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Advancements and Applications of Enzymatic Hydrolysis of Protein for Agricultural Sustainability

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

The enzymatic hydrolysis of proteins has emerged as an efficient and sustainable biotechnological method to enhance agricultural practices by converting complex proteins into bioavailable peptides and amino acids. This study explores the factors influencing enzymatic activity during the hydrolysis process, including temperature, pH and the concentrations of both the substrate and the

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enzyme. These factors are critical for optimizing the efficiency of protein breakdown, ensuring high-quality products. The resulting peptides play a vital role in plant nutrition, offering a potential solution for improving soil fertility, enhancing animal feed and developing sustainable crop fertilization methods. Genetic engineering and protein optimization can further improve enzyme performance for agricultural applications, such as enhancing nutrient uptake, promoting plant growth, and improving stress tolerance in crops. Additionally, they provide a valuable resource for enhancing animal feed, promoting nutrient cycling, and reducing the need for synthetic fertilizers. This approach not only reduces agricultural waste and mitigates environmental impacts but also enhances productivity, supporting more sustainable farming practices and contributing to the long-term sustainability of global food systems.

Keywords: Protein hydrolysis; enzymatic hydrolysis; enzyme; sustainable agriculture.

1. INTRODUCTION

Protein hydrolysis is the biochemical process of breaking down proteins into smaller peptides or individual amino acids influenced by factors such as pH, temperature, water, acid, base, enzyme (Zarei et al., 2022). Proteins from plant sources have generated interest and are in high demand by consumers as they provide good alternatives with health benefits (Amagliani, L et al., 2017). Chemical hydrolysis breaks peptide bonds using strong acids or bases at high temperatures, producing amino acids and peptides, but it lacks specificity and creates byproducts, making it suitable for laboratory use (Nelson & Cox, 2017). Enzymatic hydrolysis uses proteases under gentler conditions to break down proteins selectively, making it ideal for food, pharmaceuticals, and biotechnology (MacGregor & Varhol, 2013).

Enzymatic hydrolysis plays a crucial role in agriculture by offering sustainable solutions for utilizing food waste and processing byproducts. It enables the conversion of biomass into valuable products like bioethanol, reducing environmental liabilities and enhancing food security (Dutra et al., 2023). It is also utilized in modifying plant proteins to improve their functionality, particularly enhancing solubility near the isoelectric point, although it may lead to structural changes that affect protein aggregation (Dent et al., 2023). Enzymatic hydrolysis of proteins is a crucial process used to modify the functionality and properties of plant proteins like soy, chickpea and pigeon pea. Studies have shown that enzymatic hydrolysis can improve solubility near the isoelectric point, but it may also lead to structural changes causing aggregation, challenging the belief that hydrolysis always enhances solubility (Dent et al., 2023). Optimal hydrolysis parameters, such as enzyme-to-substrate ratios and processing times, play a significant role in determining the degree of hydrolysis and functional profiles of the resulting protein hydrolysates (Sokolov et al., 2023; Ratnayani et al 2023). Ultrasonic-assisted enzymatic hydrolysis has been found to be an efficient method for protein extraction and hydrolysis, showing increased protein extraction rates compared to traditional enzymatic hydrolysis methods (Liu et al., 2023). This review will explore recent advancements in enzymatic protein hydrolysis, with a particular emphasis on innovative enzyme technologies and their applications in sustainable agriculture.

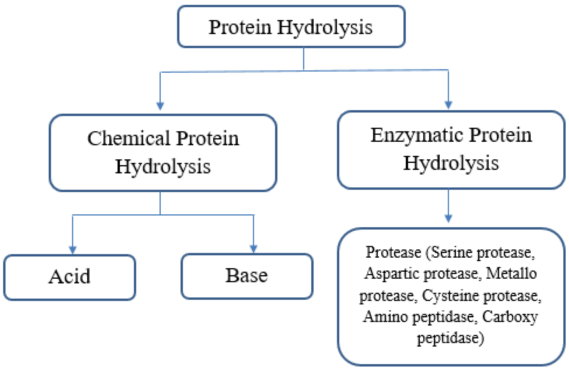

Fig. 1. Procedural classification of protein hydrolysis techniques: Chemical and enzymatic (Nelson & Cox, 2017; MacGregor & Varhol, 2013)

2. TECHNOLOGICAL ADVANCEMENTS IN ENZYMATIC HYDROLYSIS

Genetic engineering and protein optimization are powerful techniques that can help create enzymes that are more effective and selective in breaking down specific substrates. By manipulating the genetic material of organisms, scientists can design enzymes with enhanced properties, such as improved activity, stability, or specificity for certain tasks. In addition, using enzyme cocktails or conducting metagenomic screening which involves exploring genetic material from environmental samples can further boost the efficiency of hydrolysis (wang et al., 2024).

