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Protein hydrolysates as potent biostimulants: Reprogramming plant metabolism for enhanced growth and secondary metabolite production

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Abstract

Protein hydrolysates (PHs) have emerged as potent plant biostimulants capable of reprogramming plant metabolism to enhance growth, productivity, and stress resilience. Derived from animal, plant, and microbial protein sources through chemical, enzymatic, thermal, or microbial hydrolysis, PHs contain bioactive peptides and free amino acids that regulate nitrogen and carbon metabolism, hormone-like signaling pathways, reactive oxygen species (ROS) detoxification and epigenetic processes. Their ability to stimulate root development, shoot biomass accumulation, photosynthetic efficiency and the biosynthesis of secondary metabolites, including phenolics, flavonoids, carotenoids, glucosinolates and essential oils, highlights their potential to improve both crop yield and nutritional quality. Furthermore, PHs enhance plant tolerance to abiotic stresses such as salinity, drought and heavy metal toxicity by modulating physiological, biochemical and molecular responses. In line with the goals of sustainable agriculture, PHs reduce dependence on chemical fertilizers, improve nutrient use efficiency and are compatible with organic farming systems. Despite significant progress, challenges related to production standardization, molecular characterization and a comprehensive understanding of their mechanisms of action remain. Addressing these knowledge gaps will be essential for maximizing the agronomic potential and commercial adoption of PH-based biostimulants.

1. Introduction

The sustainability of agricultural production has emerged as a significant global issue owing to escalating environmental degradation, diminishing soil fertility and the overreliance on chemical fertilizers. Although, chemical fertilizers have greatly increased agricultural output, their long-term usage has resulted in nutritional imbalance, poor soil health and environmental degradation (Rouphael and Colla, 2020; du Jardin 2015). These constraints have fueled the search for eco-friendly alternatives that can boost crop performance while staying environmentally responsible.

In this context, plant biostimulants have emerged as promising solutions for increasing plant growth and production by stimulating natural physiological and biochemical processes. Biostimulants are chemicals or microorganisms that promote nutrient usage efficiency, tolerance to stress and crop quality despite their nutrient content. They constitute a burgeoning area of agricultural innovation (du Jardin, 2015). PHs have received a lot of interest because of their multiple functions in plant development and metabolism (Colla *et al.*,2015; Rouphael *et al.*, 2017). PHs are complex amino acid and peptide combinations produced by the chemical or enzymatic hydrolysis of protein-rich animal or plant substrates (Colla *et al.,* 2015). In addition to serving as direct nutrient sources, these chemicals function as signaling molecules, affecting essential metabolic processes such as nutrient absorption, enzyme activity and carbon-nitrogen metabolism (Ertani *et al.,* 2009; Colla *et al.,* 2017). Previous study has shown that PH treatment can significantly boost plant growth, yield and tolerance to abiotic stress conditions (Rouphael *et al.,* 2017).

PHs have been proven to enhance the synthesis of secondary metabolites including phenolics and other bioactive compounds, which improves plant quality (Ertani *et al.,* 2009; Colla *et al.,* 2015). Secondary metabolites are critical for plant defense, response to environmental stress and ecological interactions, as well as having antioxidant and bioactive characteristics (Isah, 2019). Despite an increasing amount of research, the molecular mechanisms underlying the involvement of protein hydrolysates in reprogramming plant metabolism and increasing secondary metabolite synthesis are not yet fully understood. Therefore, this review aims to synthesize current knowledge on protein hydrolysates as potent biostimulants, with particular emphasis on their role in regulating plant metabolic pathways and promoting secondary metabolite biosynthesis.

2. Protein hydrolysates: Sources, production and composition

Protein hydrolysates (PHs) have emerged as a key category of plant biostimulants, characterized by their complex composition and multifaceted biological activity. They are typically formed by the breakdown of protein rich materials into combinations of amino

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acids and bioactive peptides that can influence plant development, metabolism, and responses to stress (Dhanasekaran *et al.,* 2025; Malecange *et al.,* 2023). Their increasing use is closely linked to the need for sustainable agricultural inputs that improve plant performance while reducing dependency on synthetic agrochemicals (Paskovic *et al.,* 2024).

2.1 Sources of protein hydrolysates

Protein hydrolysates originate from a wide range of biological materials, broadly categorized into plant, animal and microbial sources. The raw material used has a significant impact on the amino acid content and biological activity of the finished product. Figure 1 shows numerous sources of protein hydrolysates.

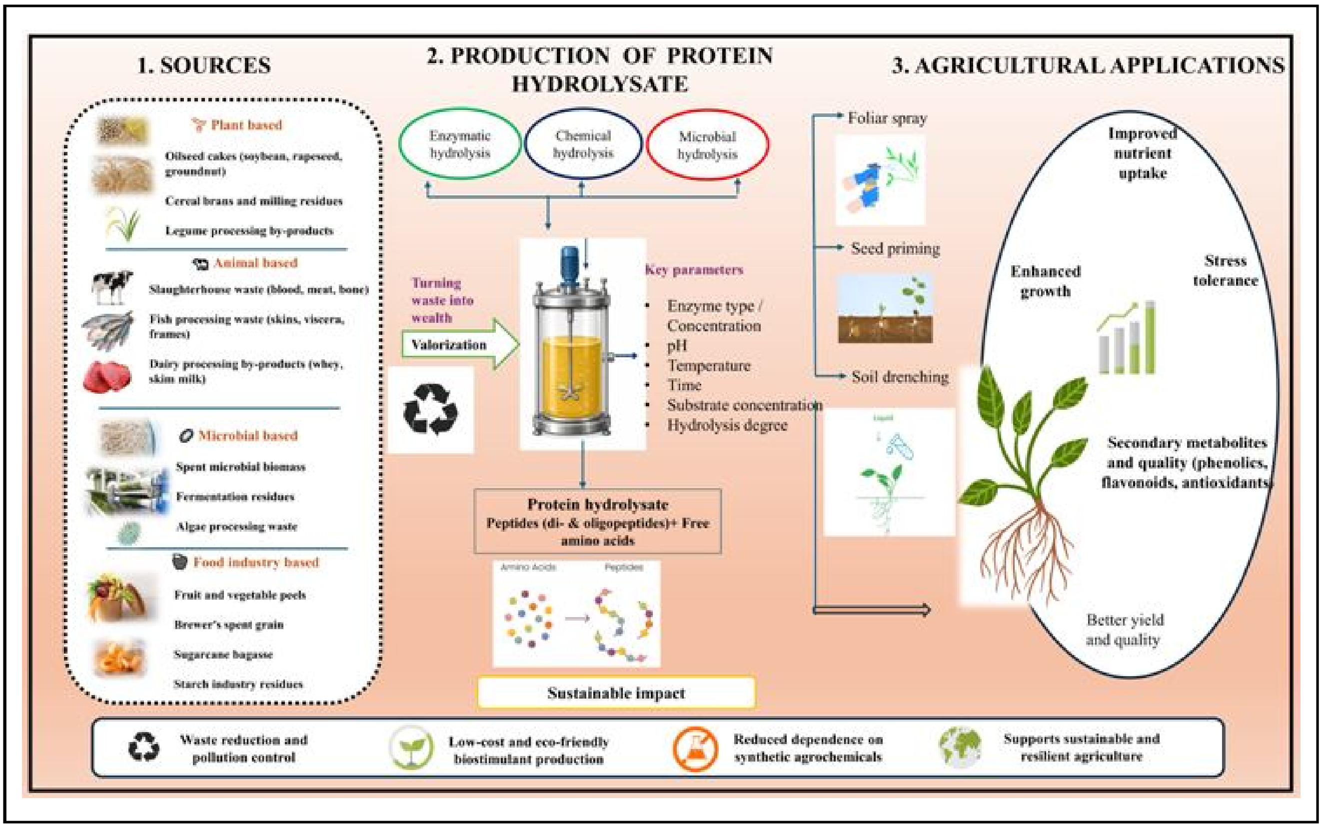

Figure 1: Valorization of agro-industrial byproducts into protein hydrolysate biostimulant.

