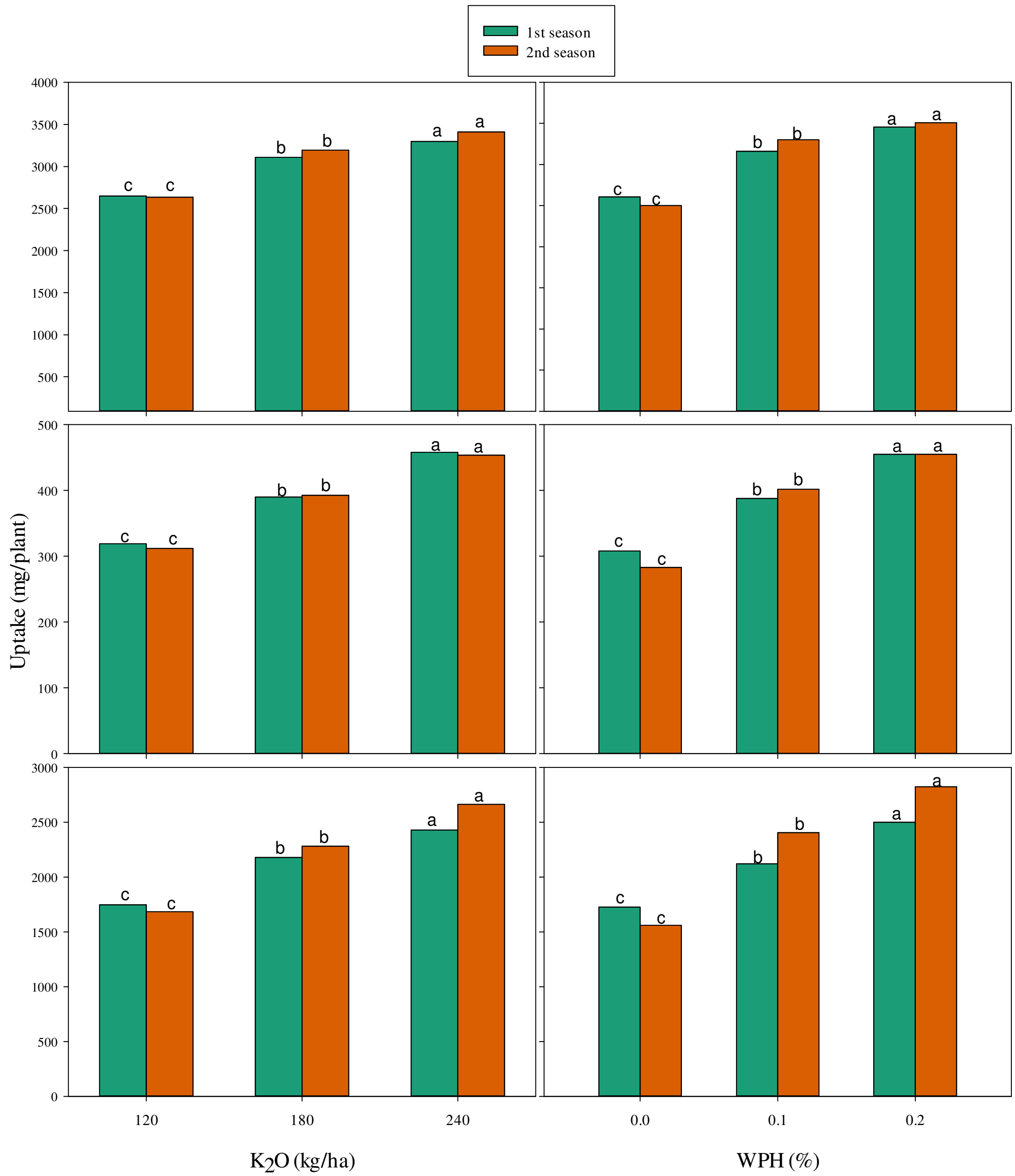


**Figure 4.** Effect of potassium fertilizer at different levels (120, 180, and 240 kg $K_2O$/ha) and foliar whey protein hydrolysate at different concentrations (0, 0.1, and 0.2%) on N, P and K uptake (mg/plant) during 2020 and 2021 seasons

*Yields and its components of sweet potato*

Effect of potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) on yield and its components (marketable, unmarketable, and total yield, weight of root and yield/plant) of sweet potato during 2020 and 2021 seasons are presented in Figure 5. Marketable yield, and total yield (ton/ha) were significantly increased with increasing potassium rates from 120 to 240 $K_2O$ (kg/ha). Increasing the K rates from 120 to 240 kg/fed increased marketable and total yield from 24 to 32 tons/ha and from 26 to 33 tons/ha, respectively in the 1st season. the same trend was observed in the 2nd season. Unmarketable yield (ton/ha) was significantly decreased with increasing potassium rates from 120 to 240 kg $K_2O$/ha in both seasons. In the 1st season, the weight of root/plant increased significantly from 115 to 127 g/plant when the K rates were increased from 120 to 240 kg /ha, while in the 2nd season increased significantly from 111.9 to 121.8 g/plant when the K rates were increased from 120 to 180 kg/ha and nonsignificant differences was observed between 180 and 240 kg $K_2O$/ha. Also, the same results were observed for yield/plant in both seasons.

