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THE APPLICATION OF A WHEY PROTEIN HYDROLISATES ENHANCE THE PRODUCTIVITY AND QUALITY OF LETTUCE UNDER NFT SYSTEM

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Abstract

Plants can synthesize amino acids, however, the biochemical process is complex and energy consuming. Therefore, the application of exogenous amino acids represents energy savings. The production of amino acids by a bioprocess using microbial sources and whey waste is promising. The objective of this research was to evaluate the effect of the foliar application of amino acids produced by biological synthesis, as a biostimulant in the lettuce crop. The plants were established in an NFT system, with a distance between plants of 30 cm at 28 days after sowing the seeds, foliar biostimulant treatments were 1.0, 2.0, 3.0, and 4.0 ml/L were applied once a week until one week before harvest. The results indicate that the variables of number of leaves and stem thickness were significant. In terms of these agronomical variables, biosynthesized amino acids exceeded the values of the commercial amino acids' applications by 12.11% for number of leaves, and 8.92% for stem thickness. The fresh and dry weight of lettuce was higher in 20.55% and 21.8% respectively, when biosynthesized amino acids were applied. The biosynthesized amino acids applied in a foliar way improves the productivity and quality of lettuce grown in the NFT system.

Key words*: biostimulants, amino acids, Aspergillus niger, fermentation broth, lettuce.*

INTRODUCTION

Lettuce (*Lactuca sativa*) is an adaptable vegetable to be grown throughout the year, coupled with the fact that it is a worldwide high-demanded vegetable. The consumption of lettuce, whether fresh or processed, brings benefits to consumers since it provides minerals (phosphorus, iron, calcium, potassium), vitamins (A, C, E, B1, B2, B3, B9 and K), has high water content, provides antioxidants and fiber that promotes digestibility (Aguilar, 2022; Valdivia & Almaza, 2016). In Mexico, the most used commercial presentations are baby leaf, Romaine (*Lactuca sativa* L. var. *longifolia*), and iceberg (*Lactuca sativa* L. var. *capitata*). To satisfy the increasing demand for this vegetable, crop production must be intensified without considering the nutritional quality of the lettuce. One of the alternatives to produce lettuce crop and, at the same time, to combat the effects of climate change and the rational use of water, is the laminar Nutrient Film Technique (NFT) (Frasetya et al., 2021). This soilless production system allows the recirculation of water and nutrient solutions, making more efficient the use of mineral elements and water, which have become a good alternative for growing lettuce.

In the production of leafy vegetables such as lettuce, it is of utmost importance to monitor the permissible ranges of nitrate for human consumption, since the nitrate content in leafy crops ranges from 3 to 5 milligrams per gram, therefore, their consumption provides between 80 and 90% of the nitrates daily needed (Bahadoran et al., 2016). On the other hand, in the nutritional management of lettuce cultivation, the nitrogen is crucial to maintain cellular metabolic activity and consequently, for its growth and development. However, the available sources of nitrogen are NO^{3-} and NH_4^+, and a combination of them generally results in better nitrogen balances. nitrogen as nitrate or ammonium in consumable leaves (Lara et al., 2019; Kappel et al., 2021).

Protein hydrolysates (PHs) are considered an important group of biostimulants, with a high content of peptides and amino acids, and therefore show a positive effect on crop performance (Colla et al., 2017). PHs are derived from raw materials of animals and plants (Colla et al., 2015), they act when they are applied in small amounts that range from 1 to 5 mL/L (Ertani et al., 2016; Kauffman et al., 2007; Kunicki et al., 2010; Zhang et al., 2003). PHs could be defined as products that help to tolerate the stress produced by the climate or by the cultivation conditions, which reflects greater assimilation of nutrients, stimulates the growth and development of plants, expressing an increase in the yield and commercial quality of the fruit (Ertani et al., 2016). Chelating and complexing activities of specific amino acids and peptides are considered to contribute to the availability and acquisition of nutrients by roots (Du Jardin, 2015). Bioactive protein hydrolysates or peptides can be obtained from different sources of dietary proteins. The most investigated sources of bioactive peptides are eggs, fish, marine species, soybeans, rice, peanuts, milk, whey, chickpea, amaranth, corn, and algae, and under different methods extraction or obtaining (Garcia et al., 2013; Stefanucci et al., 2017). Whey has been used in the preparation of organic fertilizers (Edgar, 2023; Díaz & Ovalle, 2007), in lettuce cultivation it improves germination (Maheshwari & Sood, 2021) and yield (Oña, 2018), bokashi added with acid whey, improves the weight of lettuce (Sequeira, 2019), and in other crops such as zucchini, whey promotes greater fruit set and size (Nuñez, 2002), while in oat cultivation it promotes greater growth and leaf coverage (Caiza, 2022).

