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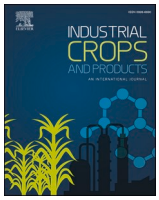

Plant-derived enzymes as sustainable biocatalysts for biosensing and industrial applications

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ABSTRACT

Plant-derived enzymes have emerged as sustainable and biocompatible alternatives to animal and microbial enzymes, with extensive applications in biotechnology, clinical diagnostics, environmental monitoring, and industrial processing. These enzymes demonstrate advantages such as low immunogenicity, environmental compatibility, substrate specificity, and operational stability under mild conditions. Additionally, plant enzymes are key biocatalysts in biosensor development, enabling real-time detection of analytes such as urea, glucose, pesticides, and pharmaceuticals through enhanced electrochemical performance. Industrially, plant enzymes replace hazardous chemicals in food, textile, and biofuel sectors, promoting greener production practices. Recent advancements in enzyme engineering and molecular farming have further enhanced enzyme yield and specificity, broadening their applicability. This review comprehensively explores the sources, extraction methods, and functional properties of various bioactive enzymes, including amylase, pectinase, cellulase, laccase, asparaginase, galactosidase, actinidin, urease, and others, derived from diverse plant tissues. By emphasizing their biochemical versatility and environmental benefits, this review highlights the growing strategic importance of plant-derived enzymes in fostering eco-friendly innovations across health, industry, and environmental domains.

1. Introduction

Enzymes are biological catalysts that enhance biochemical reactions in living organisms. They play crucial roles in diverse industries including synthesis of sweeteners, modification of antibiotics, detergents, food processing, etc. Enzymes are also essential for analytical equipment and tests with clinical, forensic, and environmental applications (Maghraby et al., 2023). While microbial and animal-derived enzymes have long dominated these fields, rising concerns about sustainability, biosafety, ethical sourcing, and biocompatibility have sparked widespread interest in plant-based enzymes (Hood, 2002; Morton, 1955; Sharma and Upadhyay, 2020). These enzymes, sourced from various plants, have notable benefits compared to their animal and microbial equivalents, possessing enhanced stability, reduced allergenicity, increased environmental sustainability and adherence to green chemistry concepts (David Troncoso et al., 2022; González-Rábade et al., 2011; Malila et al., 2024; Patel et al., 2012a, 2012b). Plant-derived enzymes are increasingly recognized for their considerable higher catalytic activity, stability under extreme conditions, and relatively straightforward extraction and purification processes. Enzymes, derived from plant parts like seeds, leaves, fruits, and roots, are crucial for plant metabolic and defensive mechanisms, and have significant industrial and medical applications (Kar et al., 2017; Tomar et al., 2014). The primary advantage of plant-based enzymes over animal-derived enzymes is their lower immunogenicity and improved biocompatibility which make them more suitable for therapeutic and diagnostic uses (Kuriakose et al., 2016; Tandon et al., 2021). Animal enzymes may sometimes trigger allergic or immunological responses, thereby limiting their clinical use (Basketter and Kimber, 2022). While plant enzymes align with dietary restrictions, religious practices, and vegan principles, thereby increasing their acceptance among diverse global communities (Gamage et al., 2024). Plants can be cultivated in

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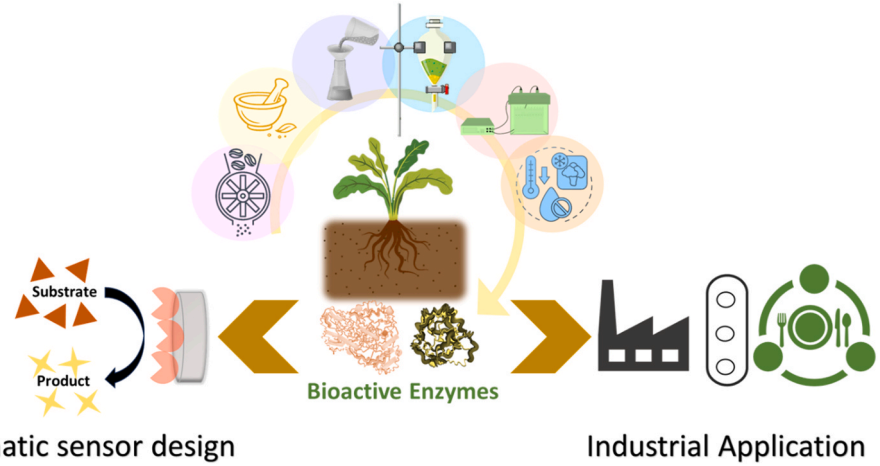