Enzymes have long been used in food processing, but native enzymes often lack the required activity, efficiency, and adaptability to harsh conditions. Advances in enzyme engineering, such as rational design and directed evolution, have led to the development of custom enzymes with improved properties. Synthetic biology, gene editing, and tools like AI and computational analysis have made precision fermentation more efficient (Boukid et al.,2023).

3. FACTOR AFFECTING ENZYMATIC ACTIVITY

Enzymatic activity is influenced by several critical factors, including temperature, pH, substrate concentration, and the presence of inhibitors or activators. Research indicates that temperature significantly affects enzyme kinetics, with optimal temperatures enhancing activity while extreme temperatures can lead to denaturation (Berger et al., 2024).

3.1 Temperature

Enzyme activity varies with temperature. It was nearly nonexistent at 9°C, moderate at 37°C, and 1.5 times greater at 41°C compared to the activity at 37°C (John, 2011). In the study, polygalacturonase enzyme activity showed a linear increase with temperature. Mannanase showed rising activity from 15°C to 40°C and protease activity increased linearly within the range of 20-40°C (Reynolds et al., 2018).

3.2 pH

Additionally, pH levels are crucial, as each enzyme has a specific pH range where it functions best; deviations can reduce activity or inactivate the enzyme (Grahame et al., 2014). The enzyme exhibited the highest activity at pH 7 (neutral), showed no activity at pH 1, and had decreased activity at pH 11 (John et al., 2011). In the case of urease from germinating chickpea seeds, optimal pH was 7.2 and temperature was 48°C (Pervin et al., 2012). Enzyme activity is sensitive to pH fluctuations, which impacts their effectiveness during the wine fermentation process. Polygalacturonase (PGU) activity improved with higher pH levels across various temperatures. In contrast, Mannanases activity diminished as the pH increased from 3.0 to 5.0 (Reynolds et al., 2018).

3.3 Substrate and Enzyme Concentration

Substrate concentration also plays a vital role, as increased substrate levels can enhance reaction rates up to a saturation point, beyond which the activity plateaus (Scanlon et al., 2017). Furthermore, the presence of specific ions or molecules can act as activators or inhibitors, modulating enzymatic function and efficiency (Pervin et al., 2012). The study examined hydrogen peroxidase from cow tissue, assessing its activity by measuring oxygen release during the breakdown of hydrogen peroxide. Enzyme concentrations of ½X, 1X, and 2X were tested, revealing a direct correlation between concentration and reaction rate. Significant activity was observed at 1X, while the 2X concentration resulted in approximately double the activity compared to 1X (John., 2011).

Table 1. Optimal conditions for protease activity: pH and temperature (Nelson & Cox., 2017)

Protein	Source	Optimal temperature	Optimal pH
Pepsin	Stomach of animal	37°C	1.5 TO 2.5
Trypsin	Secreted by the pancreas into the small intestine.	37°C	7.5 to 8.5
Chymotrypsin	secreted by the pancreas into the small intestine.	37°C	7.5 to 8.5
Bromelain	Derived from pineapple	50°C	4.5 to 5.5
Papain	Derived from papaya	60°C	6.0 to 7.0

Protein	Source	Optimal temperature	Optimal pH
Alkaline protease	Microorganism	50°C to 70°C	9.0 to 11.0
Subtilisin	*Bacillus subtilis*	50°C to 60°C	7.0 to 10.0
Thermolysin	*Bacillus thermoproteolyticus*	70°C	6.0 to 7.0
Neutrase	*Bacillus subtilis*	50°C to 60°C	7.0 to 9.0
Peptidase	Animal tissues and microorganisms	37°C to 55°C	neutral to slightly alkaline

4. SPECIFIC ENZYME FOR DIFFERENT AGRICULTURAL APPLICATION

Different enzymes play crucial roles in various agricultural applications, enhancing crop yield, pest resistance, and soil health. Enzymes such as carbohydrase break down carbohydrates, while phytases help digest phytate to release phosphorus. Proteases assist in protein digestion, and amylases aid in the breakdown of starches. In agriculture, these enzymes play a crucial role in transforming organic matter and cycling nutrients. They serve as indicators of soil health, fertility, and productivity, reacting sensitively to environmental changes. By enhancing feed digestibility, improving nutrient absorption, and supporting animal health, soil enzyme activity provides valuable insights into ecosystem disturbances. This information helps researchers and farmers assess the impact of both natural and human-induced factors on soil health and is essential for devising strategies to mitigate negative effects and promote sustainable agricultural practices (Piotrowska et al., 2018).