In horticultural crops, organic supplements are generally applied to the crops to complement the nutrition of the plants. The dose varies depending on the product and the recommendation of the formulator, and it is applied by foliar application (aspersion). However, some of the recommended doses are not necessarily adequate for a particular crop or for the system under which it grows. The previously mentioned benefits of whey imply the need to derive products based on biotechnological processes, such as protein hydrolysates, since their effect could be superior to acidic whey hydrolysis.

The addition of complex or compound nutrients, such as the use of whey applied to crops, has been reported to improve productivity in vegetables. Considering that enzymatic processes have a high specificity in the degradation processes, the hydrolysis of whey through the use of microorganisms that promote enzymatic action will allow obtaining nutrients and nitrogen compounds that are easily assimilated by the lettuce plant.

Therefore, the aim of this research was to compare two amino acid-based foliar biostimulants at different doses.

MATERIALS AND METHODS

Experimental site location

The experiment was carried out in the Fermentations and Biomolecules Lab., in the Food and Technology Department, and in a medium-technology greenhouse, located in the Plant Breeding Department, both of the Universidad Autónoma Agraria Antonio Narro (UAAAN), in Saltillo, Coahuila, Mexico. The whey was obtained from a local cheesery and mixed with a cell-free protease extract for the

Protein Hydrolysate obtention. The commercial variety Parris Island Cos romaine lettuce seeds was used. The phenotype of this lettuce seed is described as a Roman-style lettuce that grows very quickly and has a delicious flavor, with corrugated green leaves and can be harvested as baby lettuce or when fully matured. Its late flowering allows for a wide range of harvest days.

Seedling production

Polystyrene trays of 200 cavities were used and they were filled with a mixture of peat moss substrate and perlite in a 70: 30 ratio. One seed per cavity was sown with a depth of 0.3-0.5 cm and covered again with substrate. The full tray was covered with black plastic for 72 h, to stimulate germination and the emergence of the seedling. At the end of that period, they were uncovered and left in the greenhouse for growth. The sowing of the lettuce in the trays was carried out on September 18, 2019. Five days after emergence (DAE) the seedlings were nourished with a soluble triple 20 fertilizer solution at a rate of 0.5 g/L, 0.8 g/L at 10 DAE, 1 g/L at 15 DAE and until 27 DAE.

Establishment of cultivation in a hydroponic system

28 days after sowing, the seedlings were transplanted into an NFT system composed of 6 meters' length PVC tubes arranged in A-frame shape. Each level was interconnected with 16 mm tubing and elbows of the same size, and there were five levels that facilitate the light distribution for all plants. The distance between plant to plant was 30 cm. The recirculation of the system, used a submersible pump brand Master Cool® model SP-4, with a pumping capacity of 20 L/min and an energy consumption of 36 W/h. The pumping system was connected to a digital timer of 20 events (STEREN® brand model TEMP-20E), with a 120v power supply. The timer allowed us to manage an active irrigation program every 30 min every day.

Handling the recirculating nutrient solution in the NFT system

A water analysis of the deep well that provides water to the greenhouse was used to calculate the nutrient solution. This data was used to add the amount of nutrients to the solution by soluble fertilizers, where the difference between the nutrient content of the water and the units required in milliequivalents for each liter of water (meq/L) was applied. The nutrient solution used in the NFT recirculating system to nourish the lettuces is shown in Table 1, which was renewed every week, to avoid accumulation of salts in the nutrient solution.