Scheme 1. Extraction, purification, and application of plant-based enzymes in biosensor probe design and industrial related applications.

controlled environments, easy availability without any ethical and welfare concerns compared to animal use (Aït-Kaddour et al., 2024) (Chen et al., 2024). Moreover, their long shelf life, broad substrate specificity, and natural resistance to proteolytic degradation enhance their utility in complex biochemical reactions (González-Rábade et al., 2011; Ndochinwa et al., 2024). In addition, relying on animal-based enzymes like trypsin, pepsin, and rennet, as well as microbial fermentation products like bacterial amylases and fungal proteases, raises potential ethical concerns, high manufacturing costs, and the enhanced possibility of immunogenic responses (Singh et al., 2018). Plant-derived enzymes, in contrast, provide a renewable, cost-effective, and biocompatible alternative (Asadian et al., 2024; do Nascimento et al., 2025). Altogether, plant-derived enzymes may serve as excellent resources for several applications ranging from biosensors, blood clotting, wound healing, detergent, food processing and industrial biocatalysis.

Plant-based enzymes have shown significant potential in the development of cost-effective, robust, and eco-friendly biosensors for clinical diagnostics, food safety, and environmental monitoring (Seth and Meena, 2024). These bioactive enzymes are versatile catalysts with a wide range applications across multiple sectors due to their high substrate specificity, eco-friendliness, and functional compatibility with various bioprocesses (Bell et al., 2021; Radley et al., 2023). Their natural origin and ability to operate under mild conditions have led to increasing interest in their utilization for various applications. In biomedical contexts, plant enzymes offer promising opportunities for therapeutic intervention, particularly in metabolic and immune-related disorders (Salehi and Rashidinejad, 2025). Such enzymes can catalyze targeted biochemical reactions, modulate metabolic pathways, and enhance drug bioavailability. Plant enzymes are also being investigated for their potential in enzyme replacement therapies and the design of biopharmaceutical formulations. In biosensing, enzymes are increasingly integrated into analytical platforms designed for rapid and sensitive detection of clinically and environmentally relevant analytes (*Biosensing and Micro-Nano Devices*, 2022). The ability to catalyze redox or hydrolytic reactions, generates measurable signals which are fundamental to sensor development. Enzyme-based biosensors utilizing plant enzymes have shown potential in enhancing signal fidelity, operational stability, and biocompatibility. In industrial applications, plant enzymes are widely applied to optimize production processes by replacing harsh chemical treatments with more sustainable biological alternatives. They facilitate efficient substrate conversion, aid in the removal of unwanted by-products, and improve product yield and quality. Industries such as food and beverage, textiles, paper, and biofuels benefit significantly from plant-based enzymatic processing due to their specificity and cost-effectiveness. From an environmental perspective, plant enzymes' biodegradability and non-toxic nature make them ideal for bioremediation and waste treatment processes (Kumar et al., 2018). Their ability to catalyze the breakdown of organic pollutants, dyes, and xenobiotics contributes to reducing the environmental footprint of industrial effluents. Continuous advancements in biotechnology, such as enzyme engineering, molecular farming, and recombinant expression systems may enhance the yield, specificity, and stability of plant-based enzymes (Chandra and Panesar, 2022; Dkhar et al., 2022; Mahato et al., 2022; Saini et al., 2022). This broadens their functional landscape and enables their use in high-precision applications.