2.1.1 Plant-based sources

Plant-based PHs are typically produced from crops including soybeans, alfalfa, and other agricultural by-products. These amino acid-rich sources are sustainable, renewable, and compatible with eco-friendly farming techniques (Paskovic *et al.,* 2024; Sytar *et al.,* 2024). Recent research has shown that plant-based hydrolysates have great biostimulant action due to their balanced peptide composition and low environmental impact (Bulgari *et al.,* 2019).

2.1.2 Animal-based sources

Animal-derived protein hydrolysates are typically made from collagen-containing materials like leather waste, fish by-products and other protein-rich residues. These hydrolysates usually contain amino acids such as proline, arginine, and glycine, that are essential for plant metabolism and stress physiology (Perez-Aguilar *et al.,* 2024). Their manufacture contributes to the circular economy by valuing industrial byproducts (Paskovic *et al.,* 2024).

2.1.3 Microbial sources

Microbial PHs are gaining popularity as an alternative source. These originate through fermentation techniques with bacteria or fungi, resulting in the synthesis of bioactive peptides with signaling capabilities (Lu *et al.,* 2024; Sun *et al.,* 2024). Microbial sources include advantages such as controlled production conditions, uniform quality and scalability, making them interesting candidates for next-generation biostimulants (Lu *et al.,* 2024).

2.2 Production methods

The method of hydrolysis significantly influences the structure, composition and biological activity of protein hydrolysates.

2.2.1 Enzymatic hydrolysis (preferred method)

Enzymatic hydrolysis involves the use of proteolytic enzymes to break down proteins into smaller peptides and free amino acids under controlled conditions. This approach is commonly accepted because it preserves amino acid integrity while producing highly bioactive peptides with specific functional roles in plant metabolism (Dhanasekaran *et al.,* 2025). Furthermore, enzymatically synthesized hydrolysates have been found to improve plant physiological responses and metabolic activity more than chemically manufactured counterparts (Leporino *et al.,* 2024).

2.2.2 Chemical hydrolysis

Chemical hydrolysis employs acids or alkalis to break down proteins. Although this method is faster and economically viable, it is less selective and may lead to degradation of essential amino acids, thereby reducing the functional efficiency of the hydrolysate (Paskovic *et al.,* 2024). As a result, its use is gradually being replaced by enzymatic approaches in modern biostimulant production systems. The major difference between enzymatic and chemical hydrolysis were discussed in Table 1.

Table 1: Difference between chemical and enzymatic hydrolysis (Malecange *et al.*, 2023)

Feature	Chemical hydrolysis	Enzymatic hydrolysis
Main products	Free amino acids	Peptides + amino acids
Tryptophan	Destroyed	Preserved
Energy efficiency	Lower	Higher
Bioactive peptides	Few	Rich and diverse
Typical source	Animal-derived (collagen, blood)	Plant-derived (legume, corn gluten)
Phytotoxicity risk	Higher (AA imbalance)	Lower

2.3 Composition of PHs

The chemical composition of PHs, which is mostly composed of amino acids, peptides, and other nitrogen-containing substances, greatly influences their biological activity.

2.3.1 Amino acids

Free amino acids serve as readily available nitrogen sources and are directly involved in plant metabolic processes. They function as precursors for various biosynthetic pathways, including those responsible for secondary metabolites production such as phenolics and flavonoids (Sun *et al.,* 2024).

2.3.2 Peptides

Short-chain peptides are key functional components of protein hydrolysates and are increasingly recognized for their role as signaling molecules. They regulate gene expression, enzyme activity and nutrient uptake, thereby contributing to metabolic reprogramming and enhanced plant performance (Dhanasekaran *et al.,* 2025).

2.3.3 Organic nitrogen forms

The combined pool of amino acids and peptides in PHs constitutes readily assimilable organic nitrogen, which enhances nitrogen uptake efficiency and metabolic activity in plants (Rouphael and Colla, 2020).

Table 2: Physicochemical characterization of protein hydrolysates

Parameter	Description	Reported values	Key significance	References
Molecular weight distribution	range of peptides Size and oligopeptides	MW range: 75 Da (free Gly) to ~6,500 Da; four distinct FPLC peaks; smaller fractions (<1 k Da) most bioactive; native collagen 285-300 k Da reduced to 3-6 k Da hydrolysate	Low-molecular-weight peptides (<1 k Da) exhibit stronger metabolic and signaling activity	Ambrosini *et al.,* 2022
Degree of hydrolysis (DH)	Extent of protein breakdown into peptides/amino acids	DH up to 20% (enzymatic); chemical hydrolysis yields higher DH with more free AAs; enzymatic preserves broader AA diversity including tryptophan	Optimal DH enhances bioactivity; excessive hydrolysis may destroy functional peptides	Islam *et al.,* 2022
Amino acid (AA) profile	Relative abundance of amino acids AA composition from soybean enzymatic hydrolysis	Total AA content >41%; Protamex: Leucine (Leu) 1.15%, isoleucine (Ile) 0.31%, valine (Val) 0.34%; Alcalase: Leu 0.96%, Ile 0.44%, Val 0.46%; DH up to 20%	Amino acids involved in biosynthesis process	Islam *et al.,* 2022
Particle size	Colloidal particle dimensions	Potato PH: <50 nm after hydrolysis; Gelatin -stabilized PH: 179 nm particle size	Smaller particle size enhances solubility, stability and bioavailability of protein hydrolysates.	Chang *et al.,* 2020; Obeidnejad *et al.,* 2024
Solubility	Ability to dissolve in aqueous systems	Potato PH: >70% solubility at pH 3-6; lowest near isoelectric point (pH 4.5-6.5); extensively hydrolyzed fish PH: 74-99% solubility index; INSECT PH: highest solubility among animal PHs	Higher solubility improves nutrient transport and application efficiency	Chang *et al.,* 2020
Electrical conductivity (EC)	Concentration of soluble ions	Gelatin-stabilized PH: 1,197 µS/cm	Excessively high EC may induce osmotic imbalance	Obeidnejad *et al.,* 2024

Zeta potential	Surface charge and colloidal stability	Potato PH: 7.4 to 21.6 mV (pH-dependent decrease with increasing pH); Gelatin PH: –48 mV (stable colloidal system)	Stable zeta potential enhances formulation stability	Chang *et al.,* 2020; Obeidnejad *et al.,* 2024
Antioxidant activity-DPPH	Free radical scavenging (DPPH assay)	Soybean protamex PH: 62.07% DPPH scavenging; potato PPIH EC_{50}: 0.89 mg/ml vs. PPI >2.33 mg/ml; FISH and INSECT PHs strongest among animal PHs	indicates free radical scavenging potential of bioactive peptides and amino acids present in PHs	Islam *et al.,* 2022; Chang *et al.,* 2020 and Monteiro *et al.,* 2025
Viscosity	Flow resistance; inversely related to DH	Native collagen: highly viscous (triple-helix, strong electrostatic repulsion); Gelatin PH: 4.92 mPa·s; viscosity decreases with increasing DH	Lower viscosity improves solubility, sprayability and foliar absorption of protein hydrolysates.	Obeidnejad *et al.,* 2024
Emulsifying activity	Capacity to form stable oil-water emulsions	Fish PH: 61.11-108.90 m²/g; Soybean Protamex PH: 95.03 m²/g; Gelatin PH: 68.54%; emulsification improves with increasing DH	Protein hydrolysates improve emulsion formation and stability due to the active surface properties of peptides	Sharma *et al.,* 2024; Islam *et al.,* 2022 and Obeidnejad *et al.,* 2024

3. Physicochemical characterization of protein hydrolysates

The physicochemical properties of PHs are the main determinants for their biological activity, stability and efficiency as plant biostimulants. PTMs (Proteinaceous materials) are elaborate blends of peptides, amino acids and different natural atoms because of protein hydrolysis; therefore, these require in-depth characterization to determine their connection with plant metabolic frameworks. Variations in peptide size, amino acid composition, degree of hydrolysis, solubility, and charge distribution significantly influence cellular signalling potential and metabolic responses, indicating that the structural characteristics of protein hydrolysates are critical determinants of their biological activity (Tambone *et al.,* 2021). Table 2 briefly discusses the physicochemical characterization of protein hydrolysates.