Table 1. Nutrient solution used in the NFT system for the nutrition of lettuces

Nutrient	Concentration (Meq/L)	Microelement	Concentration (ppm)
Anions		Fe	1.50
NO_3^-	7	Mn	0.50
$H_2PO_4^-$	2	Cu	0.12
SO_4^{2-}	7	Zn	0.05
Cl^-	3.2	B	0.70
Cations		Mo	0.05
K^+	5		
Ca^{2+}	5.5		
Mg^{2+}	3		
NH_4^+	2		
Na^+	3		
HCO_3^-, CO_3^-	1		
EC (dS/m)	1.9		
pH	6.7		

Foliar treatments

The applied foliar treatments were 1) commercial 30% amino acids (30AA), and 2) enzymatically hydrolyzed whey protein (HWP), each one tested at doses of 0, 1, 2, 3 and 4 mL/L resulting in a factorial experiment of 2 x 5.Treatments were applicated via foliar with a 1-liter capacity sprinkler that contained distilled water, where the previously established quantities were diluted. Table 2 shows the treatments applied. The foliar applications of each treatment were carried out every Friday of the week. Doses were applied to the four repetitions established for each treatment, however, between treatments the sprinkler and nozzles were washed and cleaned.

Table 2. Foliar treatments applied to the cultivation of lettuce in the NFT system

Factor A	Factor B (Applied Doses in mL/L)
Hydrolyzed whey protein (HWP)	T0 = 0
	T1 = 1
	T2 = 2
	T3 = 3
	T4 = 4
30% commercial amino acids (30AA)	T0 = 0
	T1 = 1
	T2 = 2
	T3 = 3
	T4 = 4

Measurement of plant height, leaf length, leaf width and root length

All variables were measured with a measuring tape. For plant height the length from the origin of the radicle to the upper part of the plant was measured; for leaf length a healthy and fully developed and extended leaf of the plant was considered and located in a similar part of all the lettuces evaluated; the width of the leaf was measured from the middle part of the same leaf used for the measurement of leaf length. Regarding root length, the length was measured from the origin of the root to the end of the longest root, generally one of the main roots. It was a total of four plants for each repetition.

Quantification of the number of leaves

For this variable, the total of developed leaves was counted. The leaves with yellow color or in senescence, generally located in the lowest part of the plant, were eliminated and were not considered.

Basal stem diameter measurement

The part of the stem, located between the leaves of the basal part and the beginning of the root, was measured with the help of a Truper® brand digital vernier, and it was expressed in centimeters (cm).

Determination of fresh weight of lettuce and fresh weight of root

For the variable fresh weight of lettuce, a Torrey® brand digital scale model L-EQ 5/10, was used, in which each of the lettuces was weighed separately from the root. Then the root was weighed after draining the excess of humidity. For each variable, four plants per repetition were considered.

Determination of dry weight of lettuce and dry weight of root

For the dry weight of lettuce and root, these were subjected to drying in a Shel Lab® brand drying oven model GI2-ZZMFG, for a period of 48 h at 40°C. After which, they were introduced to a drying oven Lab Line, model 3478M at 75°C for 72 hours. Once the samples were completely dried, the dry weight of lettuce and root was obtained, using an analytical balance brand Ohaus Explorer Pro® model EP613.

Statistical analysis

The experimental arrangement was completely randomized with factorial arrangement of A x B, where: A) HWP and amino acids at 30%; B) the doses of 0, 1, 2, 3, and 4 mL/L. To assess agronomical variables, four plants were sampled from each treatment. The statistical analysis was carried out in SAS 9.0 software. For all data the one-way analysis of variance (ANOVA) was realized, and the means were compared with the least significant difference (Fisher LSD $p \leq 0.05$).

RESULTS AND DISCUSSIONS

The results of the agronomic variables for the amino acids sources (factor A) are shown in Figure 1. These results indicate that for the variables of lettuce height, leaf length, leaf width and root length there was no statistical significance. However, in three of these variables there was a slight increase using HWP. The variables that showed a statistical significance were number of leaves, and stem thickness. HWP exceeded 30AA in both, 12.11% in terms of number of leaves, and 8.92% in stem thickness. The improvements when using HWP imply a better availability of biocompounds derived from whey, probably due to the size of the molecules such as peptides, carbohydrates, amino acids, fats and micronutrients that remain after hydrolysis and the purity of the final product (Caiza, 2022), compared to 30AA that are only indicated in a general concentration of biocomposites.