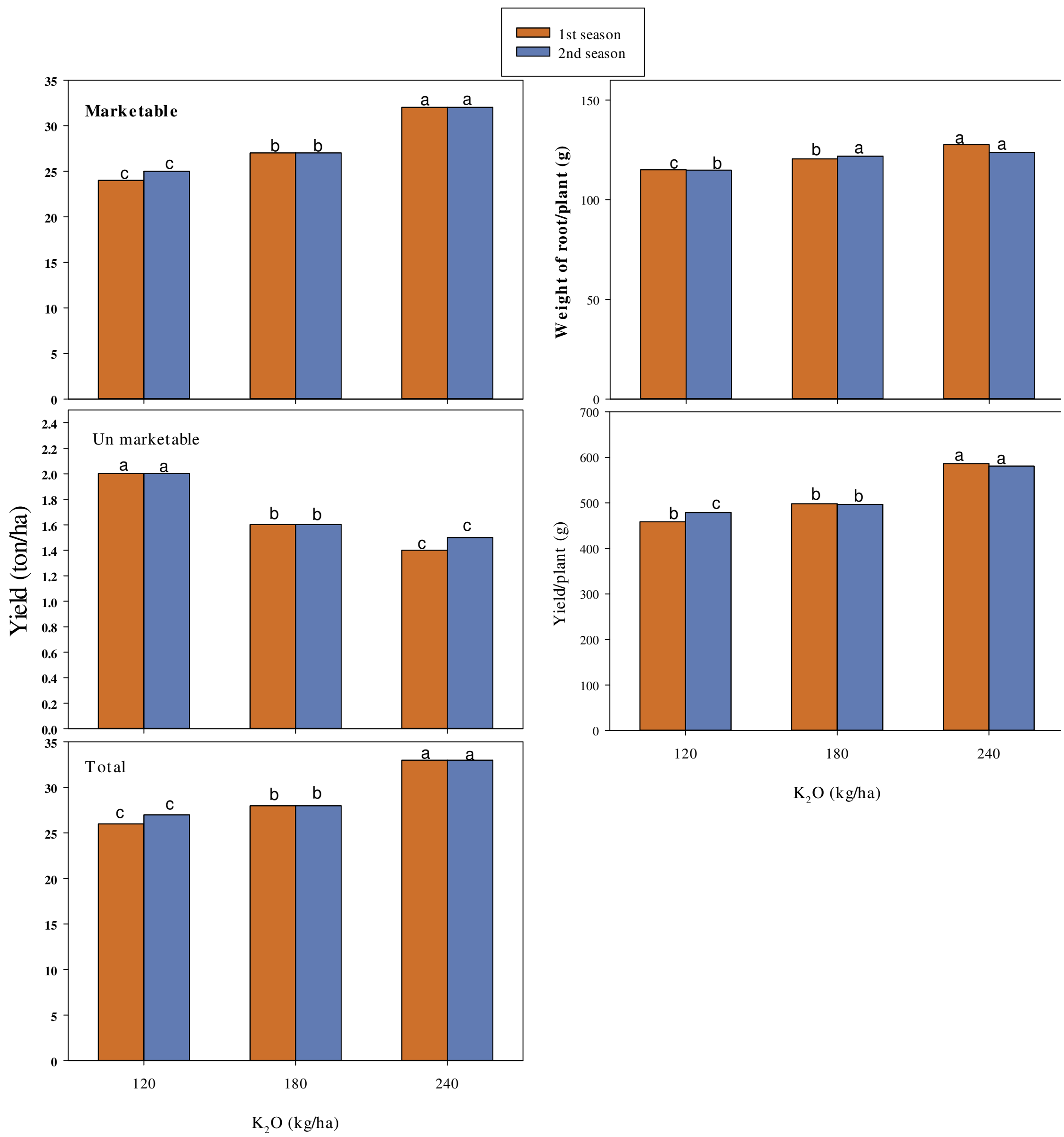


**Figure 5.** Effect of potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) on yield and its components of sweet potato during 2020 and 2021 seasons

Effect of whey protein hydrolysate at different concentrations (0, 0.1, and 0.2%) on yield and its components of sweet potato during 2020 and 2021 seasons are presented in Figure 6.