4. Biostimulant nature of protein hydrolysates

4.1 Protein hydrolysates are considered biostimulants

Protein hydrolysates (PHs) are classified as biostimulants since their major role is regulatory rather than nutritive. PHs are essential for controlling the metabolic, biochemical, and physiological processes of plants (Paskovic *et al.,* 2024; Dhanasekaran *et al.,* 2025). Upon application, bioactive peptides and amino acids enhance food uptake, photosynthetic efficiency, and total metabolic activity by interacting with plant signaling networks (Sun *et al.,* 2024). Additionally, these substances regulate the expression of genes related to stress and growth response and boost enzyme activity. As a result, PHs affect both primary metabolism involved in plant development and secondary metabolism, which is responsible for the production of phenolics, flavonoids and other bioactive metabolites (Malecange *et al.,* 2023). This extensive regulatory influence on plant metabolism clearly distinguishes protein hydrolysates from conventional fertilizers, which primarily function as nutrient providers.

4.2 Properties of protein hydrolysates as biostimulants

4.2.1 Signal molecules

Protein hydrolysates (PHs) have the ability to behave as signaling molecules. Low-molecular-weight peptides found in PHs interact with plant signaling networks, regulating gene expression involved in growth, nutrition absorption and stress-responsive pathways (Sun *et al.,* 2024). These signaling pathways stimulate physiological functions such as root development, metabolic regulation, and nutrient transport, enabling plants to react swiftly to fluctuating environmental circumstances.

4.2.2 Metabolic activators

Protein hydrolysates operate as metabolic activators in plants, stimulating critical biochemical and physiological processes. PH treatment increases enzyme activity, improves photosynthetic performance and improves nutrient assimilation, Promoting plant growth and production. (Gendaszewska *et al.,* 2025; Dhanasekaran *et al.,* 2025). In addition, amino acids supplied by PHs serve as metabolic precursors for different biosynthetic pathways, including those involved in secondary metabolite synthesis.

4.2.3 Stress modulators

Protein hydrolysates also function as stress modulators, boosting plant resilience to abiotic factors like heat and salinity. Recent study has revealed that PH application boosts antioxidant defense mechanisms, stabilizes cellular structures and promotes osmotic adjustment by accumulating compatible solutes (Leporino *et al.,* 2024; Jiang *et al.,* 2025). These defensive systems aid in the maintenance of cellular homeostasis, metabolic activity and plant development in critical environmental conditions.

5. Mechanisms of action of protein hydrolysates

Protein hydrolysates (PHs) have biostimulant effect due to a complex network of interrelated molecular, physiological and ecological processes rather than a single linear mechanism. Agronomically, PHs enhance crop productivity primarily by improving carbon, nitrogen, and sulfur uptake and assimilation. This stimulates photosynthesis and promotes the buildup of free carbohydrates, free proteins, amino acids, phenolic compounds, pigments and enzymatic antioxidants. These coordinated reactions help to promote plant growth, metabolic efficiency, and stress resilience. The main molecular pathways driving PH-mediated biostimulant effects are summarized in the following subsections, which are backed by data from multiple independent investigations (Dhanasekaran *et al.,* 2025).

5.1 Absorption and translocation of bioactive constituents

Explaining the physiological and biostimulant effects of protein hydrolysates (PHs) in plants requires an understanding of their absorption and translocation. While drench-applied PHs are absorbed by root epidermal cells and then transported through the xylem, foliar-applied PHs are absorbed through the stomata, epidermal cells and cuticle before reaching mesophyll tissues (Paul *et al.,* 2019). After being absorbed, amino acids and peptides can be stored, processed, or redistributed in shoots and other nitrogen sinks after being either directly digested or transferred via the circulatory system. The effectiveness of foliar nitrogen uptake can be further increased by the combination of amino acids and other nitrogen sources. Additionally, PHs offer easily accessible amino acids and peptides that assist cell development, metabolism and cellular defense by serving as direct sources of nitrogen and metabolic precursors for the production of complex macromolecules (Colla *et al.,* 2017).

5.2 Modulation of photosynthesis and gas exchange

PHs exert measurable effects on photosynthetic apparatus and gas exchange physiology. Using an animal-based PHs biostimulant as a foliar spray significantly improved net photosynthesis (D0.1: 22.9 μ mol CO_2 m^{-2} s^{-1} and D0.2: 22.4 μ mol CO_2 m^{-2} s^{-1}) and stomatal conductance (D0.1: 0.42 m mol H, O m^{-2} s^{-1} and D0.2: 0.39 m mol H_2O m^{-1}).

Glutamate enhances electron transfer in Photosystem II (PSII) and serves as a precursor for γ-aminobutyric acid (GABA) and proline, which are linked to plant stress tolerance at the amino acid level. Protein hydrolysate-based biostimulants have also been shown to enhance chlorophyll (a + b) content by 38-41% compared to control wheat plants, indicating improved photosynthetic efficiency (Henderson *et al.,* 2025; Gendaszewska *et al.,* 2025). In drought stress, the treatment with PH significantly increased stomatal conductance, transpiration use efficiency and leaf water potential, which indicated plant water status and water deficit tolerance. Metabolomic analyses also showed that the PH-treated plants had unique biochemical responses, such as lower cytokinin concentrations and higher salicylate, membrane lipid, sterol and terpene levels, which are related to stress resistance (Francesca *et al.,* 2021).

5.3 Hormone like activity

One of the most well-studied mechanisms of protein hydrolysate (PH) action is their ability to imitate or promote endogenous phytohormone signaling via bioactive peptides. Several commercial PH compounds include auxin- and gibberellin-like actions, which promote root growth, shoot development and agricultural productivity (Colla *et al.,* 2017). In Maize, PH treatment boosted coleoptile elongation rates in a dose-dependent manner. However, no significant differences were found between PH dosages of 0.75, 1.5, and 3.0 ml L^{-1} and IAA therapy. Similar findings were found in tomato cuttings, where PH treatment increased root length, root dry weight, shoot dry weight and root area by 24%, 35%, 21%, and 26%, respectively, in comparison with plants that were not treated (Colla *et al.,* 2015). Beyond auxins and gibberellins, PHs modulate broader phytohormonal interactions, particularly the cytokinin-auxin balance involved in root development, while also inducing a decrease in brassinosteroids, influencing plant growth regulation and adaptive responses (Ceccarelli *et al.,* 2021).

5.4 Antioxidant defense and ROS modulation

Protein hydrolysates (PHs) help plants resist oxidative stress by boosting their antioxidant defense systems. Their use enhances tolerance to abiotic stressors by stimulating protective mechanisms such as antioxidant activity and osmotic adjustment (Malecange *et al.,* 2023). ROS produced in the apoplast, mitochondria and chloroplasts are essential signaling chemicals that interact with auxin, ethylene, abscisic acid, jasmonate and cytokinin pathways through shared regulatory components like redox-sensitive transcription factors and NADPH oxidases. This network incorporates endogenous metabolites like carotenoids, glutathione, phenolics, alkaloids, terpenoids, polyamines and signaling peptides. Bioactive peptides and free amino acids contained in PHs contribute to this mechanism by changing the amplitude of ROS signaling rather than acting as direct antioxidants (Minkov, 2026). Under drought stress, pig blood-derived PH dramatically reduced oxidative damage by strengthening antioxidant defense mechanisms. Foliar application reduced stress caused by drought by modulating stomatal apertures, preserving chloroplast morphology, changing osmotic conditions, and activating antioxidant mechanisms, so enhancing overall stress tolerance (Wang *et al.,* 2022).

5.5 Osmotic adjustment and ion homeostasis

PHs enhance abiotic stress tolerance by controlling osmotic balance and ionic equilibrium. PHs trigger or improve abiotic stress defense mechanisms such as ion transport and homeostasis control, osmotic adjustment via osmolyte formation, and the stimulation of enzymatic and non-enzymatic antioxidant systems to combat secondary oxidative stress. Many PHs contain amino acids such glycine betaine and proline, which operate as compatible solutes to keep cellular structures stable amid water scarcity and salt stress (Rosas *et al.,* 2024).

