USE OF PROTEIN HYDROLYSATES IN ORGANIC AGRICULTURE

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ABSTRACT

It is known to be a real challenge to find sustainable alternatives for the use of mineral fertilizers, especially nitrogen-based ones. This is a legal requirement in organic farming.

Organic sources have a low concentration of nitrogen but have the advantage that they come from a renewable source and are found along with other compounds that have positive effects on plant nutrition. Protein hydrolysates(PHs) can improve agricultural production in terms of quality and quantity by enhancing seed germination, root development and nutrient uptake and can reduce the amount of chemical fertilizers used.

This paper presents data from the literature on the use of protein hydrolysates as a source of nitrogen for crop fertilization.

INTRODUCTION

The highest temperatures have been recorded in the last 5 years, with 1.1 °C above pre-industrial levels. Because of this, phenomena such as drought, storms and other extreme weather events are on the rise (https://eur-lex.europa.eu/legal-content).

The global population was 7.7 billion in 2019 and it is estimated that by 2050 the world's population will reach an average of 9.7 billion (https://population.un.org), and it is clear that agricultural production will it must grow continuously. This trend will lead to the increase of cultivated areas and the intensive exploitation of more and more large areas.

The increased demand for fertile soils with optimal nutrient content, especially nitrogen (N), has led to an increase in the consumption of mineral fertilizers containing this nutrient.

On the other hand, the utilization coefficient of nitrogen from mineral fertilizers is below 70%, which leads to a low efficiency compared to the nutritional needs of plants, but also to pollution phenomena(Madjar and Davidescu, 2009; Jiao et al., 2013).

In Romania, organically cultivated agricultural areas represents only 3% of the total agricultural land (https://www.madr.ro/docs/agricultura/agricultura-ecologica) while at the level of the European Union organic agriculture is 8.5%. At present, there is a target on the European Green Deal at EU level for organic farming to be practiced on 25% of the agricultural land by 2030 (Brussels, 19.4.2021 COM(2021) 141 final/2).

Protein hydrolysates can be obtained from various sources of plant or animal origin, some being by-products or waste from various industries and are an important source of nitrogen and other active biomolecules for use in organic farming.

RESULTS AND DISCUSSIONS

Nitrogen is a dominant regulator of vegetation dynamics, primary production and terrestrial carbon cycles fundamentally affecting the structure and functions of most ecosystems. As nitrogen is a major constituent of proteins, plants need to balance nitrogen investment in proteins for different biogeochemical processes to control and optimize growth and survive under specific environmental conditions (Herms and Mattson, 1992; Robertson and Vitousek, 2009; Xu et al., 2012; Zhang et al., 2020).

It is known that nitrogen-containing fertilizers are obtained through a technology invented about 100 years ago called Haber-Bosch. This process requires temperature and pressure, which means a large consumption of energy obtained from methane gas, for the most part. Globally, 450 million tonnes of nitrogen fertilizers, measured as a marketed product, are produced each year with the Haber-Bosch process. Three to five percent of global annual gas consumption is used by industry to produce nitrogen fertilizers. The cost of natural gas represents 60 - 80% of the costs for the production of nitrogen fertilizers (Fertilisers in the EU Prices, trade and use, 2019).

Approximately 500 million tonnes of NH_3 are produced annually using the Haber-Bosch process. With existing technologies, obtaining NH_3 consumes about 1% of the world's energy supply. Moreover, the Haber-Bosch process contributes significantly to carbon emissions: for every 1 tonne of NH_3 produced, it will generate 1.87 tonnes of CO2 (Wang et al., 2019).

Globally, nitrogen-containing fertilizers are only one nutrient (nitrogen-only) or multinutrient (containing nitrogen and other nutrients) as can be seen in Table 1(Fertilisers in the EU Prices, trade and use, 2019).