Although plant-derived proteins are gaining acknowledgment for their sustainability and functional capabilities, the extraction of plant enzymes poses problems that may hinder their efficient application (Karabulut et al., 2025). Significantly, poor yield persists as a major constraint owing to the intrinsically low enzyme concentrations in plant tissues and the losses sustained during mechanical disruption and purification (Bhatia, 2018). Furthermore, plant enzymes frequently demonstrate instability, especially under fluctuating pH and temperature conditions seen during processing, which might reduce their catalytic effectiveness and longevity (Robinson, 2015). Moreover, the existence of interfering chemicals, including polyphenols, alkaloids, and other secondary metabolites, might bind to enzymes or denature them, so diminishing activity and complicating subsequent purifying processes (Adrar et al., 2019; Ndochinwa et al., 2024). These challenges need the refining of extraction processes and the integration of stabilizing agents or purifying methods to guarantee the constant functioning and bioactivity of plant-derived enzymes in culinary and industrial applications. Confronting these issues is essential for completely actualizing the promise of plant proteins and enzymes in sustainable product development. Although enzymes are progressively utilized to enhance the structural and functional properties of plant-based meat, the direct extraction of these enzymes from plant sources has several intrinsic obstacles (Kleekayai et al., 2023). The enzyme output from plant tissues is frequently minimal due to their inherently restricted abundance and the challenges associated with isolating active forms without degradation (Liu et al., 2023). Secondly, enzymes produced from plants often exhibit instability under industrial processing conditions, including elevated temperature, pressure, or pH variations, resulting in a fast decline in catalytic activity (Karabulut et al., 2024). Third, the existence of interfering plant metabolites such as phenolic compounds, tannins, and alkaloids can bind to or inactivate enzymes, hence complicating purification and diminishing efficiency (Karabulut et al., 2024). These constraints constrain the repeatability and scalability of employing natural plant enzymes in food processing. Consequently, measures such as enhancing extraction efficiency, integrating stabilizers, or utilizing recombinant expression platforms (e.g., microbial or yeast hosts) are crucial for boosting enzyme stability, activity, and availability for

reliable utilization in plant-based meat production.

By exploring current advancements and identifying future research directions, we seek to highlight the significance of plant enzymes in various sectors and their role in promoting sustainable industrial practices. This review comprehensively explores the diverse enzymes extracted from various plant sources, with a special emphasis on the application of indigenously extracted enzymes. It highlights the unique biochemical properties, extraction and purification methodologies, and their integration into innovative biosensing platforms. Furthermore, the review also delves into their industrial relevance, showcasing how these eco-friendly and locally sourced biocatalysts contribute to sustainable practices in diverse industrial sectors such as food, textiles, bioremediation, and biofuels. Scheme 1

2. Plant derived bioactive enzymes: sources, extraction and purification

The increasing desire for sustainable and eco-friendly bioengineering solutions has highlighted plant-derived bioactive enzymes as feasible alternatives to traditional animal and microbial enzymes. Plants provide plentiful, renewable, ethically acceptable sources of enzyme molecules that demonstrate exceptional catalytic efficiency, operational stability, and extensive substrate specificity. The extraction and application conform to green chemistry principles, resulting in decreased environmental impact and improved biocompatibility. This section showcases specific examples of plant-derived enzymes that have been effectively extracted from diverse plant sources such as seeds, leaves, fruits, and roots using efficient and scalable techniques. Each example underscores particular extraction technique, purification methodologies, and the prospective uses of the enzymes. This section emphasizes that plant enzymes, including amylase, cellulase, and laccase, not only meet functional needs but also promote sustainable innovation across multiple sectors.

2.1. Amylase

Amylases are hydrolase enzymes that catalyze the hydrolysis of glycosidic bonds in starch, resulting in the formation of dextrins and oligosaccharides (Farooq et al., 2021). To extract plant-derived amylases, a variety of seed sources and protocols have been employed (Ben Elarbi et al., 2009; Chang et al., 2025; Nasir et al., 2025; Singh and Kayastha, 2014). For instance, in the case of barley, seeds were initially soaked and placed in trays lined with moist gauze. These were kept in a well-ventilated room at 25 °C and watered daily. Once the sprouts reached approximately 0.5 cm, the gauze was removed, and after attaining a length of 1 cm, the trays were transferred to light conditions. After 7 days, the germinated malt seeds were harvested, ground, and subjected to ultrasonication followed by filtration. The resulting filtrate was centrifuged, and the supernatant containing crude amylase was stored at 4 °C for enzymatic activity analysis (Chang et al., 2025). The extraction of β-amylase from peanut seeds (*Arachis hypogaea*) involved overnight soaking, homogenization, filtering, centrifugation, 40 % acetone fractionation, acid precipitation, glycogen-based affinity precipitation, ethanol addition, centrifugation, glycogen addition, re-suspended pellet, glycogen removal, size-exclusion chromatography, dialyzed overnight, and concentration for further use. The enzyme was then subjected to size-exclusion chromatography, dialyzed overnight, and concentrated for further use (Das and Kayastha, 2018). Similarly, for the isolation of β-amylase from fenugreek (*Trigonella foenum--graecum*) seeds, the dry seeds were surface sterilized, soaked in extraction buffer, and germinated overnight in the dark over moist filter papers at room temperature. The germinated seeds were homogenized, and the crude extract was obtained after filtration through muslin cloth and centrifugation. This supernatant was then subjected to a sequential purification process involving acetone precipitation, ion-exchange chromatography, and glycogen affinity chromatography to obtain a purified enzyme preparation (Srivastava and Kayastha, 2014).