5.6 Root morphogenesis and nutrient uptake

PHs have a substantial effect on root architecture, with treated plants frequently showing a significant increase in root hair length and root density, implying that these compounds activate a nutrient acquisition response that promotes nutrient uptake by expanding the absorptive surface available for water and mineral absorption (Costa, 2024; Santi *et al*., 2024). Gene expression research shows that application of PHs activates key amino acid transporters as well as nitrogen transporters (NRT2.1, NRT2.3) in roots and leaves. Nitrogen assimilation enzymes such as glutamine synthetase and nitrate reductase also show increased activity following application. Furthermore, peptide biostimulants can enhance micronutrient availability through chelation effects (Sestili *et al.,* 2018; Colla *et al.,* 2017).

5.7 Phenylpropanoid pathway activation and carbon flux regulation

The activation of the phenylpropanoid pathway is a key mechanism through which protein hydrolysates (PHs) regulate secondary metabolism. By enhancing the expression and activity of phenylalanine ammonia-lyase (PAL) and downstream enzymes such as cinnamate 4-hydroxylase (C4H) and chalcone synthase (CHS), PHs promote carbon flux toward the synthesis of phenolics, flavonoids, and lignin (Sestili *et al.,* 2018; Di Mola *et al.,* 2020). Overall, PH-mediated regulation of the phenylpropanoid pathway strengthens plant defense, stress tolerance, and nutritional quality by directing metabolic resources toward the production of bioactive secondary metabolites.

5.8 Indirect effects *via* the soil microbiome

Protein hydrolysates (PHs) have significant indirect effects on the plant-associated microbiota in addition to their direct physiological effects. Beneficial microbes, which increase plant fitness by improving nutrient and water intake, controlling physiological and develop mental processes and boosting resilience to external challenges, partially mediate the biostimulant effects of PHs (Colla *et al.,* 2017). PHs change the microbial populations in the phyllosphere and rhizosphere when they are applied *via* foliar spraying, drip irrigation, root drenching or root dipping. In addition to increasing nutritional availability, these stimulated microbes also release enzymes that split down peptides into smaller bioactive fragments that serve as signaling molecules to further support plant growth and development (Malecange *et al.,* 2023). The abundance of advantageous microbial taxa including *Burkholderia, Pseudomonas, Rhodanobacter* and *Trichoderma* in diverse soils and growth mediums further supports PH's ability to modify the microbiome. By improving nutrient mineralization and phytohormone synthesis, such microbial stimulation can indirectly increase plant biomass output and nutrient uptake (Costa *et al.,* 2024).

5.9 Transcriptomic and metabolomic reprogramming

Omics-based research has revealed new insights into the complex molecular pathways that underpin protein hydrolysate -mediated plant responses. Transcriptomic and proteomic analyses reveal that PHs effects are governed by interconnected pathways such as hormonal-type activity, osmotic regulation, antioxidant-mediated defense and gene expression modulation, all of which lead to better growth of plants and tolerance to stress (Dhanasekaran *et al.,* 2025). PHs application has been shown to influence complex signaling networks involving ethylene precursors, polyamines and ROS-mediated pathways, resulting in considerable metabolic reprogramming in treated plants. Metabolomic studies also revealed that PHs promote the accumulation of important secondary metabolites like alkaloids, aliphatic glucosinolates and phenylpropanoids, improving plant adaptability and resilience under stress environmental condition (Paul *et al.,* 2019; Ceccarelli *et al.,* 2021). Similarly, collagen-derived PHs (CDPHs) had a varied range of molecular effects, with transcriptomic and proteomic studies revealing variations in the expression of genes involved in nutrient acquisition, oxidative stress responses and phytohormone signaling (Ambrosini *et al.,* 2022).

6. Role of PHs in plant growth and development

6.1 Seed germination and plant establishment

Protein hydrolysate-based seed priming enhances germination and early seedling growth by stimulating reserve mobilization and stress-protective mechanisms. PH priming increases the activity of hydrolytic enzymes such as amylase and sucrose synthase, leading to improved breakdown of stored starch and proteins and higher accumulation of soluble sugars, proteins and free amino acids. Additionally, PHs promote osmotic adjustment through increased proline and soluble solute accumulation while enhancing antioxidant defense systems to reduce reactive oxygen species-induced damage during germination under stress conditions (Santi *et al.,* 2017).

In cucumber (*Cucumis sativus* L.), VPH (vegetal protein hydrolysate) treatments at lower concentrations significantly affected morphological traits in seed germination, with primed seeds showing faster germination, stronger seedlings and higher chlorophyll content even under salt stress conditions compared to water treated controls. Priming duration was discovered to be significant, as germination had a favourable effect by priming durations of 4 and 8 h, however, prolonged exposure to the identical concentration for 12 h hindered the germination process and increased stress severity in seedlings. This dose and duration dependent hormetic response suggests that VPH concentrations above a threshold shift from a biostimulatory to an inhibitory mode, likely through excessive free amino acid uptake and osmotic interference (Saadatian *et al.,* 2025).

6.2 Root architecture and morphogenesis

PHs consistently and measurably enhance root system architecture, which is fundamental to expanding the absorptive surface of the plant for water and nutrients uptake. The performance of PH treated plants has been mostly correlated with changes in root structures, including length, number, and density of lateral roots, along with an increase in available nutrients in the soil solution due to nutrient complexation by peptides and amino acids and increased microbial activity (Sun *et al.,* 2024).

PHs influence root development *via* hormone-like interactions, as revealed by a study in which five separate plant-derived enzymatic PHs increased root length by 45 to 93% and root number by 37 to 56% in tomato cuttings after fast dipping. In the same study, PHs were discovered to influence the phytohormone profile by influencing the complicated interaction between auxins and cytokinins, an interaction that is critical for root development, while also inhibiting the accumulation of brassinosteroids. Furthermore, PHs increased the accumulation of phenylpropanoids, alkaloids and aliphatic glucosinolates potentially stimulating crop resilience, confirming that PHs have hormone-like activity and modulate plant growth by interference with endogenous signaling functions (Ceccarelli *et al.,* 2021).

6.3 Shoot biomass and yield enhancement

PHs boost shoot biomass and crop output by combining nitrogen assimilation stimulation, hormone-like activity and better photosynthetic translocation. PHs may enhance plant development by increasing nitrogen absorption and assimilation. Increased synthesis of carbon skeletons may further accelerate N assimilation in plants. All of these studies suggest that PHs regulate photosynthesis, primary carbon metabolism and nitrogen absorption in a finely regulated manner, contributing to high plant biomass and growth (Colla *et al.,* 2017; Malecange *et al.,* 2023).

In maize and lettuce, increasing the dose of plant-derived PH Trainer® from 0 to 10 ml/l resulted in a significant increase in root, shoot and total dry biomass, SPAD index, and leaf nitrogen content of 27.5%,19.5%, 20.5%, 15.1%, and 21.5%, respectively. In lettuce grown at a low nutrient solution concentration (10% of standard), weekly foliar sprays at 2.5 ml/l enhanced SPAD index, fresh weight biomass and leaf nitrogen by 11%, 50% and 11%, respectively (Planques *et al.,* 2012).

6.4 Photosynthesis and chlorophyll content

PHs improve photosynthetic performance by simultaneously increasing pigment production, stomatal conductance, and electron transport efficiency. Photosynthetic activity responds to PH or amino acid supply through modified changes in cell metabolism.

PHs improve N assimilation and photosynthetic activity, which greatly influence shoot meristematic function (Paskovic *et al.,* 2024; Malecange *et al.,* 2023). In wall rocket, commercial vegetal-sourced PHs increased fresh plant biomass and leaf SPAD values by 10.6% and 25.0%, respectively, compared to the control. The peptides, amino acids, and phytohormone-like compounds present in PHs were responsible for enhanced chlorophyll content and nitrogen-related growth responses (Caruso *et al.,* 2019).