Table 1

The main fertilizers with a high nitrogen content

Fertiliser / Abbreviation	Nutrient content
Ammonium Nitrate / AN	33.5 % Nitrogen
Calcium Ammonium Nitrate / CAN	27 % Nitrogen
Ammonium Nitro Sulphate / ANS	26 % Nitrogen 14 % Sulphur
Calcium Nitrate / CN	15.5 % Nitrogen
Ammonium Sulphate / AS	21 % Nitrogen 24 % Sulphur
Monoammonium Phosphates / MAP	11 % Nitrogen, 52 % Phosphorus
Diammonium Phosphates / DAP	18 % Nitrogen 46 % Phosphorus
Urea / Urea	46 % Nitrogen
Urea Ammonium Nitrate (liquid) / UAN	30 % Nitrogen
NPK 15-15-15 / NPK	15 % Nitrogen 15 % Phosphorus 15 % Potassium

Protein hydrolysates (PHs) are an important category of biostimulants which are produced by thermal, enzymatic or chemical (with strong acid or alkali) hydrolysis or a combination thereof. Chemical hydrolysis of proteins under acid or alkaline conditions is usually preferred for producing animal-based PHs (Niculescu et al., 2009; Corte et al., 2014; Colla et al., 2015; Nardi et al., 2016; Ertani et al., 2017; Rouphael et al., 2017a, 2017b).

Protein hydrolysates, depending on the source and method of production, have an average nitrogen content ranging between 10 and 29.9% N (Corte et al., 2014).

Protein hydrolysates (PHs) can be obtained from sources of vegetable origin (seeds, alfalfa hay, vegetable by-products) or animal (skin by-products, blood meal, fish by-products, chicken feathers, casein). The rate of foliar N absorption increased as the molecular weights of amino acids decreased (Colla et al., 2015).

In Romania, certified agricultural areas or those in conversion to organic farming have increased in the last 5 years, which has led to an increase in demand for inputs, which also means for fertilizers and plant protection products. (https://www.madr.ro/docs/agricultura/agricultura-ecologica).

Under EU law, only protein hydrolysates of animal origin have application restrictions on edible parts of crops (Commission Regulation (EC) No 889/2008).

Protein hydrolysates together with algae, microorganism and humic and fulvic acids make up the category of biostimulators(Colla et al., 2015; Du Jardin et al., 2015; Nardi et al., 2016; Koleška et al., 2017; Rouphael et al., 2017a, 2017b).

The application of protein hydrolysates can be done directly on the soil (Hartz and Johnstone, 2006; Corte et al., 2014) but spraying the foliar part of the plant is preferred by farmers because the plants are able to absorb both L- and D-amino acids (Corte et al., 2014).

Peptides and amino acids in PHs form complex combinations with micronutrients (Cu, Fe, Mn and Zn), thus contributing to increasing their bioavailability for the plant (Du Jardin et al., 2015).

The study of the effects of vegetable (V-PH) versus animal (A-PH) protein hydrolyzate on the morphophysiological and metabolic characteristics of sweet basil (*Ocimumbasilicum* L.) showed different effects at the same dose compared to equivalent rates of N. Unlike A-PH, vegetable-derived PH increased photosynthesis and color status, ion content, yield, and quality in basil plants (Rouphael et al., 2021).

Due to the food crisis, fish consumption is expected to increase, and bioactive peptides derived from fish have great market potential, especially in the food industry but can also be used in agriculture in plant biostimulant products. High value of protein content was found in the skin of salmon (S. salar) (89.53%), while others ranged from 8.52 to 65.82% (FAO, 2016; Ishak and Sarbon, 2018).

The fish and chicken industry generates waste and by-products that contain a large amount of protein-rich materials that can be turned into fertilizers (Chalamaiah et al., 2012; Bhari et al., 2021).

There are studies that show the positive effect of foliar application of protein hydrolysates of fish waste on the growth of Basellaalba (Ranasingheet al., 2021).

It is known that nitrogen plays an important role in plant growth and primary production, fundamentally affecting the structure and functions of most ecosystems (Robertson and Vitousek, 2009; Rütting et al., 2018).

