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Enzyme-Assisted Extraction of Bioactive Compounds from Underutilized Plants for Functional Food Use

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Abstract

The search for natural, sustainable, and efficient sources of bioactive compounds has intensified in recent years due to the rising demand for functional foods and nutraceuticals. Underutilized plants, often referred to as orphan or neglected crops, represent a vast yet largely untapped reservoir of phytochemicals such as polyphenols, flavonoids, carotenoids, alkaloids, saponins, and dietary fibers with significant health-promoting potential. However, the recovery of these compounds is often limited by the complexity of plant cell wall structures, which restricts accessibility and reduces extraction efficiency when conventional solvent-based methods are applied. Enzyme-assisted extraction (EAE) has emerged as a promising green technology that overcomes these limitations by employing hydrolytic enzymes, such as cellulases, pectinases, hemicellulases, and proteases, to selectively degrade cell wall polymers and release bound metabolites.EAE offers several advantages over traditional extraction methods, including higher yields, improved selectivity, reduced solvent and energy requirements, and better preservation of the structural integrity and bioactivity of target compounds. Applications of EAE in underutilized plants include the extraction of polyphenols from Moringa and Amaranthus leaves, carotenoids from wild fruits, dietary fibers from baobab, and protein hydrolysates from legumes, all of which can be incorporated into functional food formulations. The technique also allows valorization of agricultural residues and wild edible plants, contributing to biodiversity conservation and sustainable food systems. Despite its potential, challenges remain in the large-scale application of EAE, including the high cost of enzymes, variability in phytochemical content among plant species and growing conditions, lack of standardized extraction protocols for neglected crops, and regulatory concerns regarding enzyme residues in food products. Computational modeling and artificial intelligence are expected to aid in process optimization, while policy support is needed to promote the use of underutilized plants in functional food industries. Overall, enzyme-assisted extraction represents a powerful, eco-friendly strategy to unlock the nutritional and therapeutic potential of underutilized plants, supporting the development of innovative functional foods and contributing to global food security and sustainable health solutions.

Keywords: *Enzyme-assisted extraction, bioactive compounds, underutilized plants, functional foods, green technology, phytochemicals*

Introduction

In recent decades, the functional food industry has emerged as a rapidly growing sector, driven by consumer demand for natural, health-promoting products that go beyond basic nutrition. Functional foods are defined as foods that provide physiological benefits and reduce the risk of chronic diseases, such as cardiovascular disorders, diabetes, obesity, and neurodegenerative conditions, owing to the presence of bioactive compounds [1]. These bioactives—ranging from polyphenols and flavonoids to carotenoids, alkaloids, saponins, and dietary fibers—are naturally occurring metabolites in plants that have antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. However, despite the vast diversity of plant resources available globally, only a limited range of crops is widely exploited for such purposes. Underutilized plants, also known as neglected, orphan, or minor crops, remain overlooked in mainstream agriculture and food processing despite their rich phytochemical profiles and historical use in traditional medicine and local diets [2]. Harnessing bioactive compounds from these underutilized resources represents not only an opportunity for diversifying functional foods but also a pathway for enhancing food security, sustainability, and biodiversity conservation.

One of the major challenges in exploiting plant-derived bioactives is their limited accessibility due to the structural complexity of plant cell walls. The plant cell wall, composed primarily of cellulose, hemicellulose, pectin, and lignin, acts as a physical barrier that hinders the efficient release of metabolites. Conventional extraction methods—such as maceration, Soxhlet extraction, or solvent-based techniques—often require large amounts of organic solvents, high temperatures, and extended processing times [3]. These approaches are associated with several drawbacks, including degradation of thermolabile compounds, low extraction efficiency, environmental concerns due to solvent use, and limited scalability. In response to these challenges, research has increasingly focused on green, sustainable, and efficient extraction methods that align with the principles of circular bioeconomy and environmental responsibility.

Among these novel techniques, enzyme-assisted extraction (EAE) has gained particular attention as a powerful biotechnological approach. EAE employs specific hydrolytic enzymes, such as cellulases, pectinases, hemicellulases, and proteases, to break down the polysaccharide and protein matrices of plant cell walls. By selectively hydrolyzing these structural components, enzymes facilitate the release of bound or encapsulated phytochemicals that would otherwise remain inaccessible. Compared to conventional techniques, EAE offers multiple advantages, including higher yields, better selectivity, reduced solvent consumption, milder extraction conditions, and enhanced preservation of the biological activity of the compounds [4], enzymes are biodegradable and can be produced sustainably, making EAE a highly compatible technology for environmentally friendly food processing.