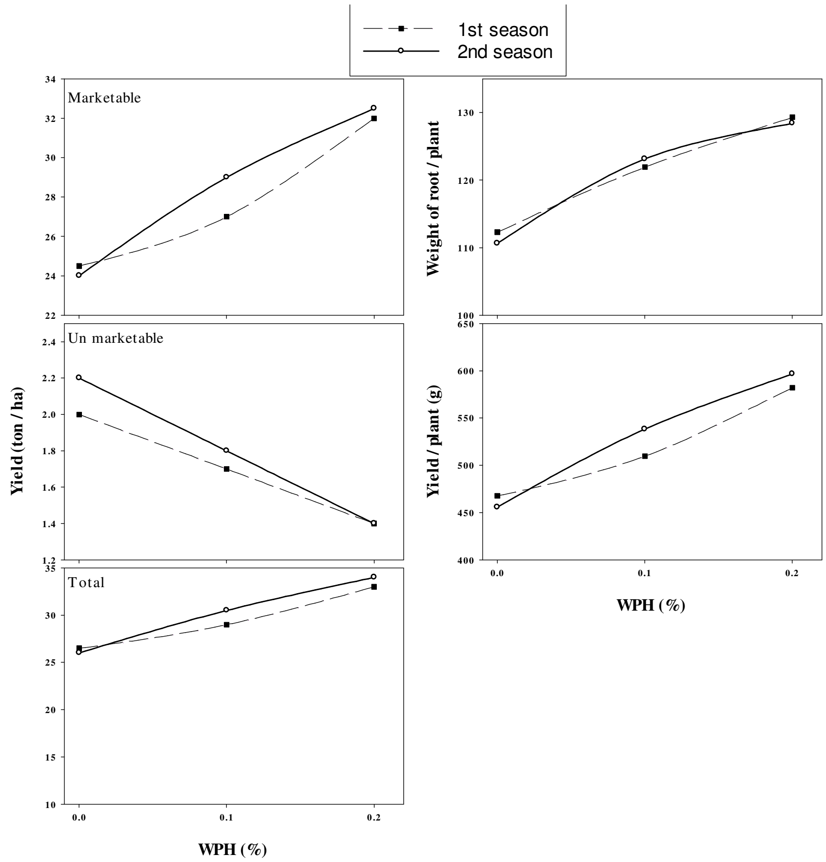


**Figure 6.** Effect of whey protein hydrolysate at different concentrations (0, 0.1, and 0.2%) on yield and its components of sweet potato during 2020 and 2021 seasons

The results illustrated in Figure 6 presented that WPH at different concentrations (0.1 and 0.2%) had a positive effect on marketable yield, total yield, the weight of root/plant, and yield/plant when compared to control. On average, foliar application of WPH up to 0.2% significantly increased marketable yield from 24 ton/ha to 32 tons/ha compared to control in both seasons. The same trend was observed in the total yield, the weight of root/plant, and yield/plant. Unmarketable yield (ton/ha) was significantly decreased with increasing WPH levels from 0 to 0.2% in both seasons.

The effect of interaction between potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) and foliar application of WPH at different concentrations (0, 0.1, and 0.2%) on yield and its components of sweet potato during 2020 and 2021 seasons are presented in Table 4. Results reported that in Tables 4 showed that the highest total yield, the weight of root/plant, and yield/plant was recorded with when K was applied at the rate of 240 Kg/ha. With WPH at rate of 0.2% in both seasons.

**Table 4.** Effect of interaction between potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) and foliar application of WPH at different concentrations (0, 0.1, and 0.2%) on yield and its components of sweet potato during 2020 and 2021 seasons

| Treatments | | Yield / plant (g) | | yield (ton/ha) | | | | | | Average tuber root weight (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Marketable | | Unmarketable | | Total | | | |
| $K_2O$ (kg/ha) | WPH (%) | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season |
| 120 | 0 | 415.1i | 428.4gh | 21i | 22f | 2.7a | 2.7a | 23.6g | 24.4gh | 110.42gh | 112.44gh |
| | 0.1 | 442.6h | 486.1f | 23h | 26e | 2bc | 2.2b | 25.2f | 27.7f | 120.94d | 126.92abc |
| | 0.2 | 521.4d | 549.0cd | 28d | 29c | 1.6de | 1.9c | 29.8d | 31.4c | 129.38bc | 131.82a |
| 180 | 0 | 486.2ef | 425.9h | 27fg | 22f | 1.9c | 2b | 27.5e | 24.3h | 110.51h | 104.66i |
| | 0.1 | 476.1fg | 523.6de | 25g | 28d | 1.6de | 1.5e | 27e | 29.6e | 114.19efg | 118.21ef |
| | 0.2 | 573.8c | 589.7bc | 31c | 32b | 1.4e | 1.3f | 32.8c | 33.5b | 122.87d | 121.34cde |
| 240 | 0 | 502.1de | 513.2ef | 27e | 28d | 1.5de | 1.7d | 28.5de | 29.2e | 115.96ef | 114.81fgh |
| | 0.1 | 610.2b | 605.4b | 33b | 33b | 1.4e | 1.6de | 34.6b | 34.4b | 130.68b | 124.33bcd |
| | 0.2 | 651.4a | 651.3a | 36a | 36a | 1.2f | 0.9g | 37.2a | 37a | 135.72a | 132.12a |