6.5 Flowering and fruit setting

PHs improve flowering and fruit set by enhancing pollen viability, promoting phytohormone-mediated reproductive development, improving nutrient uptake, and mitigating stress-induced damage, thereby increasing fruit formation and yield. PH application mitigated the negative effects of water deficit stress by increasing pollen viability by 51% compared with untreated plants. Biostimulant-treated plants produced 70% more fruits per plant, while the average fruit weight increased by 95% under reduced irrigation conditions. Furthermore, under 50% irrigation, PH treatment resulted in an approximately six-fold increase in final yield compared with untreated controls (Francesca *et al*., 2021).

7. Role of protein hydrolysates in secondary metabolite biosynthesis

Secondary metabolites are crop-specific bioactive chemicals that influence plant nutrition, sensory quality and defense capacity. PHs have repeatedly been shown to increase the accumulation of these chemicals across multiple metabolite groups and crop systems. This section lists, by compound class and crop, the specific secondary metabolites that are created or improved as a direct result of PH application, with a focus on measurable metabolite outputs rather than the underlying mechanisms driving their production. Figure 2 shows the role of protein hydrolysates in secondary metabolite production.

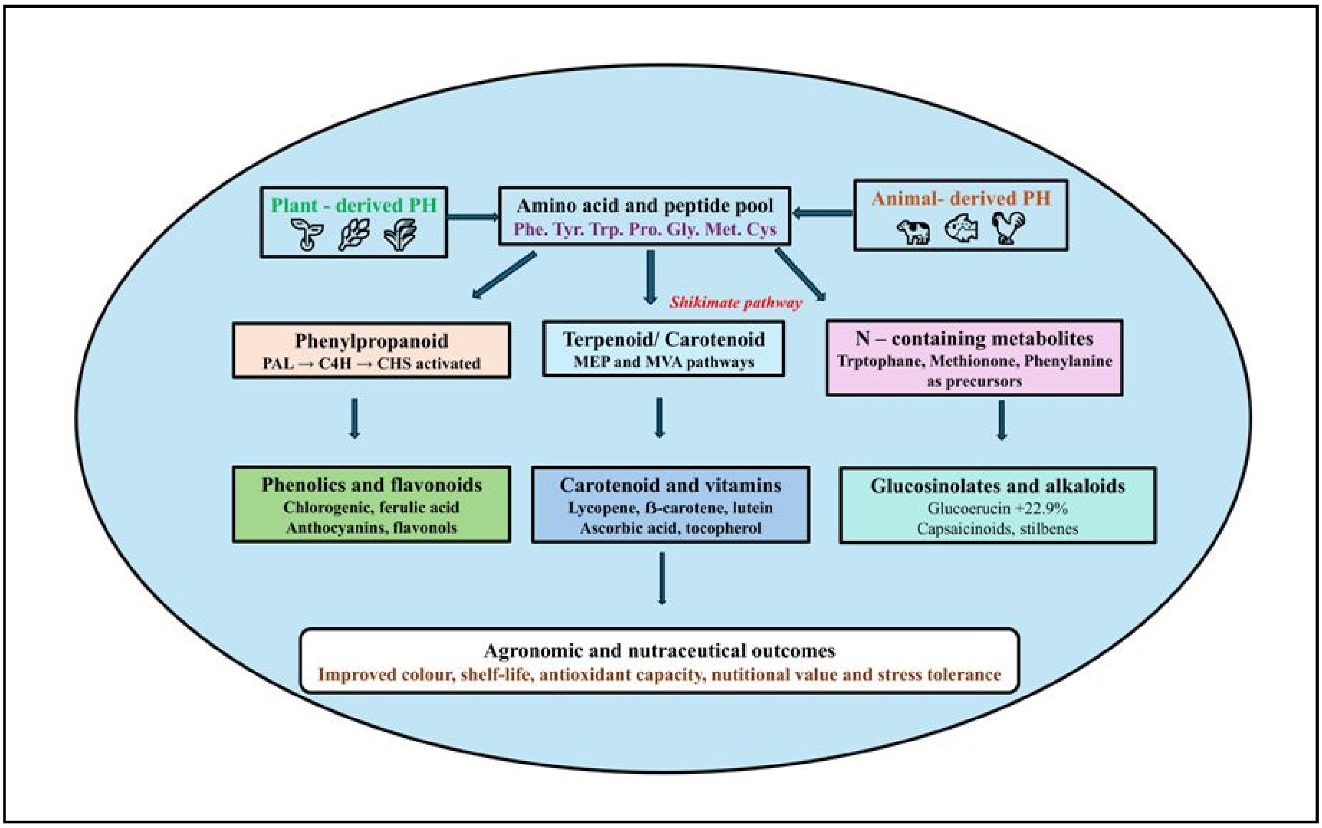

Figure 2: Role of protein hydrolysate in secondary metabolite production.

7.1 Phenolic acids and hydroxycinnamic derivatives

Phenolic acids are the simplest and most extensively distributed family of phenylpropanoid-derived secondary metabolites and applying protein hydrolysate (PH) has repeatedly resulted in detectable increases in these chemicals crosswise a varied range of vegetable crops. In organic arugula (*Diplotaxis tenuifolia*), foliar application of a vegetal-base PHs, as evaluated by UHPLC-Q-Orbitrap HRMS (ultra-high-performance liquid chromatography coupled with quadrupole-orbitrap high-resolution mass spectrometry), increased total phenolic acid and glucosinolates derivatives by 17.2% and 15.2%, respectively. Significant increases in glucocheirolin (76.8%), glucoerucin (22.9%) and ferulic acid (94.1%) were observed, confirming PHs ability to enhance the nutraceutical quality and commercial value of vegetables (Ciriello *et al.,* 2022). Similarly, in *Diplotaxis tenuifolia*, combining a legume-resultant protein hydrolysate with extracts from tropical plants increased leaf dry matter, organic acid composition, ascorbic acid content, mineral accumulation, phenolic concentration and antioxidant activity while not increasing nitrate accumulation. Furthermore, the winter-spring cultivation cycle resulted in stronger antioxidant buildup than winter cultivation alone (Caruso *et al.,* 2019).

In basil (*Ocimum basilicum* L. cv. Genovese) grown on a floating raft system, cichoric acid, total phenols, chlorogenic acid, FRAP (ferric reducing antioxidant power) and DPPH (2,2-Diphenyl-1-picrylhydrazyl) values had strong positive associations with PC1(first principal component), accounting for 36.1% of the cumulative variance among biostimulant-treated plants. These findings suggest that PH treatment results in a coordinated augmentation of the polyphenolic and antioxidant profiles (Ciriello *et al.,* 2022).

7.2 Flavonoids and anthocyanins in fruits and vegetables

Flavonoids, such as flavonols, flavanones and anthocyanins, are among the most commercially relevant secondary metabolites that benefit from protein hydrolysate (PH) application, contributing to better fruit colour, shelf life, antioxidant capacity and nutritional value**.** Treatments with alfalfa-derived protein hydrolysates, seaweed extracts, and B-group vitamins improved the appearance and functionality of "Jonathan" apples by increasing red pigmentation, phenolic substances, anthocyanin synthesis, and antioxidant capacity (Soppelsa *et al.,* 2018). Similarly, in Annurca apple, the PH biostimulant "Sunred" increased secondary metabolite production in fruit skin, boosting anthocyanin concentration, antioxidant activity and soluble solids after harvest and reddening and cold storage (Graziani *et al.,* 2020). Vegetable-derived PHs increased strawberry (*Fragaria* × *ananassa*) fruit firmness, soluble solids, mineral composition and yield. They also stimulated beneficial metabolites linked with fruit quality enhancement (Cardarelli *et al.,* 2024; Ciriello *et al.,* 2025). PH treatment additionally promoted vegetative growth, chlorophyll content, antioxidant compounds, and tissue mineral accumulation by activating microbial activity and key enzymes in primary and secondary metabolism (Mounaimi *et al.,* 2024).