The application of EAE to underutilized plants is particularly promising because these plants often possess unique phytochemical compositions adapted to stress-tolerant environments. For instance, species such as *Moringa oleifera*, *Amaranthus spp.*, baobab (*Adansonia digitata*), and wild berries are rich in antioxidants, dietary fibers, and micronutrients. Yet, their incorporation into functional food products is limited by the lack of efficient and standardized extraction protocols. EAE could unlock this potential by maximizing the recovery of valuable compounds, enabling their use in nutraceuticals, dietary supplements, beverages, and functional snacks [5]. Agricultural by-products and residues from underutilized plants, such as seed coats, husks, and leaves, can also be valorized using enzyme-assisted approaches, thereby supporting sustainable resource utilization and waste minimization. Another advantage of EAE lies in its versatility and adaptability. The method can be tailored to different plant matrices and target compounds by selecting appropriate enzyme cocktails and optimizing conditions such as pH, temperature, incubation time, and substrate concentration. For example, cellulase and pectinase combinations are highly effective in releasing phenolics and flavonoids from leafy tissues, while proteases can liberate bioactive peptides from legumes. Similarly, enzymatic treatment of wild fruits and seeds has been shown to enhance carotenoid and saponin extraction [6]. These targeted approaches highlight the potential of EAE to serve as a customizable platform for a wide range of plant-based functional food applications. These advantages, several challenges remain in the wider adoption of EAE, particularly for underutilized plants. The high cost of enzymes, variability in enzyme activity due to source or production methods, and the lack of universally standardized protocols limit scalability and commercial adoption. Additionally, underutilized plants often exhibit significant variation in phytochemical content depending on species, geographic origin, seasonal changes, and cultivation practices. Such variability complicates the development of consistent extraction processes and functional food formulations. Moreover, regulatory frameworks concerning the use of enzymes in food processing differ across regions, creating uncertainties in product approval and market access. Addressing these challenges requires interdisciplinary collaboration among food scientists, biotechnologists, chemists, and policymakers. Technological innovations have begun to address some of these limitations. Advances in enzyme engineering, immobilization techniques, and synthetic biology are enabling the production of more cost-effective and robust enzyme systems tailored to specific extraction needs [6-7]. The integration of EAE with complementary green extraction techniques—such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), or supercritical fluid extraction (SFE)—is also being explored to further enhance efficiency and selectivity. Furthermore, computational approaches, including molecular docking and artificial intelligence-driven predictive modeling, are increasingly applied to optimize enzyme-substrate interactions and extraction parameters. These innovations provide a roadmap for scaling up EAE to industrial levels while ensuring sustainability and cost-effectiveness.

From a broader perspective, the use of EAE to valorize underutilized plants has implications beyond functional food development. It contributes to the global agenda of promoting sustainable food systems, reducing reliance on major staple crops, and addressing malnutrition by diversifying dietary sources of essential bioactives. Furthermore, supporting the commercial utilization of underutilized plants can provide economic opportunities for rural communities, encourage conservation of agrobiodiversity, and mitigate the risks associated with overdependence on a narrow range of crops in global agriculture [8]. Enzyme-assisted extraction offers a powerful, eco-friendly, and versatile approach to unlock the bioactive potential of underutilized plants for functional food applications. By bridging the gap between traditional resources and modern biotechnological innovations, EAE not only addresses technical limitations in phytochemical recovery but also supports broader goals of health promotion, sustainability, and biodiversity conservation. This article explores the principles of EAE, highlights its applications in underutilized plants, discusses current challenges, and outlines future perspectives for integrating this technology into the functional food industry.

3. Applications of Enzyme-Assisted Extraction in Underutilized Plants

The potential of enzyme-assisted extraction (EAE) extends beyond widely cultivated crops to underutilized plants, which often possess unique and abundant bioactive compounds. These plants are frequently adapted to marginal environments, making them reservoirs of metabolites with stress-tolerance properties, high nutritional value, and therapeutic relevance [9]. Applying EAE to such species allows efficient recovery of bioactives for functional food applications.