Chitinase has versatile uses in agriculture, waste management, food safety, and biotechnology. It degrades chitin, aiding in pest control and waste reduction, and enhances food preservation by preventing spoilage. Sourced from plants and microbes, it supports sustainable farming by managing pests and reducing the need for chemical pesticides. In biotechnology, chitinase is valuable for studying chitin metabolism and its ecological roles (Srivastava et al., 2023).

Enzymes like pectinase and cellulase are employed to decrease bitterness in fruit juices by breaking down pectin and other polysaccharides, resulting in clearer and more enjoyable juice. Amylases and glucoaylases are essential for converting starch into sweeteners. Additionally, proteases are used to tenderize meat, and lipaes play a key role in generating flavor compounds in dairy products (John et al., 2011).

5. APPLICATION OF ENZYMATIC HYDROLYSIS IN AGRICULTURE SECTOR

Table 2. Applications of protease enzymes different sectors (Razzaq et al., 2019)

S/ No	Enzyme		Application in agriculture	Additional application	Reference
1.	Serine protease	Trypsin	Enhance animal feed, Process crop protein	Cell culture	(Yamaya et al. 2015)
		Chymotrypsin	Improve cattle digestion, Process plant protein, Enhance feed efficiency	Pharmaceutical, Food industry	(Razzaq et al., 2019)
2.	Cysteine protease	Papain	Enhance animal feed, Processing plant material, Composting	Meat tenderization, Clarifying beer	(Razzaq et al., 2019)
		Bromelain	Enhance animal feed Composting Waste management	Meat tenderization, Medical application (Inflammation and digestive disorder)	(Razzaq et al., 2019)

S/ No	Enzyme		Application in agriculture	Additional application	Reference
3.	Aspartic protease	Pepsin	Enhance animal feed	Cheese production Digestive enzyme supplements	(Nair et al., 2019)
		Renin	Produce high quality animal feed	Cheese production	(Nair et al., 2019)
4.	Metalloprotease	Matrix metalloproteinase	Composting Soil fertility	Medicine Tissue remodeling Wound healing	(Razzaq et al., 2019)
		Thermolysin	Processing animal feed Composting	Food industry Biotechnological application	(Razzaq et al., 2019)
5.	Metalloendopeptidases	Endoproteinase	Enhance feed quality Composting	Food industry	(Razzaq et al., 2019)
6.	Alkaline protease	Subtilisin	Enhance feed quality	Laundry detergent Food industry	(Razzaq et al., 2019)
		Alcalase	Enhance feed quality Composting by breaking down organic material	Detergent formulation	(Razzaq et al., 2019)

6. ENHANCING ANIMAL FEED QUALITY

6.1 Enzyme Hydrolysis and Feed Digestibility

Enzymatic hydrolysis enhances alternative feed ingredients by lowering crude fiber content and improving digestibility, which promotes better animal growth without adverse effects, despite its dependence on temperature and pH. Optimal enzyme hydrolysis can reduce the crude fiber content in coconut cake by 67.8%. Additionally, enzyme hydrolysis in alternative feed ingredients can positively influence animal growth due to the increased digestibility of the feed (Pratiwy et al., 2022). In the animal feed industry, enzymes such as phytase, protease, alpha-amylase, and others play crucial roles. They help eliminate anti nutritional factors and improve feed digestibility, enhancing animal growth and health (Singh et al., 2017). Enzymatic hydrolysis from fungal co-cultivation of Bermuda grass and corn cob enhances animal feed quality by improving ruminal digestibility and offering economical on-site enzyme production (Aldo et al., 2016).

6.2 Soya Bean Meal Processing

Soybean meal (SM), a vital protein source in the food and feed industries due to its balanced amino acid profile, cost-effectiveness, and stable supply, underwent fermentation using *Lactobacillus plantarum*, *Lactobacillus casei*, *Bacillus subtilis*, *Bacillus licheniformis*, and *Aspergillus oryzae* for 24 hours. This was followed by a 15-minute hydrolysis with Alcalase, which effectively reduced the antigenicity of β-conglycinin and glycinin in SM. This approach also led to a higher concentration of low molecular weight peptides (<10 kDa), enhancing its nutritional value and digestibility (Yang et al., 2020).