7.3 Carotenoids: Lycopene, β-carotene and xanthophylls

Carotenoids are terpenoid-derived pigments that play a significant role in describing the nutritional and commercial value of horticultural crops and protein hydrolysate (PH) application has consistently increased their concentration. In tomato (*Solanum lycopersicum*), the application of PHs and tropical plant extracts significantly improved ascorbic acid, phenols, antioxidant activity, lycopene content, fruit number and yield in the cultivar 'Pomodorino del Piennolo del Vesuvio PDO' compared with non-treated plants. Similarly, protein gel-based PH treatments increased vitamin C and lycopene contents by 1.29 and 1.44 times, respectively, with the biostimulant effect heavily influenced by amino acid composition, implying that phytochemical enhancement is PH source and composition dependent (Graziani *et al.,* 2020). In lettuce (*Lactuca sativa*), peptide fractions generated from commercial plant PHs increased total ascorbic acid, lutein and β-carotene levels, particularly under low nitrate circumstances. They also enhanced flavonoid molecules including disinapoylgentiobiose and kaempferol derivatives. These results show that various peptide fractions can selectively modulate carotenoid and flavonoid accumulation (Cristofano *et al.,* 2022).

7.4 Glucosinolates and their hydrolysis products in Brassicaceae

Glucosinolates are sulphur-containing, amino acid-derived secondary metabolites found in the Brassicaceae family and their hydrolysis products, such as sulforaphane and erucin, exhibit significant anticancer and chemoprotective activity. PH treatment has been demonstrated to increase glucosinolate accumulation and boost the nutraceutical potential of Brassica crops. The application of a vegetable-derived PH to arugula enhanced overall glucosinolate concentration by 5.2%, with significant increases in glucoerucin (22.9%) and glucocheirolin (76.8%). Ferulic acid also demonstrated a remarkable 94.1% increase, demonstrating that PH therapy preferentially enhances the accumulation of health-promoting bioactive substances with significant nutritional and functional effects (Ciriello *et al.,* 2025).

7.5 Stilbenes and salicylate-derived compounds in grapevine

In grapevine (*Vitis vinifera*), protein hydrolysate (PH) application regulates the biosynthesis of anthocyanins, stilbenes and other phenolic compounds during berry ripening. Maize gluten-derived PH upregulated several transcription factors associated with ripening, including *Vitis vinifera* MYB transcription factor 5B (VviMYB5B) and MYB anthocyanin regulator 2 (MYBA2) involved in anthocyanin biosynthesis and *Vitis vinifera* MYB transcription factor 15 (VviMYB15), a key regulator of stilbene synthesis. PH treatment also enhanced the expression of salicylic acid carboxyl methyltransferase (SAMT), suggesting improved biotic stress tolerance through methyl salicylate (MeSA)-mediated systemic acquired resistance (SAR) and accelerated berry ripening (Peli *et al.,* 2025). Moreover, PHs rich in bioactive peptides and amino acids improved grapevine tolerance to water as well as heat stress, increased chlorophyll content and phenolic derivative accumulation, and modulated defense-associated gene expression, all of which contributed to improved grape quality and stress resilience (Alfonso *et al.,* 2025).

7.6 Capsaicinoids and pungency related compounds in pepper

Capsaicinoids, including capsaicin and dihydrocapsaicin, are alkaloid-like secondary metabolites responsible for the pungency and pharmaceutical importance of Capsicum species. Protein hydrolysate (PH)-based biostimulants have demonstrated excellent results in terms of increasing bioactive chemicals and improving pepper fruit quality. A PH biostimulant produced from wine lees was developed for green pepper (*Capsicum annuum* L.) to boost phenolics, vitamins, carotenoids, capsaicinoids and vital minerals, hence promoting sustainable nutrient management and better nutritional quality (Tejada *et al.,* 2025). Similarly, in *Capsicum baccatum*, amino acid-based biostimulants increased phenylalanine ammonia lyase and capsaicin synthase activity within an hour, followed by a slow drop over 72 hours. This suggests that amino acid-containing PHs can cause a quick but transient rise in capsaicinoid production, which is one of the fastest secondary metabolite reactions recorded after PH application (Zamljen *et al.,* 2022).

7.7 Ascorbic acid and tocopherols

Ascorbic acid (vitamin C) and tocopherols (vitamin E) are crucial non-enzymatic antioxidants that are typically increased by protein hydrolysate (PH) application in horticultural crops. These antioxidants, like carotenoids and glutathione, are essential for protecting plants from oxidative stress. Despite nutrient solution treatments, combining PHs and microbial bio-stimulants in lettuce maintained higher SPAD index values and increased marketable fresh production by sustaining chlorophyll content and antioxidant pools (Cristofano *et al.,* 2022). Similarly, PH treatment in tomato boosted vitamin C content by 1.29-fold when compared to untreated plants, recommending that amino acids and bioactive peptides found in PHs significantly enhance ascorbic acid accumulation in fruit tissues (Luta *et al.,* 2023).

7.8 Volatile organic compounds (VOCs) and aroma Quality

The VOCs such as monoterpenes, sesquiterpenes and ester derivatives play a vital role in fruit scent and sensory quality. The use of protein hydrolysate (PH) can be shown to be beneficial in altering the VOC composition in addition to the quality of the horticultural product in terms of the sensory and nutritional quality. The commercial products of hydrolyzed plant proteins (organic biostimulants) were applied to strawberries during the blooming and fruit ripening periods and resulted in increased harvest of carbohydrates, minerals, vitamin C, phenolic compounds and VOCs. These changes enhanced the aroma, nutritional attributes and quality of the fruits, as reported by Garcia-Cano *et al.* (2025).

7.9 Crop quality improvement: A multi-metabolite Perspective

Crop biostimulants have a variety of effects, which includes improving visual quality, stimulating plant immune responses, inducing the biosynthesis of plant defensive biomolecules, improving crop performance, enhancing root growth and germination of seeds, stimulating tolerance to biotic and abiotic stressors, and elevating nutrient use efficiency. PHs can reduce stress from the environment, improve plant growth, and boost crop yields, with amino acids offering numerous advantages such as higher yields and components of yield, improved nutrient assimilation and stress tolerance and quality characteristics (Dhanasekaran *et al.,* 2025).

Foliar treatments with alfalfa protein hydrolysate, B-group vitamins and microalgae extract elevated the phenolic content and antioxidant capacity in apples, confirming that PH-based biostimulants are among the most effective tools for improving multi-metabolite quality in fruit tree production (Soppelsa *et al.,* 2018). Table 3 summarizes the effects of different protein hydrolysates on secondary metabolite accumulation.

Table 3: Effect of protein hydrolysates on secondary metabolite biosynthesis

Crop species	Protein hydrolysate type	Major metabolite(s) enhanced	Increase (%)
Arugula (*Diplotaxis tenuifolia*)	Vegetal-derived PH	Phenolic acids, glucosinolates, ferulic acid	15.2-94.1
Apple (*Malus domestica*)	Alfalfa-derived PH	Anthocyanins, phenolics	Increased
Tomato (*Solanum lycopersicum*)	Protein-based PH	Lycopene, vitamin C	29-44
Lettuce (*Lactuca sativa*)	Plant-derived peptide fractions	β-carotene, lutein, ascorbic acid	Increased
Grapevine (*Vitis vinifera*)	Maize gluten-derived PH	Anthocyanins, stilbenes	Increased
Pepper (*Capsicum* spp.)	Wine lees/amino acid-based PH	Capsaicinoids, phenolics, carotenoids	Increased
Strawberry (*Fragaria* × *ananassa*)	Plant-derived PH	Phenolics, VOCs	Increased

8. Protein hydrolysates in abiotic stress tolerance

Protein hydrolysates (PHs) help plants tolerate abiotic conditions such drought, salt, temperature fluctuations, and nutrient deprivation. PH-derived peptides and amino acids function as signaling molecules that activate antioxidant defense systems, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), thereby reducing oxidative damage caused by reactive oxygen species. They also promote the accumulation of osmoprotectants such as proline and soluble sugars, which help maintain cellular water balance under drought and salinity stress. In addition, PHs contribute to ion homeostasis by improving K^+ retention and limiting Na^+ toxicity, while supporting photosynthetic performance and chlorophyll stability under adverse environmental conditions. Transcriptomic and physiological studies further suggest that PHs modulate hormone signaling and stress-responsive gene expression, enabling plants to better adapt to environmental constraints and sustain productivity (Colla *et al.,* 2017; Rouphael and Colla, 2020). Table 4 illustrates the protein hydrolysates in abiotic stress tolerance.