Mean values in the same column for each trait followed by the same lower-case letter is not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

*Tuber root quality*

Data in Table 4 showed that there are significant increases in dry matter by application of K fertilization at rats of 120, 180, and 240 Kg $K_2O$/ha. The dry matter of tuber root was increased from 21.17 g at 120 kg/ha to 23.19 g, and 24.99 g for 180, and 240 kg/ha, respectively for the 1st growing season and from 23.55 g to 26.99 g and 28.4 g for the same treatments at the second growing season. Data also showed that application of K fertilization significantly increased starch, total sugar, carotene, and K content for the two growing seasons. In the 2nd season, total protein was significantly increased with increasing K fertilization rats while, in the 1st season nonsignificant differences was observed between 180 and 240 kg/ha. Quality of sweet potato tubers expressed as dry matter, total protein, starch, total sugar, carotene, and K content was increased with increasing WPH foliar application rats (Table 5).

**Table 5.** Effect of potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) and foliar whey protein hydrolysate at different concentrations (0, 0.1, and 0.2%) on tuber root quality during 2020 and 2021 seasons

| Treatments | Total protein (%) | | Dry matter (%) | | Starch (%) | | Total sugars (%) | | Carotene (mg/gFW) | | K contents (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season |
| $K_2O$ (kg/ha) | Effect of potassium rates (kg $K_2O$ /ha) | | | | | | | | | | | |
| **120** | 6.42b | 6.63c | 21.17c | 23.55c | 39.67c | 42.30c | 6.09c | 5.81b | 4.9c | 6.4c | 1.41c | 1.68c |
| **180** | 8.65a | 8.22b | 23.19b | 26.99b | 42.74b | 45.92b | 6.49b | 6.10a | 6.1b | 8.0b | 2.07b | 2.28b |
| **240** | 8.70a | 9.04a | 24.99a | 28.40a | 43.82a | 47.83a | 6.84a | 6.32a | 7.0a | 8.8a | 2.15a | 2.54a |
| **WPH (%)** | Effect of WPH concentrations | | | | | | | | | | | |
| **0** | 6.92c | 7.25c | 21.53c | 24.22b | 39.60b | 42.49c | 6.12b | 5.75b | 5.2c | 6.8b | 1.71b | 1.85c |
| **0.1** | 7.52b | 7.64b | 22.62b | 26.06a | 42.12a | 44.62b | 6.53a | 6.02a | 5.4b | 6.9b | 1.74b | 2.09b |
| **0.2** | 8.12a | 8.04a | 23.85a | 26.62a | 41.58a | 45.00a | 6.70a | 6.23a | 6.0a | 7.4a | 1.93a | 2.20a |

Mean values in the same column for each trait followed by the same lower-case letter is not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

The effect of interaction between potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) and foliar application of WPH at different concentrations (0, 0.1, and 0.2%) on tuber root quality during 2020 and 2021 seasons are presented in Table 6. The highest total protein was recorded with when K was applied at the rate of 180 Kg/ha. With WPH at rate of 0.2% in both seasons. The highest values of dry matter, starch, and total sugar were recorded when K were applied at 240 kg/ha with WPH at 0.2% in both seasons. The highest value of carotene was obtained at 240 kg/ha with WPH at 0.1, and 0.2% in both seasons. The highest amount of K content was recorded at 180 kg/ha with WPH at 0.2% in the 1st season, and at 240 kg/ha with WPH at 0.2% in the second season.