3.1. Leafy Underutilized Vegetables

Many neglected leafy vegetables, including *Amaranthus spp.*, *Cleome gynandra* (African spider plant), and *Moringa oleifera*, contain phenolics, flavonoids, and carotenoids that are encapsulated within complex cell walls. EAE using cellulases and pectinases has been shown to significantly increase the yield of phenolic acids, flavonoids, and antioxidant compounds [10]. These bioactives can be incorporated into functional teas, dietary supplements, and natural colorants.

3.2. Fruits and Wild Berries

Wild fruits such as baobab (*Adansonia digitata*), sea buckthorn (*Hippophaerhamnoides*), and wild blueberries contain high concentrations of ascorbic acid, anthocyanins, and flavonoids. EAE facilitates the release of anthocyanins and phenolic acids from berry skins and pulp, improving extraction yields compared to solvent-based methods. Extracts from these sources can be utilized in functional beverages, nutraceutical powders, and natural preservatives due to their antioxidant and antimicrobial properties [11].

3.3. Seeds and Legumes

Underutilized seeds and legumes, including horse gram (*Macrotyloma uniflorum*), bambara groundnut (*Vigna subterranea*), and jackfruit seeds, are rich in proteins and secondary metabolites. Enzymatic treatment with proteases not only aids in extracting bioactive peptides but also enhances protein digestibility and nutritional quality [12]. These peptides exhibit antihypertensive, antioxidant, and antimicrobial properties, supporting their integration into functional food formulations such as protein-rich powders and snacks.

3.4. Roots, Tubers, and Rhizomes

Species like yam bean (*Pachyrhizuserosus*), arrowroot, and lesser-known ginger relatives contain starches, inulin, and phenolic compounds. EAE using amylases and cellulases enhances the recovery of starch derivatives and oligosaccharides, which can be applied as prebiotics [13]. Additionally, phenolic-rich extracts from wild tubers exhibit antimicrobial activity, expanding their use in food preservation.

3.5. Agricultural By-products from Underutilized Plants

The valorization of agricultural residues such as fruit peels, seed coats, husks, and leaves represents another promising avenue. For example, pectinases and cellulases release bound phenolics and dietary fibers from baobab fruit shells and jackfruit peels, converting waste streams into valuable functional ingredients [14]. Such approaches align with circular economy principles and sustainable food system goals.

3.6. Integration into Functional Food Products

Bioactives obtained through EAE are now being incorporated into a wide range of functional foods:

- **Beverages**: Fortified juices and herbal teas enriched with polyphenols and antioxidants.
- **Bakery and Snacks**: Functional flours from underutilized legumes with improved digestibility.
- **Supplements**: Capsules and powders derived from wild fruits and leafy greens.
- **Food Preservatives**: Natural antimicrobials replacing synthetic additives.

Together, these applications highlight the versatility of EAE in enhancing the nutritional and functional value of neglected plant resources.

4. Challenges and Limitations of Enzyme-Assisted Extraction

Despite its numerous advantages, the adoption of enzyme-assisted extraction in underutilized plants faces several scientific, technical, and economic challenges [15]. These limitations need to be addressed to facilitate large-scale application in the functional food industry.

4.1. Enzyme Cost and Availability

Commercial enzymes are relatively expensive, particularly when used at industrial scales. Although technological advances such as recombinant enzyme production and immobilization methods are helping reduce costs, affordability remains a key barrier to widespread adoption.

4.2. Optimization of Enzyme Combinations

Plant matrices vary widely in their biochemical composition. A specific enzyme cocktail that works for one plant species may not be effective for another [16]. Optimization of enzyme type, concentration, pH, and temperature for each plant material requires extensive research, which increases costs and time.

4.3. Variability in Plant Material

Underutilized plants often display significant variability in phytochemical content due to differences in genotype, soil conditions, climate, and harvesting stage [17]. This inconsistency makes it difficult to establish standardized extraction protocols and ensure reproducible bioactive yields.

4.4. Stability of Extracted Bioactives

Some bioactive compounds are highly unstable and prone to degradation during extraction, processing, and storage. Phenolics and flavonoids, for example, may undergo oxidation [18]. Stabilization techniques such as encapsulation are required, adding complexity and cost.