7. ENHANCEMENT OF SOIL FERTILITY

7.1 General Benefits of Enzyme-Rich Fertilizers

The organic green fertilizer enriched with bio enzymes enhances enzymatic hydrolysis in the soil, leading to improved crop yields, better soil quality, and more efficient nutrient utilization. It also helps decompose heavy metals and creates favorable temperature conditions for plant growth (Xue, 2013). The soil enzyme urease accelerates the hydrolysis of urea in fertilizers, releasing NH4. Its activity rises with increasing substrate concentration, reaching a maximum and leveling off at approximately 30 mM, which influences soil enrichment processes (Kumari et al., 2020).

7.2 Organic Waste as Bio-Stimulants

Okara (OK), a by-product of soy milk production, is processed through enzymatic hydrolysis and fermentation to produce different soil bio stimulants. Soil analysis indicated that all treatments enhanced β-glucosidase, phosphatase, and dehydrogenase activities, with the Enzymatic Hydrolysate showing particularly strong effects on dehydrogenase and phosphatase (Orts et al., 2018). Wheat-condensed distiller solubles (WCDS) are transformed into WCDS-Enzymatic Extract (Hydrolysate product). Physicochemical analysis revealed that the main difference lies in protein size, with WCDS-EE containing peptides instead of the original proteins found in WCDS. Our investigation showed that both products quickly boosted soil dehydrogenase (DHA) and phosphatase (APA) activity, as well as ATP production (Garcia-Martinez et al., 2001).

7.3 Combined Microbial-Enzyme Formulations

To boost plant growth, soil fertility, and microbiome health, an innovative blend of *Bacillus pumilus* AR57 and chicken feather protein hydrolysate (CFPH) was introduced. This combination led to a notable rise in soil fertility, enriching the microbial community with heterotrophic bacteria (42.23 × 10^6 CFU/g), nitrogen fixers (2.45 × 10^4 CFU/g), phosphate solubilizers (0.48 × 10^4 CFU/g), and potassium solubilizers (0.33 × 10^4 CFU/g) (Jagadeesan et al., 2023).

8. EFFECTIVE SOLUTIONS FOR WASTE MANAGEMENT AND COMPOSTING

Enzymes like xylanase, proteases, hydrolases, cellulose, peroxidases, chitinases, and laccases are increasingly used in waste management due to their eco-friendly and sustainable properties. They break down waste materials into biodegradable forms, enabling recycling, reuse, and conversion into valuable products (Rath et al., 2023; Jagadeesh and Muthura., 2022). To enhance environmental sustainability and resource use, new technologies are developed to convert waste into biofuels, notably bioethanol, which is derived from food waste rich in carbohydrates and fermentable sugars. This process supports the circular bioeconomy and food security by adding value to waste and reducing environmental impact. Effective bioethanol production involves pretreatment and enzymatic hydrolysis, which require optimization of pH and temperature conditions to maximize efficiency in converting biomass to bioethanol. Understanding these enzymatic processes is key to improving biofuel production (Dutra et al., 2023).

9. SYNTHESIS OF PEPTIDES WITH BIOACTIVITY

The enzymatic hydrolysis of bovine hemoglobin using electro dialysis with bipolar membrane produced bioactive peptides with antimicrobial, antifungal, and antioxidant properties, beneficial for agriculture (Nelson and Cox, 2017). Hydrolyzing dairy proteins releases a variety of bioactive peptides beneficial for human health. Innovative, eco-friendly technologies like ultrasound-assisted processing (UAP), microwave-assisted processing (MAP), and high-pressure processing (HPP) offer promising pretreatments. While generally less effective alone than traditional methods, these novel technologies, when combined with fermentation and enzymatic hydrolysis, can enhance peptide profiles, improve yields, and increase the release of bioactive peptides more effectively than conventional techniques (MacGregor & Varhol, 2013).

10. CONCLUSION

Optimizing agricultural operations can be achieved by the diverse and effective approach of enzymatic hydrolysis of proteins. Proteins can be broken down into smaller, easier-to-access components with this process, which also helps with efficient waste management and adds vital nutrients to the soil. A viable strategy for improving resource utilization and raising the general sustainability of farming operations is enzymatic hydrolysis. There are several advantages of using enzymatic hydrolysis into agricultural systems. Enzymatic hydrolysis is an essential part of contemporary agricultural innovation because it supports both ecological and economic goals by generating bioactive peptides and maximizing the use of agricultural waste.

Future directions include the development of tailored enzyme cocktails for efficient conversion of diverse agro-industrial wastes into bioactive compounds. Emphasis will be placed on integrating these processes into circular bioeconomy models to reduce reliance on synthetic inputs and enhance nutrient recycling.

Identifying functional peptides will enable next-generation biostimulants, while advances in enzyme engineering and process optimization will support scalable, sustainable agricultural applications.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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