There are numerous studies that prove that PHs stimulate metabolism and nutrient assimilation (Ertani et al., 2009; Baglieri et al., 2014; Calvo et al., 2014).Plant protein hydrolysates contain soluble carbohydrates and phenols, which play an important role in energy metabolism and defense against oxidative stress (Colla et al., 2015;Parađiković et al., 2019)

Protein hydrolyzatebiostimulators are products that have proven effective in some horticultural crops with benefits on growth, yield, product quality, resource efficiency and tolerance to various sources of stress(Colla et al., 2015; Parađiković et al., 2019).From this product category, pepper plants treated with Radifarm® and Megafol® showed a higher antioxidant activity and fruit yield increased by up to 55%, and the incidence of non-marketable fruits decreased (Parađiković et al., 2010; Tkalec et al., 2010).Positive effects on metabolic profiling have also been observed and crop performance of lettuce grown under saline conditions (Lucini et al., 2015; Rouphael et al., 2017a).

Foliar applications of PHs to tomatoes can lead to growth, mineral composition of leaves, yield and fruit quality(Colla et al., 2017; Rouphael et al., 2017b; Paul et al., 2019)

Depending on their composition and expected results, biostimulators with PHs can be applied to soil or leaves and can also be applied as seed treatments, especially for field crops such as wheat, maize and soybeans (Rouphael et al., 2018)

Most studies on the application of products based on PHs are foliar. Only about 5% of HPs are directly absorbed by roots, with the rest being primarily metabolized by soil micro-organisms and then made available to plants (Schiavon et al., 2008).

CONCLUSIONS

Although there are many studies showing the beneficial effects of the use of protein hydrolysates on cultures, the mechanisms of action to better control the parameters that determine the variability of responses are not fully understood.

The products based on PHs can be used in organic agriculture according to the European legislation in force.

It is recognized that PHs products are applied to plants in order to increase plant resistance and also to improve nutrient absorption and translocation.

Agriculture is one of the sectors that contributes greatly to the world's greenhouse gas emissions. The use of PHs obtained from the recycling of by-products from agricultural and industrial activities allows the return to the production chain. It can be beneficial both economically and ecologically and is part of the concept of circular economy.

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BIBLIOGRAPHY

1. Baglieri, A., Cadili, V., Mozzetti Monterumici, C., Gennari, M., Tabasso, S., Montoneri, E., Nardi, S., Negre, M., 2014. *Fertilization of bean plants with tomato plants hydrolysates. Effect on biomass production, chlorophyll content and N assimilation*. Sci. Hortic. 176, 194–199.
2. Bhari, R., Kaur, M. and Singh, R.S., 2021. *Chicken feather waste hydrolysate as a superior biofertilizer in agroindustry*. Current Microbiology, pp.1-19.
3. Calvo, P., Nelson, L., Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. Plant Soil 383, 3–41.
4. Chalamaiah, M., Hemalatha, R. and Jyothirmayi, T., 2012. *Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review*. Food chemistry, 135(4), pp.3020-3038.
5. Colla, G., Cardarelli, M., Bonini, P., and Rouphael, Y. 2017. *Foliar applications of protein hydrolysate, plant and seaweed extracts increase yield but differentially modulate fruit quality of greenhouse tomato*. HortScience 52, 1214–1220. doi: 10.21273/hortsci12200-17.
6. Colla, G., Nardi, S., Cardarelli, M., Ertani, A., Lucini, L., Canaguier, R. and Rouphael, Y., 2015. *Protein hydrolysates as biostimulants in horticulture*. Scientia Horticulturae, 196, pp.28-38.
7. Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control.

8. Corte, L., Dell'Abate, M.T., Magini, A., Migliore, M., Felici, B., Roscini, L., Sardella, R., Tancini, B., Emiliani, C., Cardinali, G. and Benedetti, A., 2014. *Assessment of safety and efficiency of nitrogen organic fertilizers from animal-based protein hydrolysates—a laboratory multidisciplinary approach*. Journal of the Science of Food and Agriculture, 94(2), pp.235-245.

9. 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.