**Table 6.** Effect of interaction between potassium fertilizer at different levels (120, 180, and 240 Kg $K_2O$/ha) and foliar application of WPH at different concentrations (0, 0.1, and 0.2%) on tuber root quality during 2020 and 2021 seasons

| Treatments | | Total protein (%) | | Dry matter (%) | | Starch (%) | | Total sugars (%) | | Carotene (mg/g FW) | | K contents (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $K_2O$ (kg / ha) | WPH (%) | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season | 1st season | 2nd season |
| 120 | 0 | 5.63g | 6.00f | 19.90h | 20.53f | 36.67h | 39.53i | 5.69f | 5.41h | 3.8f | 4.8f | 1.16g | 1.27h |
| | 0.1 | 6.19f | 6.44ef | 19.90h | 23.39e | 39.61f | 40.85h | 5.92ef | 5.63gh | 4.1f | 5.4e | 1.32f | 1.62g |
| | 0.2 | 6.38f | 6.31ef | 21.68g | 24.74d | 38.68g | 42.15g | 6.09def | 5.90d-g | 5.0e | 6.1d | 1.45e | 1.65g |
| 180 | 0 | 7.44 e | 7.44cd | 21.54g | 26.05c | 42.54cd | 45.45e | 6.38b-e | 5.99c-g | 5.6d | 7.9b | 1.84d | 1.84f |
| | 0.1 | 8.00cd | 7.56c | 22.94f | 27.37b | 44.63a | 48.39b | 6.91a | 6.34ab | 5.1e | 6.3d | 1.87d | 2.20 e |
| | 0.2 | 9.31a | 8.69ab | 23.75def | 26.11c | 44.49a | 47.86bc | 6.93a | 6.28abc | 5.9cd | 7.0c | 2.24a | 2.42cd |
| 240 | 0 | 7.69de | 8.31b | 23.16f | 26.10c | 40.86e | 45.27e | 6.31cde | 5.86efg | 6.2bc | 7.9b | 2.15c | 2.46bc |
| | 0.1 | 8.38bc | 8.94ab | 25.02bc | 27.42b | 43.25bc | 47.54bc | 6.78abc | 6.09c-f | 7.2a | 9.1a | 2.03c | 2.46bc |
| | 0.2 | 8.69b | 9.13a | 26.13a | 29.03a | 44.19a | 49.48a | 7.09a | 6.51a | 7.3a | 9.1a | 2.12b | 2.53ab |

Mean values in the same column for each trait followed by the same lower-case letter is not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

## Discussion

Sweet potatoes (*Ipomoea batatas* [L.] Lam.) are a common food for human use. Due to its nutrition qualities, adaptability to various edaphoclimatic conditions, and high yield within a short period of time, this crop is of significant socioeconomic value (Da Silva *et al.,* 2022). Fertilization with potassium (K) is an essential step in the formation of sweet potatoes. K is necessary for photosynthesis, transport of sugar, movement of water and nutrients, synthesis of protein, and formation of starch in sweet potatoes (Harvey *et al*., 2022). This study found that increasing the potassium fertilizer rate promoted vegetative growth as measured by leaf area and shoot dry weight. This can be accounted for by the idea that raising K improves N uptake. Previous reports by El-Baky *et al*. (2010) and Cecílio Filho *et al.* (2016) noted the increase in vegetative development and strong yield response to K fertilization. In the present study, marketable, total yield, weight of root and yield/plant were increased with increasing the rate of potassium fertilizer. This result could be as a result of potassium's crucial involvement in increasing photosynthate synthesis and delivery to roots (Mengel and Kirkby, 1987). According to El-Baky *et al*. (2010), the critical function of potassium in the energy status of the plant, the translocation and storage of assimilates, and the maintenance of tissue water relations can all be ascribed to rising roots production of plants as a result of increasing potassium application rates. In the current study, increased potassium levels significantly increased the uptake of N, P, and K by sweet potato shoots during both seasons. According to the research of Clarkson and Hanson (1980) this might be caused by the plant's high K nutrient mobility. Based on the beneficial impact of translocation of assimilates, it is possible to explain the observed improvement in the fruit quality metrics (total protein, starch, total sugar, carotene, and K content) as altered by potassium nutrition (Mengel, 1997). Today's agriculture must continually overcome obstacles to produce more and better food for a population that is expanding in a climate that is changing. Agricultural practices must develop novel approaches to managing crop nutrition, crop productivity and plant health. It is also required to develop novel chemicals or biological agents that can enhance plant yield (by controlling plant physiology, metabolism, and crop performance) as well as agro-product quality. Agents having these qualities have been proposed for sustainable agriculture throughout the past ten years. It has been demonstrated that these substances, which are referred to as biostimulants, enhance plant nutrition, quality, yield, and abiotic tolerance in a variety of agricultural crops (Moreno-Hernández *et al*., 2020). In the present study, whey protein was hydrolysed with trypsin for 4 h at an enzyme/substrate ratio (1/300, w/w). The obtained whey protein hydrolysates (WPH) were chemically characterized, and their antioxidant activity was estimated. WPH, which was produced using trypsin for 4 hours and presented the highest antioxidant activity, Therefore, it was selected as a biostimulant with potassium fertilization for enhancing the productivity and tuber quality of sweet potatoes (Kosaric and Asher, 2005). Trypsin hydrolysis of whey proteins produced peptides having biological properties (Ballatore *et al*., 2020). With 6%–7% total solids, whey is a considerable by-product of the cheese-making process. Whey proteins, a soluble by-product of cheese production, make up almost 20% of all milk proteins (Kosaric and Asher, 2005). In the present study, the responses of vegetative growth, yield and roots quality of sweet potato were statistically significant with increasing WPH foliar application. Protein hydrolysates have been shown experimentally to increase plant root and shoot biomass, increasing crop productivity in both greenhouse and open field settings for a variety of crops, including tomato, lettuce, kiwifruit, papaya, pepper, lily, passionfruit, pea, wheat, and corn (Colla *et al.,* 2017; Rouphael *et al*., 2018; El-Sanatawy *et al*., 2021; Osman *et al*., 2021b). By improving nutrient use efficiency, protein hydrolysates can assist plants in performing their functional functions more effectively when nutrients are scarce (Planques *et al.,* 2012). As a result, it has been demonstrated that the foliar application of protein hydrolysates from various sources (animal and plant) increases the vegetative development and productivity of various fruit trees. Recently, it was suggested that protein hydrolysates could be a novel and effective way to bio-stimulate plant metabolism and production by improving nutrient absorption and inducing physiological and molecular processes that lessen the effects of various abiotic stresses (Sitohy *et al*., 2020). The following are effects of biostimulation activity may be due to: (i) the release of key enzymes involved in N assimilation and C metabolism (citrate synthase, malate, and isocitrate dehydrogenase); (ii) increased promoted auxin and gibberellin-like activities; and (iii) increased antioxidant enzymatic activity, pigment biosynthesis, and production of secondary metabolites (Rouphael *et al*., 2018; Sestili *et al*., 2018).