Table 4: Protein hydrolysate in abiotic stress tolerance

Section	Stress type	Crop	PH type	Key outcome	References
7.1	Drought	Tomato	Animal-derived PH (enzymatic)	Pollen viability +51%; fruit number +70%; fruit weight +95% under 50% irrigation	Francesca *et al.,* 2021
7.2	Salinity	Tomato and Lettuce	Vegetal PHs (Malvaceae, Poaceae, legume-derived)	Na^+/K^+ ratio reduced by 73% (lettuce); proline accumulation +200-fold; antioxidant enzymes (CAT, SOD, APX) significantly upregulated	Alzate Zuluaga *et al.,* 2023
7.3	Heat + Drought (combined)	Soybean, Chilli, Chickpea	Vegetal protein hydrolysates	Yield increases in off-season; PS-II photochemical efficiency maintained under high temperature + water deficit	Mamatha *et al.,* 2023
7.4	Fe Deficiency	Maize	Bovine collagen-derived PH (CDPH)	Faster recovery from Fe starvation than $FeCl_3$ alone or FeEDTA; higher SPAD and leaf Fe; PH confirmed Fe chelatorby circular dichroism	Ambrosini *et al.,* 2021

9. Protein hydrolysates and sustainable agriculture

9.1 Reduction of chemical fertilizer dependency

Protein hydrolysates stimulate plant growth and nutrient uptake, reducing dependence on synthetic fertilizers. The amino acids and peptides present in PHs promote root development, promote nutrients absorption efficiency, and improve whole metabolic performance, thus allowing crops to sustain adequate output with lesser fertilizer inputs (Colla *et al.,* 2015).

9.2 Nutrient use efficiency

PHs improve nutrient use efficiency by enhancing nitrogen metabolism, nutrient transporter activity, and root development, thereby increasing nutrient absorption and translocation within the plant (Schiavon *et al.,* 2015). In addition to their direct effects on plant physiology, PHs can influence the rhizosphere microbiome by serving as readily available carbon and nitrogen sources for beneficial microorganisms. Enhanced microbial activity can stimulate nutrient mineralization, nutrient cycling, and nutrient availability in the rhizosphere, further contributing to improved nutrient acquisition and resource-use efficiency by plants (Colla *et al.,* 2017; Costa *et al.,* 2024).

9.3 Organic farming compatibility

Protein hydrolysates are ideal for organic farming systems since they are sourced from natural protein sources and promote environmentally responsible crop development. Their application improves crop quality, stress tolerance and soil biological activity without relying on synthetic agrochemicals (du Jardin, 2015).

9.4 Circular economy and agricultural by-Products

Many commercial PHs are made from agro-industrial residues, such as slaughterhouse waste, fish-processing byproducts, agricultural residues and protein-rich industrial effluent. Using waste streams as biostimulants increases resource value, reduces environmental impact and conforms with circular bioeconomy principles by converting low-value organic materials into high-value agricultural inputs (Avila-Pozo *et al.,* 2023; Perez-Aguilar *et al.,* 2023).

10. Factors influencing the efficiency of protein hydrolysates

The agronomic performance of protein hydrolysates (PHs) is not consistent under all application conditions. Numerous studies have found that their biostimulant activity is controlled by both inherent product qualities and external agronomic and environmental variables. Because of their multifaceted nature, the efficacy of biostimulants cannot be attributed to a single component, making evaluation difficult. The primary parameters that influence their effectiveness are outlined below (Sun *et al.,* 2024).

10.1 Protein source and feedstock origin

The biological activities of protein hydrolysates are highly related to the amino acid composition and thus to the source protein used for their preparation. More than 90% of the goods utilized in the horticultural market are chemically hydrolyzed animal proteins (mostly collagen derived from leather sector byproducts from India, Europe and China, as well as fish-based byproducts from the United States). Plant-derived PHs that are produced through enzymatic hydrolysis have a small market share, but it is growing due to their greater variety of amino acids and suitability for organic agriculture (Kadam *et al.,* 2024). To corroborate this, the most recent investigations on collagen and keratin-derived hydrolysates have demonstrated that the physiological and metabolic response exhibited by plants to these distinct active chemicals can differ, such as in stress tolerance and seed vigour (Skwarek-Fadecka *et al.,* 2026). PHs derived from fish tend to be especially effective in stimulating photosynthesis. Fish based PH applied to lettuce increased the chlorophyll content (from 9.6 to 12.2 mg/g DW), stomatal conductance, photosynthetic rate (from 12.2 to 16.9 µmol CO_2 m^{-2} s^{-1}) and transpiration rates. The hormone like activity of PHs, improvement of water use efficiency and the increase of potassium concentrations were hypothesized to rise stomatal reactivity (Xu and Mou, 2017).

10.2 Hydrolysis method and phytotoxicity risk

The mode of production has a critical impact on the preservation of amino acids and the possible phytotoxic effect. The bulk of commercial PH biostimulants are generated by chemical or enzymatic hydrolysis of animal or plant proteins. In such products, phytotoxicity is said to be due to "general amino acid inhibition," which involves the excessive assimilation of free amino acids, which leads to intracellular amino acid imbalance, to the reduction of cellular ability to uptake nitrate and to an increased susceptibility of the cells to apoptosis (Malecange *et al.,* 2023). Supporting this concern, results from the winter wheat showed that foliar application of PHs based bio-stimulants with Herbal Retrieves had the greatest impact on root elongation stimulation at the lowest concentration of the extract, which then decreased with increasing extract concentrations. This indicates that formulation strategy and application rates are essential factors to consider when determining whether protein hydrolysates support or hinder plant growth (Gendaszewska *et al.,* 2025). As a whole, the findings suggest that, even when using more than one biostimulant simultaneously, phytotoxicity is a dose-dependent concern and further calls for careful dosage optimization prior to field-scale implementation of combinatorial approaches.

10.3 Molecular weight and peptide Size

Peptide fractions with low molecular weight always show the greatest biostimulant activity. A commercial fractionated protein hydrolysate from collagen induced using fast protein liquid chromatography demonstrated that the lower molecular weight compounds were most active at inducing lateral root formation in maize, while all the fractions were active in in vitro grown tomatoes and liquid chromatography–tandem mass spectrometry identified a consensus sequence common to all the bioactive peptides (Ambrosini *et al.,* 2022).

10.4 Application strategy and physiological targeting

Application method (foliar vs. soil) determines the physiological pathways affected by PHs. Most foliar treatments work primarily via the effects on photosynthesis, redox metabolism and the biosynthesis of secondary metabolites, while soil treatments primarily on the effects on root growth and nutrient uptake and rhizosphere interactions. Recent research suggests that timing and frequency of application are important factors to consider, as they affect the plant's metabolic response based on the application stage (Dlacic *et al.,* 2026).

For example:

- Early-stage application – root development + nutrient uptake
- Later-stage application – secondary metabolite accumulation

The effects of fertilizer rate and application method (foliar vs. soil drench) on the growth of tea plants were shown to be significantly higher when CFPH (Chicken Feather Protein Hydrolysate) was applied foliarly as compared to soil drench (Raguraj *et al.,* 2022). Applying the biostimulant *via* either of the two methods (*i.e*., PH routes) and at the right moment (*i.e*., PH timing) has a significant impact on the reaction of the biostimulant. During soybean growth, more tests need to be conducted at more frequent intervals throughout development to determine when application of the PH should occur, because the activity of nitrogen metabolic enzymes (nitrate reductase, urease and asparagine synthetase) changed at the R5.1 (seed-filling) phenological stage, meaning that the time of application could dictate which enzymes are activated (Engel *et al.,* 2024).