Amylases play a pivotal role in starch saccharification by catalyzing the breakdown of complex polysaccharides into simpler sugars such as glucose and fructose (Nwagu et al., 2021). These saccharified products, particularly fructose syrups, are widely utilized as artificial sweeteners in the beverage industry due to their high sweetness index and cost-effectiveness. In the baking industry, amylases serve as anti-staling agents, preserving the softness and texture of bread and thereby extending the shelf life of various baked goods (Giannone et al., 2016). In detergent formulations, amylases enhance cleaning efficacy by breaking down starchy stains (Herrera-Márquez et al., 2019). In the textile industry, starch is used to strengthen warp yarns during weaving, and amylases are later employed to remove this starch without damaging the fabric (Farooq et al., 2021). Beyond industrial applications, amylases also hold significant relevance in human health and nutrition (Jothyswarupha et al., 2024). Since amylases are crucial for the digestion and absorption of dietary polysaccharides, their activity directly influences postprandial glycemic responses (Warren et al., 2015). Therefore, inhibiting amylase activity can delay carbohydrate digestion and absorption and blunt the glycemic peak, making amylase as a therapeutic target for glycemic control and weight management (Peddio et al., 2022). In addition to their metabolic significance, β-amylases also exhibit noteworthy antioxidant properties, attributed to their ability to hydrolyze polysaccharides and release bound phenolic compounds, which are known for their free radical scavenging activity (Das and Kayastha, 2019; Smith et al., 2002). This dual functionality as a digestive enzyme and a source of bioactive molecules underscores the clinical potential of plant-derived β-amylase in the development of functional foods and nutraceuticals (Das and Kayastha, 2018).

2.2. Pectinase

Pectinases are a group of enzymes involved in the degradation of pectin, a structural polysaccharide found in plant cell walls (Voragen et al., 2009). Such enzymes are widely studied for its applications in food, pharmaceutical, and waste valorization industries. Various plant sources, including fruit peels and pulps, have been explored for the extraction of pectinase due to their rich enzyme content and accessibility (Riyamol et al., 2023; Roman-Benn et al., 2023). For instance, lemon peels were processed to obtain pectinase by initially separating the albedo portion, which was then chopped into fine pieces and dried at 40 °C for 12 h. The dried material was ground into a coarse powder and subsequently subjected to centrifugation. The resulting supernatant was collected and used for the analysis of pectinase activity, representing a straightforward method for crude enzyme extraction (Bakshi and Ananthanarayan, 2022). In another study, the pulp of soursop (*Annona muricata*) and cherimoya (*Annona cherimola*) fruits was used as the enzymatic source. After manually removing the seeds, the pulps were mixed with phosphate and acetate buffers and homogenized for 1 h in an ice bath under constant stirring to preserve enzyme integrity. The homogenate was then centrifuged, and the supernatant was filtered to remove particulate matter. The crude extract was stored at 4 °C in amber bottles to prevent enzyme degradation by light. For purification, acetone precipitation was carried out at 4 °C, and the resulting precipitate was dried at room temperature in a desiccator. Further purification was achieved using ion-exchange chromatography (IEC), allowing for the isolation of highly active pectinase fractions from the fruit pulp sources (García et al., 2024).

Pectinases are widely employed in both industrial and biomedical sectors due to their ability to degrade complex pectic polysaccharides into bioactive and functional components (Barrera-Chamorro et al., 2025). In the pharmaceutical domain, pectinase has been utilized to derive valuable therapeutic products from fruit and vegetable peels. This enables the production of colon-targeted drug delivery systems such as colon-specific tablets, where pectin serves as a biodegradable matrix to ensure site-specific release in the large intestine (Barrera-Chamorro