## Conclusions

K plays a vital role in sweet potato production. In the current study, increased potassium levels significantly increased the uptake of N, P and K by sweet potato shoots during both seasons as well as vegetative growth and tuber quality. Whey protein hydrolysates as a bio stimulant with potassium fertilization was subjected for enhancing the productivity and tuber quality of sweet potatoes. The recommended dose for enhancing sweet potato vegetative growth, yield, and tuber quality was recorded at 240 Kg $K_2O$/ha with 0.2% WPH.

## Authors' Contributions

Conceptualization, EE, HI, EA; methodology, EE, HI, EA, AO; software, HA, KhA and WO; validation, EE, HI, EA; investigation, EE, HI, EA, HA; resources, EE, HI, EA; data curation EE, HI, EA; writing—original draft preparation, EE, HI, EA, AO and WO; writing—review and editing, EE, HI, EA, AO, KhA; funding acquisition, SA, NA, WO; All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

## Acknowledgements

The researchers would like to acknowledge Deanship of Scientific Research, Taif University for funding this work.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

## References

Abdel-Hamid M, Otte J, De Gobba C, Osman A, Hamad E (2017). Angiotensin I-converting enzyme inhibitory activity and antioxidant capacity of bioactive peptides derived from enzymatic hydrolysis of buffalo milk proteins. International Dairy Journal 66:91-98. *https://doi.org/10.1016/j.idairyj.2016.11.006*

Almadi L, Paoletti A, Cinosi N, Daher E, Rosati A, Di Vaio C, Famiani F (2020). A biostimulant based on protein hydrolysates promotes the growth of young olive trees. Agriculture 10(12):618. *https://doi.org/10.3390/agriculture10120618*

Al-Mohammadi A-R, Osman A, Enan G, Abdel-Shafi S, El-Nemer M, Sitohy M, Taha M (2020). Powerful antibacterial peptides from egg albumin hydrolysates. Antibiotics 9(12):901. *https://doi.org/10.3390/antibiotics9120901*

Amaro GB, Fernandes FR, Silva GO, Mello AF, Castro L (2017). Performance of sweet potato cultivars in the Alto Paranaíba region. Horticultura Brasileira 35(2):286-291. *https://doi.org/10.1590/S0102-053620170221*

Anitha K (2020). Role of biostimulants in uptake of nutrients by plants. Journal of Pharmacognosy and Phytochemistry 9:563-567.