10. Ertani, A., Cavani, L., Pizzeghello, D., Brandellero, E., Altissimo, A., Ciavatta, C., Nardi, S., 2009. *Biostimulant activities of two protein hydrolysates on the growth and nitrogen metabolism in maize seedlings*. J. Plant. Nutr. Soil. Sci. 172, 237–244.

11. Ertani, A., Schiavon, M., and Nardi, S. 2017. *Transcriptome-wide identification of differentially expressed genes in Solanum Lycopersicon L. in response to an alfalfa-protein hydrolysate using microarrays*. Front. Plant Sci. 8:1159. doi: 10.3389/fpls.2017.01159.

12. FAO, 2016. Global aquaculture production (fish stat).http://www.fao.org/3/a-i5555e.pdf.

13. Fertilisers in the EU Prices, trade and use, no 15, June 2019, European Commission.

14. Hartz, T.K. and Johnstone, P.R., 2006. *Nitrogen availability from high – nitrogen-containing organic fertilizers*. HortTechnology, 16(1), pp.39-42.

15. Herms D.A., Mattson W.J., 1992. The dilemma of plants: to grow or defend. The Quarterly Review of Biology 67: 283–335.

16. Ishak, N.H. and Sarbon, N.M., 2018. *A review of protein hydrolysates and bioactive peptides deriving from wastes generated by fish processing*. Food and Bioprocess Technology, 11(1), pp.2-16.

17. Jiao, F., Wu, J., Yu, L. and Zhai, R., 2013. *15 N tracer technique analysis of the absorption and utilisation of nitrogen fertiliser by potatoes*. Nutrient cycling in agroecosystems, 95(3), pp.345-351.

18. Koleška, I., Hasanagić, D., Todorović, V., Murtić, S., Klokić, I., Parađiković, N. and Kukavica, B., 2017. *Biostimulant prevents yield loss and reduces oxidative damage in tomato plants grown on reduced NPK nutrition*. Journal of Plant Interactions, 12(1), pp.209-218.

19. Lucini, L., Rouphael, Y., Cardarelli, M., Canaguier, R., Kumar, P., and Colla, G. 2015.*The effect of a plant-derived protein*

hydrolysate on metabolic profiling and crop performance of lettuce grown under saline conditions. Sci. Hortic. 182, 124–133. doi: 10.1016/j.scienta.2014.11.022.

20. Nardi, S., Pizzeghello, D., Schiavon, M., & Ertani, A. 2016. *Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism.* Science in Agriculture, 73, 18–23. https://doi. org/10.1590/0103-9016-2015-0006.

21. Niculescu, M., Bajenaru, S., Gaidau, C., Simion, D., and Filipescu, L. 2009. *Extraction of the protein components as amino-acids hydrolysates from chrome leather wastes through hydrolytic processes*. Rev. Chim. 60, 1070–1078.

22. Parađiković, N., Teklić, T., Zeljković, S., Lisjak, M. and Špoljarević, M., 2019. *Biostimulants research in some horticultural plant species—A review*. Food and Energy Security, 8(2), p.e00162.

23. Parađiković, N., Vinković, T., Vinković Vrček, I., Teklić, T., Lončarić, R., & Baličević, R. 2010. *Antioxidative activity and BER appearance in pepper fruits under influence of biostimulant treatment and hybrid*. Agriculture 16, 20–24.

24. Paul, K., Sorrentino, M., Lucini, L., Rouphael, Y., Cardarelli, M., Bonini, P., Reynaud, H., Canaguier, R., Trtílek, M., Panzarová, K. and Colla, G., 2019. *Understanding the biostimulant action of vegetal-derived protein hydrolysates by high-throughput plant phenotyping and metabolomics: A case study on tomato*. Frontiers in plant science, 10, p.47.

25. Ranasinghe, R.H.A.A., Kannagara, B.T.S.D.P. and Ratnayake, R.M.C.S., 2021. *Hydrolysis of fish waste using fruit wastes of Ananas comosus and Carica papaya for the formulation of liquid fertilizers*. International journal of recycling organic waste in agriculture, 10(2), pp.129-143.