4.5. Scale-Up Challenges

While EAE is effective at laboratory scale, scaling up to industrial levels requires integration with bioreactors, continuous systems, and downstream purification steps [6]. The lack of industrial-scale demonstrations for underutilized crops limits commercialization.

4.6. Regulatory and Safety Concerns

The regulatory acceptance of enzyme use in food processing differs between regions [9]. For underutilized plants, safety evaluations of extracted compounds and enzymes are required to ensure compliance with food safety laws. Delays in regulatory approval can hinder product development.

4.7. Limited Research on Underutilized Species

Most research on EAE has focused on major crops such as soybean, wheat, and apples. In contrast, scientific data on underutilized plants remain limited, particularly in terms of enzyme-substrate specificity, phytochemical characterization, and bioactivity validation [17]. Bridging this knowledge gap requires dedicated funding and collaborative research.

5. Optimization Strategies for Enzyme-Assisted Extraction (EAE)

The efficiency and reproducibility of enzyme-assisted extraction (EAE) largely depend on the optimization of multiple parameters that govern enzyme activity, substrate accessibility, and mass transfer. A systematic optimization not only improves yields of bioactive compounds but also ensures cost-effectiveness and scalability for industrial applications [7].

5.1. Enzyme Selection

The choice of enzyme(s) is central to EAE success. Depending on the plant matrix and target compounds, either single enzymes or synergistic enzyme cocktails may be employed:

- **Single enzymes** (e.g., cellulase, pectinase, hemicellulase) are effective when the substrate composition is well understood and relatively simple.
- **Enzyme cocktails** can provide complementary action on multiple structural components of the cell wall, improving the release of phenolics, flavonoids, carotenoids, and other bioactives. For example, combining cellulase and pectinase enhances extraction from fruit peels by targeting both cellulose fibrils and pectin-rich middle lamella.

5.2. Process Parameters

Optimal enzymatic activity is strongly influenced by reaction conditions:

- **Temperature and pH:** Each enzyme has an activity optimum, typically ranging from 30–55°C and pH 4–6 for plant cell-wall-degrading enzymes. Deviation from these ranges can lead to enzyme denaturation or reduced activity.
- **Enzyme concentration:** While higher concentrations may increase extraction efficiency, they also raise costs. Optimization ensures a balance between yield and economic feasibility.
- **Incubation time:** Longer exposure increases the release of compounds but risks the degradation of sensitive metabolites. Thus, fine-tuning is required depending on compound stability [12].

5.3. Pretreatment of Plant Material

Pretreatment improves enzyme accessibility by disrupting physical barriers:

- **Mechanical methods,** such as milling or grinding, reduce particle size and increase surface area for enzymatic action.
- **Ultrasonication** creates cavitation effects that weaken plant cell walls, enhancing enzyme penetration.
- **Microwave pretreatment** disrupts structural integrity and partially hydrolyzes polysaccharides, making subsequent enzymatic hydrolysis more efficient.

5.4. Solid-to-Liquid Ratio

The ratio of plant material to extraction solvent affects mass transfer efficiency:

- **A low solid-to-liquid ratio** enhances diffusion of compounds but may require more solvent, increasing downstream processing costs.
- **A high ratio** may reduce efficiency due to saturation effects and reduced enzyme-substrate interactions.

5.5. Integration with Green Extraction Technologies

Recent studies highlight the benefits of combining EAE with environmentally friendly extraction methods to achieve synergistic outcomes:

• **Ultrasound-Assisted Extraction (UAE):** Ultrasonication enhances cell disruption, reducing extraction time and enzyme requirement.

• **Microwave-Assisted Extraction (MAE):** Microwaves accelerate enzyme-substrate interactions by enhancing molecular mobility and local heating.

• **Supercritical Fluid Extraction (SFE):** Coupling with EAE can selectively extract lipophilic compounds while maintaining enzyme efficiency for polar compounds [15].

5.6. Process Modeling and Optimization Tools

Modern optimization techniques such as Response Surface Methodology (RSM), Artificial Neural Networks (ANNs), and genetic algorithms are increasingly applied to fine-tune EAE parameters. These tools enable the prediction of optimal conditions with fewer experimental trials, saving time and resources.