Ballatore MB, del Rosario Bettiol M, Braber NLV, Aminahuel CA, Rossi YE, Petroselli G, … Montenegro M (2020). Antioxidant and cytoprotective effect of peptides produced by hydrolysis of whey protein concentrate with trypsin. Food Chemistry 319:126472. *https://doi.org/10.1016/j.foodchem.2020.126472*

Cecílio Filho AB, Nascimento S, Silva AS, Vargas P (2016). Agronomic performance of sweet potato with different potassium fertilization rates. Horticultura Brasileira 34:588-592. *https://doi.org/10.1590/s0102-053620160421*

Clark R (1982). Plant response to mineral element toxicity and deficiency. Breeding-plants-for-less-favorable-environments. USA, pp 71-142

Clarkson DT, Hanson J (1980). The mineral nutrition of higher plants. Annual Review of Plant Physiology 31(1):239-298. *https://doi.org/10.1146/annurev.pp.31.060180.001323*

Colla G, Hoagland L, Ruzzi M, Cardarelli M, Bonini P, Canaguier R, Rouphael Y (2017). Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. Frontiers in Plant Science 8:2202. *https://doi.org/10.3389/fpls.2017.02202*

Colla G, Nardi S, Cardarelli M, Ertani A, Lucini L, Canaguier R, Rouphael Y (2015). Protein hydrolysates as biostimulants in horticulture. Scientia Horticulturae 196:28-38. *https://doi.org/10.1016/j.scienta.2015.08.037*

Da Silva L, Oliveira A, Cruz J, Sousa V, Silva A, Silva M (2022). Sweet potato yield in response to different potassium sources and splitting of fertilization. Revista Brasileira de Engenharia Agrícola e Ambiental 26:527-532. *https://doi.org/10.1590/1807-1929/agriambi.v26n7p527-532*

Du Jardin P (2015). Plant biostimulants: Definition, concept, main categories and regulation. Scientia Horticulturae 196:3-14. *https://doi.org/10.1016/j.scienta.2015.09.021*

Duncan D (1955). Multiple range and Multiple F test. Biometrices 11: 1. *https://doi.org/10.2307/3001478*

El-Baky A, Ahmed A, El-Nemr M, Sciences B (2010). Effect of potassium fertilizer and foliar zinc application on yield and quality of sweet potato. Research Journal of Agriculture and Biological Sciences 6(4):386-394.

El-Sanatawy AM, Ash-Shormillesy SM, El-Yazied AA, El-Gawad HGA, Azab E, Gobouri AA, … Osman A (2021). Enhancing grain yield and nitrogen accumulation in wheat plants grown under a Mediterranean arid environment by foliar spray with papain-released whey peptides. Agronomy 11(10):1913. *https://doi.org/10.3390/agronomy11101913*

Ertani A, Pizzeghello D, Francioso O, Sambo P, Sanchez-Cortes S, Nardi S (2014). Capsicum chinensis L. growth and nutraceutical properties are enhanced by biostimulants in a long-term period: Chemical and metabolomic approaches. Frontiers in Plant Science 5:375. *https://doi.org/10.3389/fpls.2014.00375*

Göçer H, Gülçin İ (2011). Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. International Journal of Food Sciences and Nutrition 62(8):821-825. *https://doi.org/10.3109/09637486.2011.585963*

Harvey LM, Shankle MW, Morris CJ, Hall MA, Chatterjee A, Harvey K (2022). Sweet potato (Ipomoea batatas L.) response to incremental application rates of potassium fertilizer in Mississippi. Horticulturae 8(9):831. *https://doi.org/10.3390/horticulturae8090831*

Hasanah Y, Rahmawati N, Nasution K (2021). Growth and production of Cilembu sweet potatoes (*Ipomoea batatas* L.) varieties in the highlands with potassium fertilizer and pruning treatments. IOP Conference Series: Earth and Environmental Science pp 042027.

Hoshmand AR (2001). Statistical methods for environmental & agricultural sciences. CRC press: Boca Raton pp 212-212.

Hoyle NT, Merrltt J (1994). Quality of fish protein hydrolysates from herring (*Clupea harengus*). Journal of Food Science 59(1):76-79. *https://doi.org/10.1111/j.1365-2621.1994.tb06901.x*

Kosaric N, Asher Y (2005). The utilization of cheese whey and its components. Agricultural Feedstock and Waste Treatment and Engineering. Springer Berlin Heidelberg pp 25-60.

Mengel K (1997). Impact of potassium on crop yield and quality with regard to economical and ecological aspects. In: Johnston AE (Ed). Food security in the WANA region, the essential need for balanced fertilization. Proceedings of the regional workshop of the international potash institute held at Bomova, Izmir, Turkey, 26-30 May 1997.