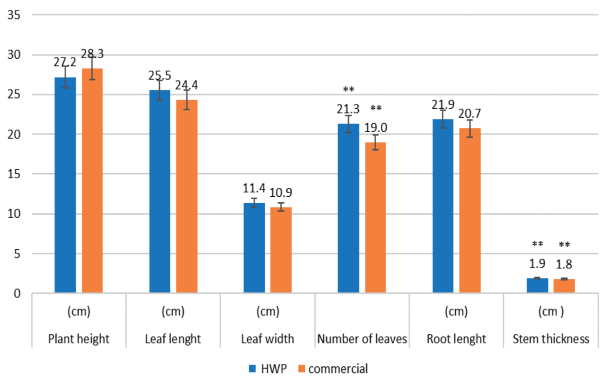

Figure 1. Lettuce leaf area at 27 days after transplant, with foliar application of hydrolyzed whey protein and 30% commercial amino acids. ** = mean between treatments is statistically different using LSD Fisher $p \leq 0.05$

The results of the ANOVA and test of means (LSD Fisher $p \leq 0.05$) indicated statistical significance in the variables of fresh and dry weight of lettuce, as well as fresh and dry weight of the root (Figure 2) with regard factor A.

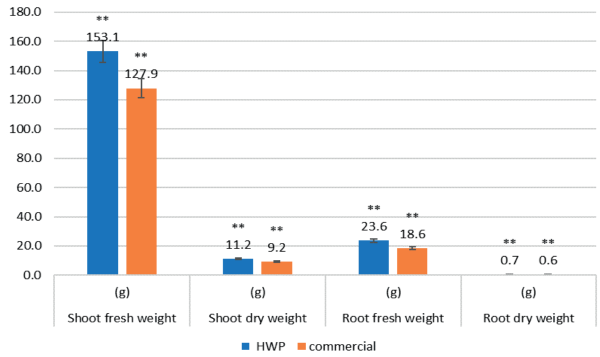

Figure 2. Lettuce weight accumulation at 27 days after transplant, with foliar application of hydrolyzed whey protein and 30% commercial amino acids. ** = mean between treatments is statistically different using LSD Fisher $p \leq 0.05$

These results showed that the fresh and dry weight of lettuce is superior with HWP in 20.55 and 21.8%, respectively. Similar effects could be observed in fresh and dry root weight, with an increase of 26.9 and 19.5%, respectively, when applying HWP. This means a greater accumulation of weight in lettuces and probably a greater assimilation of mineral nutrients when applying HWP compared to 30AA. Since it has been proven that whey also provides microelements Fe (3 ppm), Zn (0.20 ppm) and macroelements, Na (791 ppm), K (1547 ppm), Ca (362 ppm), P (468 ppm), Mg (78 ppm), which are directly incorporated into the plant's metabolism (Caiza, 2022). Protein hydrolysates can also provide amino acids such as alanine, arginine, glycine, proline, glutamate, glutamine, valine and leucine, and also components such as fats, and carbohydrates to the plant (Colla et al., 2015). This can be explained by Azcon & Talon (2000) who mention that 50% of the nitrogen absorbed by the plant becomes part of compounds with high molecular weight, such as proteins and nucleic acids. Therefore, the effect of the application of nitrogenous biostimulants such as protein and amino acid hydrolysates can generate an increase in performance that is expressed through fresh weight in addition to dry weight and other variables of interest (Aruani et al., 2008; Baturcca et al., 2022; Del Bono, 2021; De Pinheiro & Marcelis, 2000).

The results of the weight variables of the foliar applications dose (factor B) are shown in Figure 3 (A and B). No significance between applied foliar doses of amino acids at 30% or HWP, for the variables of root length were shown. By other part, there was statistical significance in plant height, leaf length, and root length. The increase trend of these variables is probably due to the contribution of additional nutrients (Fe^{2+}, Zn^{2+}, Na, K, Ca^{2+}, P and Mg^{2+}) contained in the protein hydrolysates as the dose of the product increases, as descrybed and substantiated (Caiza, 2022; Colla et al., 2015). The no statistical changes in root length are due to the fact that the protein hydrolysates were applied via foliar spray, not via root, consequently the results are more noticeable in the aerial part, since absorption and transport over long distances have been reported when amino acids are absorbed from the soil, via specific transporters, and when they are applied via foliar application, they generally accumulate in the vacuoles (Tegeder, 2014).