et al., 2025; Liu et al., 2003). In the food and beverage industry, pectinase plays a critical role in juice clarification, where it eliminates colloidal pectin-induced cloudiness, resulting in clear, visually appealing juices. Similarly, in winemaking, pectinase enhances the clarity, aroma, and taste of the final product by breaking down haze-forming pectic substances (Shrestha et al., 2021). In the textile industry, pectinase is employed in the bio-scouring of cotton, where it removes non-cellulosic impurities from cotton fibers (Aggarwal et al., 2020). This enzymatic treatment not only improves the aesthetic appearance and dye uptake of the fabric but also increases its water absorption capacity, offering an eco-friendly alternative to harsh chemical scouring methods. Emerging research has also revealed the potential of pectinase in biomedical applications through the extraction of bioactive polysaccharides. For instance, pectinase-assisted digestion of citrus peel facilitated the isolation of a bioactive fraction known as CPE-II, which demonstrated a significant anti-metastatic effect (81.3 %) in lung cancer models (Park et al., 2017). Similarly, pectinase-mediated extraction of lotus leaf polysaccharide (LLP) exhibited remarkable antioxidant activity, effectively reducing the levels of malondialdehyde (MDA) and reactive oxygen species (ROS), while enhancing the activities of catalase (CAT) and superoxide dismutase (SOD). Additionally, LLP displayed anti-inflammatory potential by upregulating the production of anti-inflammatory cytokines, highlighting its therapeutic promise in oxidative stress and inflammatory conditions (Xu et al., 2024).

2.3. Cellulase

Cellulases are a group of hydrolytic enzymes that catalyze the breakdown of cellulose into glucose units, playing a vital role in biomass conversion, textile processing, and the production of biofuels and functional food ingredients. In a recent study, cellulase was extracted from mulberry (*Morus alba*) leaf powder using ultrasound-assisted techniques to enhance enzyme recovery and activity. Initially, the mulberry leaf powder was dissolved in distilled water, and the pH was adjusted to 5.0, an optimal condition for cellulase stability and performance. Three different ultrasound-assisted extraction approaches were investigated: single-frequency ultrasonic extraction (SUE), dual-frequency ultrasonic extraction (DUE), and multi-frequency ultrasonic extraction (MUE). In the SUE method, the sample was sonicated at 40 kHz, facilitating the disruption of plant cell walls and improving enzyme release. Protein precipitation was then carried out via the alkaline solubilization and acid precipitation method, a widely used technique that involves solubilizing proteins under alkaline conditions and subsequently precipitating them by acidifying the solution. The DUE method employed the same extraction principle but used a dual-frequency setup (28 kHz + 40 kHz), which generated more intense acoustic cavitation for enhanced extraction efficiency. In the MUE method, a combination of three ultrasonic frequencies (22 kHz + 28 kHz + 40 kHz) was applied, providing a synergistic effect that maximized cellulase release. Following sonication in each method, the solution's pH was re-adjusted to 5.0, and enzymatic treatment was conducted at 40 °C for 30 min, maintaining a final enzyme concentration of 0.66 g/L. A control extraction, using traditional alkaline methods without ultrasound, was also performed for comparative assessment. The results highlighted that multi-frequency ultrasonication significantly improved cellulase yield and activity, demonstrating its potential as an efficient and sustainable extraction strategy for plant-derived enzymes (Zhao et al., 2023).

Cellulase is an industrially significant enzyme that catalyzes the hydrolysis of β-1,4-glycosidic bonds in cellulose, leading to the breakdown of cellulosic biomass into fermentable sugars, primarily glucose (Ranjan et al., 2023). This enzymatic conversion is a cornerstone in the production of second-generation bioethanol, where the sugars released from lignocellulosic waste are fermented to generate ethanol, offering a sustainable alternative to fossil fuels (Roy Choudhury et al., 2024). In the pulp and paper industry, cellulases are employed in multiple stages including pulping, refining, deinking, dewatering, and bio-bleaching (Barrios et al., 2024). Their applications improve fiber modification, brightness, and paper quality while reducing the need for harsh chemicals. Particularly in kraft pulp bio-bleaching, cellulase treatment facilitates the removal of hemicellulose and surface impurities, thereby improving the efficiency of downstream bleaching processes (Wang et al., 2015). In the brewing industry, cellulase plays a crucial role during malting, a key step in beer production (Chakraborty et al., 2016). Its application ensures uniform malt quality regardless of seasonal variations in barley crops, improving fermentability and yield. The textile industry also benefits from cellulase applications in eco-friendly and sustainable processing techniques such as cotton softening, denim finishing, and biopolishing (de Souza Moreira et al., 2016). These processes enhance the texture, appearance, and durability of fabrics by removing surface microfibrils without damaging the underlying fibers. Furthermore, cellulase-mediated degradation of plant cell walls facilitates the release of intracellular bioactive compounds such as flavonoids, chlorophyll, carotenoids, and other phytochemicals (Singh et al., 2021). Such compounds hold substantial commercial value in the food, pharmaceutical, and cosmetic industries due to their antioxidant, antimicrobial, and health-promoting properties (Singh et al., 2021).