Moreno-Hernández JM, Benítez-García I, Mazorra-Manzano MA, Ramírez-Suárez JC, Sánchez E (2020). Strategies for production, characterization and application of protein-based biostimulants in agriculture: A review. Chilean Journal of Agricultural Research 80(2):274-289. *https://doi.org/10.4067/S0718-58392020000200274*

Mengel K, Kirkby E (1987). Principles of Plant Nutrition. 4th Edn. International Potash Institute, Horgen, Switzerland. International Potash Institute, Horgen, Switzerland, pp 687-695.

Mukhongo RW, Tumuhairwe JB, Ebanyat P, AbdelGadir AH, Thuita M, Masso C (2017). Combined application of biofertilizers and inorganic nutrients improves sweet potato yields. Frontiers in Plant Science 8:219. *https://doi.org/10.3389/fpls.2017.00219*

Nedunchezhiyan M, Byju G, Dash S (2010). Effects of organic production of orange fleshed sweet potato (*Ipomoea batatas* L.) on root yield, quality and soil biological health. International Research Journal of Plant Science 1(6):136-143.

Osman A, Imbabi TA, El-Hadary A, Sabeq II, Edris SN, Merwad A-R, Azab E, Gobouri AA, Mohammadein A, Sitohy M (2021a). Health aspects, growth performance, and meat quality of rabbits receiving diets supplemented with lettuce fertilized with whey protein hydrolysate substituting nitrate. Biomolecules 11(6):835. *https://doi.org/10.3390/biom11060835*

Osman A, Merwad A-RM, Mohamed AH, Sitohy M (2021b). Foliar Spray with pepsin-and papain-whey protein hydrolysates promotes the productivity of pea plants cultivated in clay loam soil. Molecules 26(9):2805. *https://doi.org/10.3390/molecules26092805*

Planques B, Colla G, Svecová E, Cardarelli M, Rouphael Y, Reynaud H, Canaguier R (2012). Effectiveness of a plant-derived protein hydrolysate to improve crop performances under different growing conditions. In: Proceedings of the I World Congress on the Use of Biostimulants in Agriculture 1009:175-179. *https://doi.org/10.17660/ActaHortic.2013.1009.21*

Ramadan MF, Osman A, El-Akad H (2008). Total antioxidant potential of juices and beverages-Screening by DPPH in vitro assay. Wissenschaftliche Verlagsgesellschaft Stuttgart 104:235-239. *https://doi.org/10.3989/gya.2008.v59.i3.516*

Reynolds HL (1989). Association of Official Analytical Chemists: 1964–1988. Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews 109-136.

Rós AB, Narita N, Araújo H (2015). Biostimulant effect on the initial growth and yield of sweet potato. Revista Ceres 62:469-474.

Rouphael Y, Giordano M, Cardarelli M, Cozzolino E, Mori M, Kyriacou MC, Bonini P, Colla G (2018). Plant-and seaweed-based extracts increase yield but differentially modulate nutritional quality of greenhouse spinach through biostimulant action. Agronomy 8(7):126. *https://doi.org/10.3390/agronomy8070126*

Rouphael Y, Spíchal L, Panzarová K, Casa R, Colla G (2018). High-throughput plant phenotyping for developing novel biostimulants: from lab to field or from field to lab? Frontiers in Plant Science 9:1197. *https://doi.org/10.3389/fpls.2018.01197*

Sestili F, Rouphael Y, Cardarelli M, Pucci A, Bonini P, Canaguier R, Colla G (2018). Protein hydrolysate stimulates growth in tomato coupled with N-dependent gene expression involved in N assimilation. Frontiers in Plant Science 9:1233. *https://doi.org/10.3389/fpls.2018.01233*

Shahrajabian MH, Cheng Q, Sun W (2022). The effects of amino acids, phenols and protein hydrolysates as bostimulants on sustainable crop production and alleviated stress. Recent Patents on Biotechnology 16(4):319-328. *https://doi.org/10.2174/1872208316666220412133749*

Sitohy MZ, Desoky E-SM, Osman A, Rady M (2020). Pumpkin seed protein hydrolysate treatment alleviates salt stress effects on *Phaseolus vulgaris* by elevating antioxidant capacity and recovering ion homeostasis. Scientia Horticulturae 271:109495. *https://doi.org/10.1016/j.scienta.2020.109495*

Taffouo VD, Nono GV, Simo C (2017). Evaluation of different sweet potato varieties for growth, quality and yield traits under chemical fertilizer and organic amendments in sandy ferralitic soils. Journal of Agricultural Research 12(48):3379-3388. *https://doi.org/10.5897/AJAR2017.12675*

Uwah D, Undie U, John N, Ukoh G (2013). Growth and yield response of improved sweet potato (*Ipomoea batatas* (L.) Lam) varieties to different rates of potassium fertilizer in Calabar. Nigeria. Journal of Agricultural Science 5(7):61. *https://doi.org/10.5539/jas.v5n7p61*