10.5 Dose response and application frequency

The fish-derived hydrolysate treatments had a clear dose-window for the best biostimulant efficacy, and the use of anchovy-derived PH for primrose resulted in substantial spikes in leaf area and dry weight at 1.5 g/l, while the treatment with 1.0 g/l had the highest leaf chlorophyll content (Tutuncu, 2024). Further evidence of the importance of treatment rate was provided with tea nursery plants. In perennial crops, rate of application is a very important point for effectiveness of biostimulants, sub-optimal rates of application showing negligible responses as observed in growth of tea nursery plants under different rates of chicken feather PHs (Raguraj *et al.,* 2022).

10.6 Crop species and cultivar genotype

The intrinsic identity of the crop, at both species and cultivar level, is a key determinant for the efficacy of protein hydrolysate biostimulants; same protein hydrolysate applied at the same dose can yield significantly different results according to the plant species, genotype and cultivar that are studied.

10.6.1 Species-level differences

When evaluating the four vegetal PHs (Malvaceae-derived, Poaceae-derived and two commercial PHs of legume origin: Trainer® and Vegamin®) in lettuce and tomato plants under high salinity conditions, Alzate Zuluaga *et al.* (2023) observed significant differences among the species in terms of their responsiveness to PH. The study found that biostimulant treatments and salinity had both a positive and negative effect on biomass production of the two plant species, but to varying extents. The biostimulants were more efficient at reducing salinity in lettuce than in tomato plants, which are naturally more tolerant of salinity. Among the four biostimulants examined, D (legume-derived Trainer®) and P (Poaceae-derived) showed the most favorable effects for salt stress alleviation in both species, whereas the use of biostimulants in salt-stressed plants resulted in a differential induction of antioxidant enzyme function based on the plant species and the nature of the PH applied.

10.6.2 cultivar-level differences

Francesca *et al.* (2022) established cultivar-level variability within the same species by investigating PH responses in two tomato genotypes (‘E42’ and ‘LA3120’) exposed to drought, heat or combination stress. The reaction to biostimulants in plants was genotype-specific, with ‘E42’ responding more strongly to protein hydrolysate treatment than ‘LA3120’. Biostimulant being applied improved net photosynthesis and highest efficiency of PSII photochemistry under drought in ‘E42’, probably related to the existence of glycine betaine and aspartic acid in the PH, while elevated antioxidant levels and reduced accumulation of proline, soluble sugars and hydrogen peroxide, in plants that were treated under drought and combined stress further confirmed that the biostimulant mitigated the negative effects of abiotic stressess in a genotype dependent manner

10.7 Combination with other biostimulants

Combining PHs with other biostimulant groups improves their effectiveness. The advances in science underlying the complementary and beneficial effects of microbial and non-microbial plant biostimulants are compiled, which reveals that over the last decade, commercial enterprises have invested in improving the efficacy of their product formulations through blends of microbial and non-microbial biostimulants such as nitrogen-fixing bacteria, mycorrhizal fungi, protein hydrolysates and seaweed extracts (Rouphael and Colla, 2018). A two-year open-field study on eggplant demonstrated the synergistic interaction between plant protein hydrolysates and arbuscular mycorrhizal (AM) fungi in improving tolerance to iodine stress. The combined application of these biostimulants provided greater protection against nutrient toxicity compared to individual treatments, highlighting that the integration of PHs with microbial biostimulants can be an effective approach to enhance PH efficiency under adverse soil conditions (Consentino *et al.,* 2024).

10.8 Environmental conditions and stress intensity

The effectiveness of protein hydrolysate biostimulants is greatly influenced by environmental conditions, including the nature, intensity and seasonal occurrence of stresses. The positive effects of PH applications are often enhanced through repeated use and depend on several interacting factors such as crop species or cultivar, growth stage and surrounding environmental conditions. Many PHs exhibit auxin- and gibberellin-like activities that promote seed germination, root development, shoot growth and fruit production. In addition, PH applications have been shown to reduce or prevent yield losses caused by unfavourable soil and environmental conditions, including drought, alkalinity, salinity and nutrient deficiency (Zhang *et al.,* 2025).

11. Limitations, economic feasibility and commercial adoption challenges

11.1 Variability and standardisation of protein hydrolysates

Protein hydrolysates (PHs) are extremely varied in composition due to changes in protein sources, hydrolysis methods, and processing conditions. Such changes affect peptide patterns, amino acid content, and biological activity, resulting in uneven plant responses. The absence of established production techniques and characterisation methods hampers product comparison and reproducibility among research. As a result, the creation of quality standards and analytical benchmarks is critical to ensuring consistent efficacy and facilitating the wider adoption of PH-based biostimulants (Colla *et al.,* 2015; Malecange *et al.,* 2023).

11.2 Economic feasibility and cost considerations

Although PHs can improve crop productivity and quality, their economic sustainability is contingent on production costs, application rates, and crop value. The production of PHs incurs costs for raw material acquisition, hydrolysis procedures, formulation, and quality control. Enzymatic hydrolysis, which produces highly bioactive molecules, is frequently more expensive than chemical hydrolysis. As a result, the profitability of PH application varies depending on the crop and production method. Comprehensive cost-benefit assessments are thus required to support large-scale commercialization (Rouphael and Colla, 2020; Sun *et al.,* 2024).

11.3 Challenges in regulatory and quality control

Biostimulants are regulated differently in different countries, posing obstacles for product registration, labeling, and market access. Although recent rules have improved the classification of plant biostimulants, consistent standards for efficacy evaluation and quality assessment are still lacking. The complex and varied character of PHs complicates regulatory approval and quality assurance processes. Internationally established guidelines can enhance transparency, product reliability, and consumer confidence (Du Jardin, 2015; Paskovic *et al.*, 2024).

11.4 Commercial adoption and farmer acceptance

The successful implementation of PH-based biostimulants is contingent on constant field performance and economic benefits. However, crop genotype, weather circumstances, soil features, and application methods can all have an impact on their performance. Such unpredictability may result in inconsistent agronomic outcomes, lowering farmer faith in these products. Furthermore, low awareness and technical expertise of biostimulant uses may impede their broad use. Increased extension activities and field demonstrations are required to increase farmer acceptance (Bulgari *et al*., 2019; Rouphael and Colla, 2020).

11.5 Research gaps and future needs

Despite tremendous progress, the particular mechanisms underlying many PH-mediated plant responses remain unclear. Additional research is needed to identify particular bioactive peptides and their interactions with plant metabolic and signaling pathways. Future research should combine advanced omics techniques with long-term field evaluations to improve mechanistic knowledge and validate agronomic performance. Standardized formulations and sustainable production procedures will also be important in the future development of PH-based biostimulants (Malecange *et al*., 2023; Dhanasekaran *et al*., 2025).

12. Conclusion

Protein hydrolysates (PHs) have emerged as potent biostimulants that can reprogramme plant metabolism to improve growth, nutrient utilization efficiency, stress tolerance, and secondary metabolite biosynthesis. The evidence presented in this article shows that PH-derived amino acids and bioactive peptides regulate a variety of physiological and molecular processes, including carbon and nitrogen metabolism, phytohormone signalling, antioxidant defense, root architecture development, and phenylpropanoid pathway activation. These responses help to boost the accumulation of important secondary metabolites such phenolics, flavonoids, anthocyanins, carotenoids, glucosinolates, and volatile compounds, which improves crop production, nutritional quality, and resilience to severe environmental circumstances. Furthermore, the use of agro-industrial byproducts in PH production promotes circular bioeconomy concepts and sustainable agriculture practices. However, significant knowledge gaps exist in the discovery of individual bioactive peptides, peptide-plant interactions, molecular signalling pathways, and the consistency of responses across crops and settings. Variability in feedstock sources, hydrolysis processes, and product composition further hinders standardization and commercial scalability. Future study should concentrate on sophisticated multi-omics techniques, peptide characterization, crop-specific formulation creation, and long-term field validation to establish solid structure-function connections. Addressing these problems will help to accelerate the development of next-generation PH-based biostimulants and strengthen their role in sustainable, climate-resilient crop production.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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