Table 1. Examples of bioactive compounds from underutilized plants extracted using EAE

Plant Source	Enzyme(s) Used	Major Bioactive Compounds Extracted	Reported Benefits/Applications
Moringa oleifera leaves	Cellulase + Protease	Flavonoids, phenolic acids	Antioxidant, anti-inflammatory
Tomato peels	Pectinase + Cellulase	Carotenoids (lycopene, β-carotene)	Nutraceuticals, natural colorants
Grape pomace	Pectinase	Phenolic compounds, tannins	Functional beverages, antioxidants
Banana peels	Hemicellulase + Cellulase	Dietary fibers, polyphenols	Food fortification, gut health
Quinoa husks	Amylase + Protease	Proteins, saponins	Nutraceutical supplements, feed

Table 2. Comparison of conventional extraction vs. enzyme-assisted extraction

Parameter	Conventional Methods (Solvent, Heat)	Enzyme-Assisted Extraction (EAE)
Yield of bioactive compounds	Moderate to low	Higher (20–60% improvement)
Selectivity	Low	High (compound-specific release)
Energy and solvent consumption	High	Low (eco-friendly)
Compound stability	Risk of degradation	Better preservation of bioactivity
Scalability	Established, but not green	Emerging, scalable with optimization

Table 3. Optimization factors influencing EAE efficiency

Factor	Influence on Extraction Efficiency	Notes/Examples
Enzyme type	Determines specificity of cell wall breakdown	Pectinase for fruits, cellulase for leaves
Enzyme concentration	Too low = poor yield; too high = uneconomical	Requires optimization
Temperature & pH	Affects enzyme activity and stability	Optimum varies per enzyme
Solid-to-liquid ratio	Influences mass transfer and solubilization efficiency	Balanced ratio essential
Pretreatment methods	Enhance cell wall accessibility	Milling, ultrasonication, microwave

Table 4. Challenges and opportunities in EAE from underutilized plants

Challenges	Opportunities/Strategies
High enzyme cost	Use of recombinant enzymes; enzyme recycling
Variability in plant biomass	Standardization of raw material sourcing
Regulatory concerns on food safety	Clear guidelines for enzyme residues
Lack of standardized protocols	Development of plant-specific optimized methods
Limited industrial adoption	Integration with green extraction (UAE, MAE, SFE) technologies

6. Comparative Efficiency: EAE vs. Conventional Methods

Traditional extraction techniques such as maceration, Soxhlet extraction, and refluxing with organic solvents have been widely applied for recovering bioactive compounds from plant materials. While effective to some extent, these methods typically suffer from major drawbacks, including long extraction times, high solvent and energy consumption, low selectivity, and potential degradation of heat-sensitive compounds. In contrast, enzyme-assisted extraction (EAE) provides a sustainable and efficient alternative by leveraging enzymatic hydrolysis to disrupt plant cell wall barriers and liberate bioactive molecules [13].

6.1. Yield Enhancement

Numerous studies report significantly higher extraction yields with EAE compared to conventional solvent-based techniques. For example:

- **Phenolic compounds:** Pectinase-assisted extraction of grape pomace resulted in 40–60% higher recovery of phenolic compounds compared to ethanol-based solvent extraction.
- **Flavonoids:** A cellulase–protease cocktail improved flavonoid extraction from *Moringa oleifera* leaves by 35% over conventional refluxing methods.
- **Carotenoids:** Enzymatic pretreatment of tomato peels and carrot by-products enhanced carotenoid recovery by disrupting chromoplast membranes, yielding up to 45% higher levels than solvent extraction alone.

These findings highlight that EAE not only improves yield but also promotes the release of compounds that are otherwise inaccessible with conventional techniques due to rigid cell-wall matrices [14].

6.2. Solvent and Energy Efficiency

Conventional extractions often require large volumes of organic solvents (ethanol, methanol, acetone) and extended heating, raising environmental and safety concerns. EAE, by contrast, generally operates under milder conditions (30–55°C, aqueous buffers, shorter times), thus:

- Reducing solvent consumption by up to 50–70%.
- Lowering energy requirements due to reduced heating and shorter extraction durations.
- Minimizing environmental footprint, aligning with green chemistry principles.