2.4. Laccase

Laccases are multicopper oxidases capable of catalyzing the oxidation of a wide range of phenolic and non-phenolic substrates using molecular oxygen, with water as the only by-product (Mayolo-Deloisa et al., 2020). These enzymes are extensively studied for their applications in bioremediation, textile dye decolorization, biosensors, and pharmaceutical industries (Jeyabalan et al., 2023; Yang et al., 2017). Although fungi are well-known producers of laccase, recent efforts have focused on plant-derived laccases as sustainable and eco-friendly alternatives (Sodhi et al., 2024). In one study, laccase was extracted from Malva sylvestris by homogenizing the plant material in acetate buffer, followed by cooling and centrifugation to obtain a crude enzyme extract. For purification, the extract was subjected to Sephadex G-150 gel filtration chromatography, an effective method for size-based separation of proteins. The Sephadex G-150 matrix was initially hydrated in distilled water at 90 °C for 3 h, then cooled at 4 °C overnight to ensure complete swelling. The column was equilibrated with phosphate buffer, and the laccase extract was carefully loaded. Elution was carried out at a flow rate of 20 mL/hour, and 3 mL fractions were collected. The protein content of each fraction was quantified spectrophotometrically at 280 nm, and laccase activity was measured using standard oxidation assays (e.g., ABTS or guaiacol as substrates). Active fractions were pooled, concentrated, and stored at –20 °C for subsequent biochemical and kinetic characterization (Aziz et al., 2023). Other plant sources of laccase include *Syzygium cumini*, *Zea mays*, *Triticum aestivum*, and *Pisum sativum*, where laccases have been isolated predominantly from roots, stems, and germinating seeds (Bai et al., 2023). In *Zea mays*, for instance, laccase-like activity was detected in coleoptile segments during seedling development and is believed to be involved in lignification and stress response mechanisms (Gao et al., 2012; Xie et al., 2020).

Plant laccases are copper-containing oxidases that play a central role in lignin biosynthesis by catalyzing the oxidation of monolignols, leading to their polymerization into lignin, a critical structural component of plant cell walls (Bai et al., 2023). Lignin provides mechanical strength, hydrophobicity, and resistance against pathogens, thereby supporting plant growth, vascular development, and responses to abiotic and biotic stresses(Bai et al., 2023). Beyond their physiological role in plants, laccases have garnered considerable interest for their biotechnological potential, particularly in environmental remediation. Through their ability to catalyze one-electron oxidation reactions, laccases can break down a wide range of synthetic dyes, including azo, anthraquinone, and triphenylmethane dyes, which are otherwise recalcitrant and persistent

in the environment (Bhardwaj et al., 2022; Saha and Mukhopadhyay, 2020). The efficacy of dye degradation by laccase is highly dependent on the chemical structure of the dye, with lower molecular weight and simpler configurations typically being more susceptible to enzymatic oxidation (Jeyabalan et al., 2023; Othman and Flaifil, 2025). Importantly, crude plant laccase extracts have often demonstrated superior performance compared to purified enzymes in practical applications (Aziz et al., 2023). This is attributed to the presence of natural stabilizers and synergistic cofactors in crude extracts, which enhance enzyme robustness and functional stability under industrial conditions (Aziz et al., 2023). Moreover, the lower production costs and minimal processing requirements make crude extracts a more economically viable and scalable option for large-scale bioremediation efforts. By combining broad substrate specificity, operational simplicity, and eco-compatibility, plant-derived laccases offer a promising and sustainable tool for the treatment of dye-contaminated industrial effluents, contributing significantly to green chemistry and environmental protection (Aziz et al., 2023).