26. Robertson, G.P. and Vitousek, P.M., 2009. *Nitrogen in agriculture: balancing the cost of an essential resource*. Annual review of environment and resources, 34, pp.97-125.

27. Rouphael, Y., Cardarelli, M., Bonini, P., and Colla, G. 2017a. *Synergistic action of a microbial based biostimulant and a plant-*derived protein hydrolysate enhances lettuce tolerance to alkalinity and salinity. Front. Plant Sci. 8:131. doi: 10.3389/fpls.2017.00131.

28. Rouphael, Y., Carillo, P., Cristofano, F., Cardarelli, M. and Colla, G., 2021. *Effects of vegetal-versus animal-derived protein*

hydrolysate on sweet basil morpho-physiological and metabolic traits. Scientia Horticulturae, 284, p.110123.

29. Rouphael, Y., Colla, G., Giordano, M., El-Nakhel, C., Kyriacou, M. C., and De Pascale, S. 2017b. *Foliar applications of a legume-derived protein hydrolysate elicit dose dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars*. Sci. Hort. 226, 353–360. doi: 10.1016/j.scienta.2017.09.007.
30. Rouphael, Y., Spìchal, L., Panzarova, K., Casa, R., and Colla, G. 2018. *High-throughput plant phenotyping for developing novel biostimulants: from lab to field or from field to lab?* Front. Plant Sci. 9:1197. doi: 10.3389/fpls.2018.01197.
31. Rütting, T., Aronsson, H. and Delin, S., 2018. *Efficient use of nitrogen in agriculture*, Nutr Cycl Agroecosyst 110:1–5.
32. Schiavon, M., Ertani, A. and Nardi, S., 2008. *Effects of an alfalfa protein hydrolysate on the gene expression and activity of enzymes of the tricarboxylic acid (TCA) cycle and nitrogen metabolism in Zea mays L.* Journal of agricultural and food chemistry, 56(24), pp.11800-11808.
33. Tkalec, M., Vinković, T., Baličević, R., & Parađiković, N. 2010. *Influence of biostimulants on growth and development of bell pepper (Capsicum annuum L.).* Acta Agriculturae Serbica, 15, 83–88.
34. Wang, Y. and Meyer, T.J., 2019. *A route to renewable energy triggered by the Haber-Bosch process*. Chem, 5(3), pp.496-497.
35. Xu C., Fisher R., Wullschleger S.D., Wilson C.J., Cai M., McDowell N.G.2012. *Toward a Mechanistic Modeling of Nitrogen Limitation on Vegetation Dynamics*. PLoS ONE 7(5): e37914. https://doi.org/10.1371/journal.pone.0037914.
36. Zhang, X., Ward, B.B. and Sigman, D.M., 2020. *Global nitrogen cycle: critical enzymes, organisms, and processes for nitrogen budgets and dynamics*. Chemical Reviews, 120(12), pp.5308-5351.
37. Madjar Roxana, Davidescu Velicica Agrochimie, 2009. http://www.horticultura-bucuresti.ro/images/pdf/Agrochimie.pdf.
38. https://eur-lex.europa.eu/legal-content/RO/TXT/PDF/?uri=CELEX:52020DC0562&from=EN

Brussels, 17.9.2020 COM(2020) 562 final, Stepping up Europe’s 2030 climate ambition Investing in a climate-neutral future for the benefit of our people, Communication from the Commission to the

European Parliament, the Council, the European economic and social committee and the committee of the regions EU.
39. https://population.un.org/wpp/Publications/Files/WPP2019_Highlights.pdf, United Nations, 2019. World Population Prospects 2019 Highlights.
40. https://www.madr.ro/docs/agricultura/agricultura-ecologica/
41. ***Brussels, 19.4.2021 COM(2021) 141 final/2: Communication from the Commission to the European Parliament, the Council, the European economic and social committee and the committee of the regions on an action plan for the development of organic production.