6.3. Preservation of Bioactivity

Many phytochemicals, such as polyphenols, flavonoids, and carotenoids, are sensitive to high temperatures and harsh solvents. EAE preserves structural integrity by releasing these compounds under gentle, enzyme-optimized conditions, thereby maintaining their antioxidant, antimicrobial, and nutraceutical properties [15].

6.4. Scalability and Industrial Feasibility

One of the major advantages of EAE is its compatibility with large-scale bioprocessing. Advances in industrial enzyme production, cost reduction strategies, and integration with other green extraction techniques (e.g., ultrasound or microwave-assisted extraction) have made EAE more scalable. Pilot-scale trials in fruit juice, wine-making, and nutraceutical industries demonstrate its potential for commercial adoption [9].

6.5. Limitations Compared to Conventional Methods

While EAE shows superior efficiency, certain limitations must be acknowledged:

- Enzyme costs can be higher than solvents, especially if specialized cocktails are required.
- Enzymatic activity is highly sensitive to pH and temperature fluctuations.
- Conventional solvent extraction may still outperform EAE for certain highly nonpolar compounds (e.g., essential oils), unless combined with hybrid methods.

6.6. Comparative Summary

Overall, EAE demonstrates clear advantages in terms of yield, eco-friendliness, and product quality over conventional extraction. Nevertheless, integrating EAE with complementary technologies (e.g., supercritical fluid extraction for lipophilic compounds) often provides the best balance between efficiency, cost, and scalability [12].

7. Challenges in EAE of Underutilized Plants

Despite the clear advantages of enzyme-assisted extraction (EAE), several challenges continue to limit its widespread application, particularly in the context of underutilized plants. One of the foremost barriers is the high cost of enzymes, which becomes a significant concern when scaling up to industrial levels. Although advances in biotechnology and microbial fermentation are gradually lowering enzyme production costs, affordability remains a key obstacle to broader adoption. In addition, the inherent variability in the biochemical composition of underutilized plants—caused by seasonal, geographical, and environmental differences—makes it difficult to establish standardized extraction protocols [2-7]. This variability not only affects extraction efficiency but also complicates reproducibility and quality assurance in functional food development. Another critical issue is the lack of optimized protocols tailored specifically for underutilized species, since most EAE research has traditionally focused on well-studied crops. Moreover, regulatory hurdles pose additional challenges, as the presence of enzyme residues, food-grade safety compliance, and labeling requirements must be carefully addressed before EAE-derived products can reach the consumer market. Sustainability concerns also arise, both in terms of ensuring responsible sourcing of underutilized plant biomass and in reducing the ecological footprint of enzyme production itself.

Addressing these challenges requires a multidisciplinary approach, involving biotechnologists to engineer cost-effective enzymes, food scientists to develop standardized protocols, and policymakers to establish supportive regulations that encourage innovation while ensuring consumer safety.

8. Conclusion

Enzyme-assisted extraction (EAE) has emerged as a promising and sustainable technology for unlocking the vast reservoir of bioactive compounds present in underutilized plants. Unlike conventional solvent-based techniques, EAE enhances the yield, purity, and stability of phytochemicals while preserving their functional integrity, making it highly relevant for the development of functional foods and nutraceuticals. Through targeted enzymatic hydrolysis, cell wall barriers can be efficiently disrupted, thereby releasing valuable compounds such as polyphenols, flavonoids, carotenoids, and dietary fibers that play critical roles in promoting human health and wellness.The integration of EAE into food processing holds particular significance in addressing current global challenges. By valorizing underutilized plants, this approach supports dietary diversification, reduces dependence on staple crops, and provides new avenues for the discovery of unique bioactives with therapeutic potential. Furthermore, adopting EAE can contribute to more sustainable agricultural practices by minimizing waste and enhancing the economic value of neglected plant species. However, widespread industrial application is still hindered by challenges such as enzyme costs, variability in raw material composition, and regulatory barriers related to food safety and standardization. Advancements in biotechnology, synthetic biology, and green processing technologies are expected to reduce these barriers and improve the scalability of EAE. Multidisciplinary collaboration among scientists, industry stakeholders, and policymakers will be essential to unlock its full potential. Ultimately, leveraging underutilized plants through EAE not only enriches human diets but also contributes to sustainable food systems and global food security.

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