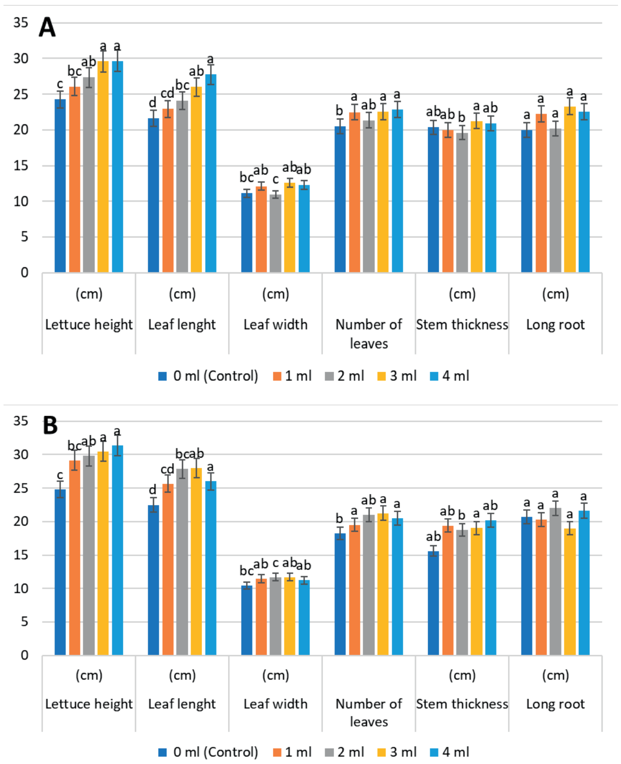

Figure 3. Agronomical variables corresponding to leaf area of lettuce crop at 27 days after transplant, in response of the doses of foliar application of A) hydrolyzed whey protein and B) commercial amino acids at 30%

The results of the applied foliar dose are shown in Figure 4. The fresh weight of lettuce can be observed a common denominator in which the application of protein hydrolysate and commercial amino acids exceed the control treatment, however, the hydrolysates exceed commercial amino acids by more than 7%, a similar response trend It was observed in the dry weight of the lettuce and in the fresh weight of the root where the control treatment was lower than the different doses of protein hydrolysates and commercial amino acids. It has been documented that whey generates benefits to lettuce in a 3:1 ratio of water and whey, and even better, using 100% whey even as a nutrient solution (Siqueira, 2019).

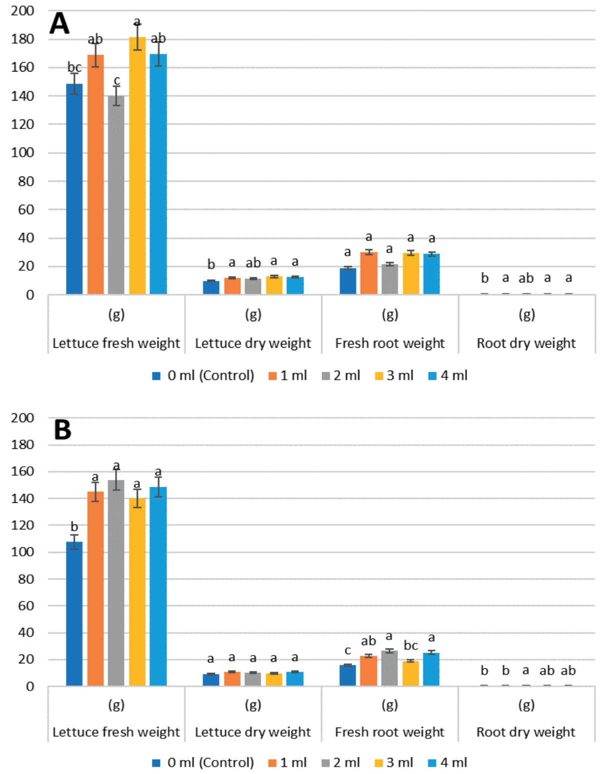

Figure 4. Lettuce weight accumulation at 27 days after transplant, with foliar application of A) hydrolyzed whey protein and B) commercial amino acids at 30%

CONCLUSIONS

The HWP applied in a foliar way increases the accumulation of fresh and dry weight of lettuce and roots in lettuces grown in the NFT system. In general, HWP causes similar effects as 30AA, so it could have potential use in the cultivation of lettuce.

The proven doses of HWP and 30AA do not generate significant beneficial effects in the lettuce cultivation in the NFT system. So, it is recommended in future research to try higher doses.

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