2.5. Asparaginase

L-asparaginase is a therapeutic enzyme that catalyzes the hydrolysis of L-asparagine into aspartic acid and ammonia (Verma et al., 2007). It has gained significant attention due to its application in the treatment of acute lymphoblastic leukemia (ALL) and potential use in the food industry to reduce acrylamide formation (Patel et al., 2022). To extract L-asparaginase from *Asparagus racemosus*, fresh plant tissue was thoroughly homogenized using a mortar and pestle. The homogenate was then transferred to a magnetic stirrer and gently stirred for 10 min to ensure proper solubilization of the enzyme. Following this, the mixture was filtered to remove coarse plant debris and centrifuged at 10,000 × g for 15 min at 4 °C to further clarify the extract. The resulting supernatant was designated as the crude plant extract and stored at 4 °C for subsequent L-asparaginase activity assays and protein quantification (Beulah and Hemalatha, 2019). In another method, dried soybean (*Glycine max*) debris was processed to obtain L-asparaginase through a more buffered approach. The dried biomass was first ground into a fine powder to increase the surface area for extraction. The powdered material was homogenized in a potassium phosphate buffer (pH 7.5) supplemented with sodium chloride, phenylmethylsulfonyl fluoride (PMSF) as a protease inhibitor, ethylenediaminetetraacetic acid (EDTA) to chelate metal ions, and glycerol as a stabilizer. The homogenate was subjected to centrifugation at 12,000 × g for 20 min at 4 °C, and the resulting supernatant was collected as the crude L-asparaginase enzyme extract for further purification or biochemical analysis (Al-Hazmi and Naguib, 2022).

L-asparaginase is a clinically significant enzyme widely utilized in the treatment of hematological malignancies, particularly ALL (Kumar et al., 2014; Shrivastava et al., 2016). Its therapeutic potential lies in its ability to catalyze the hydrolysis of L-asparagine into L-aspartic acid and ammonia, thereby depriving rapidly proliferating tumor cells of a critical nutrient. Cancerous cells, especially leukemic lymphoblasts, exhibit low or absent expression of asparagine synthetase, rendering them incapable of synthesizing L-asparagine de novo (Grima-Reyes et al., 2022)(Radadiya et al., 2020). As a result, they rely heavily on extracellular sources for survival and proliferation. The administration of L-asparaginase leads to a systemic depletion of circulating L-asparagine, causing metabolic stress in tumor cells, disrupting protein synthesis, and ultimately inducing apoptosis (Feng et al., 2025). This selective mechanism of action allows L-asparaginase to target malignant cells while sparing normal cells that can synthesize asparagine. Owing to these properties, L-asparaginase has been established as a cornerstone in chemotherapeutic regimens for ALL and is being investigated for its potential application in treating other solid tumors and reducing acrylamide formation in food processing (Al-Hazmi and Naguib, 2022). These approaches highlight the potential of diverse plant species as eco-friendly and low-cost sources of L-asparaginase, which may offer fewer immunogenic side effects compared to bacterial counterparts and thus warrant deeper exploration for pharmaceutical and industrial applications.

2.6. Glycosyl hydrolases (GHs)

The glycosyl hydrolase enzymes specifically hydrolyze various oligosaccharides, polysaccharides, and their conjugates. Based on amino acid sequence resemblance there are presently 190 glycosyl hydrolase classes available on the regularly updated CAZY web server (CAZy: http://www.cazy.org/), indicating its diversity for substrate and need of selective cleavage of the glycosidic linkage. They serve a crucial role in several biological processes across nearly all living creatures. In mammals, liver cytosolic β-glucosidase is an enzyme that metabolizes xenobiotics by hydrolyzing flavonoid glucosides, whereas defects in specific glycosidases can lead to inherited lactose intolerance (Berrin et al., 2003), demonstrating potential benefits for individuals with Gaucher and Krabbe illnesses (Schiffmann and Brady, 2002). The different glycosyl hydrolase enzymes that are potentially important are described in the sub-section below:

2.6.1. α-Galactosidase

α-Galactosidase is an important enzyme involved in the hydrolysis of α-galactosidic bonds, which are present in various oligosaccharides and polysaccharides, including raffinose and stachyose (Gote et al., 2004). To extract α-galactosidase from tomato (*Solanum lycopersicum*), the peels and seeds were first removed. The remaining tissue was then homogenized in citrate buffer. The homogenate was subjected to filtration using two layers of cheesecloth to remove coarse particles. The resulting suspension was placed on a magnetic stirrer for gentle mixing before being centrifuged. The supernatant, containing soluble proteins, was collected and subjected to ammonium sulfate precipitation to selectively isolate the enzyme. The mixture was stirred overnight at 4 °C, followed by centrifugation to collect the precipitate. The resulting pellet was dissolved in citrate buffer and dialyzed overnight against the same buffer to remove small molecular contaminants. The dialysate was then centrifuged again to eliminate any remaining insoluble particles. The supernatant obtained after centrifugation represented the crude tomato α-galactosidase extract. To further purify the enzyme, the crude extract underwent three-phase partitioning. In this process, the extract was mixed with ammonium sulfate and t-butanol, vortexed thoroughly, and then centrifuged. The interfacial layer, which contained the α-galactosidase enzyme, was carefully collected, dissolved in citrate buffer, and analyzed for enzymatic activity. The optimum duration for this three-phase partitioning step was determined to be 1 h (Çalci et al., 2009). In a similar manner, α-galactosidase was extracted from the seeds of white chickpea (*Cicer arietinum*). The seeds were soaked in extraction buffer, then homogenized in a blender. The homogenate was passed through a muslin cloth, and the filtrate was centrifuged to obtain a clear supernatant. This supernatant was subjected to acid precipitation and ammonium sulfate fractionation to concentrate the enzyme. The obtained pellet was dialyzed to remove salts and small molecules. To further purify the enzyme, the dialyzed extract was subjected to a series of chromatographic techniques, including hydrophobic interaction chromatography, ion-exchange chromatography, and affinity chromatography. The active fractions were pooled, concentrated, and analyzed for α-galactosidase activity (Singh and Kayastha, 2012).

One of the primary nutritional challenges associated with legume consumption, particularly in soybeans and other pulses, is the presence of raffinose family oligosaccharides (RFOs) (Elango et al., 2022). These oligosaccharides are not digestible in human gastrointestinal tract due to the absence of endogenous α-galactosidase activity (Gasiński et al., 2022). As a result, undigested RFOs reach the colon, where they undergo fermentation by intestinal microbiota, leading to the production of gases that cause gastrointestinal discomfort and flatulence (Mutuyemungu

et al., 2023). The enzymatic application of α-galactosidase provides a biotechnological solution to this problem. It effectively hydrolyzes terminal α-1,6-linked galactose residues from RFOs under mildly acidic conditions and moderate temperatures, making it suitable for integration into food processing workflows (Bangoria et al., 2023). This not only enhances the digestibility of legume-based products but also improves consumer acceptance by reducing post-consumption discomfort. The enzyme can be applied during pre-soaking or fermentation steps to enzymatically reduce RFO levels in food matrices (Falkoski et al., 2024).

Beyond nutritional applications, α-galactosidase also finds extensive utility in industrial bioprocessing (Xavier et al., 2018). It is employed to hydrolyze galactose-containing polysaccharides such as galactomannans, which are widely used as thickening and gelling agents in the food, pharmaceutical, and cosmetic industries (Singh and Sambyal, 2023; Yang and Xu, 2018). Controlled enzymatic degradation by α-galactosidase leads to a reduction in viscosity, texture modification, and improved processability of plant-derived extracts (Çalci et al., 2009). In the sugar industry, raffinose poses a significant challenge by interfering with sucrose crystallization, especially during the processing of sugar beet. The incorporation of α-galactosidase enables the enzymatic breakdown of raffinose, thereby enhancing sucrose recovery efficiency and improving yield and purity in downstream processing (Çalci et al., 2009; Martins et al., 2023).

2.6.2. *β-glucosidase*

β-D-glucosidase enzymes facilitate the hydrolysis of β-glycosidic bonds in β-D-glucosides, resulting in the release of glucose. It is essential in the last stage of cellulose breakdown, transforming cellobiose along with other short-chain oligosaccharides into monomeric glucose, and has considerable commercial benefits. The predominant flavorful chemicals, monoterpenes, and those produced from the shikimate pathway, accumulate as odorless β-D-glucosides and diglycosidic conjugates in fruits as well as plant tissues. These enzymes may be employed to improve flavor by enzymatic hydrolysis and have applications throughout a broad aromatic spectrum in wine, fruit juice, and tea. Furthermore, β-glucosidase serves as a valuable resource for augmenting the nutritional quality of many food products by transforming isoflavone glycosides into their aglycones, facilitating biomass conversion and bioethanol generation. β-galactosidases hydrolyze lactose into glucose and galactose, which are extensively utilized in the food business, especially in dairy, for the manufacturing of lactose-free or low-lactose milk and the creation of probiotic food products such as galactooligosaccharides. While several β-galactosidases have been described, attention has recently switched to thermostable β-galactosidase. The hydrolysis of lactose at elevated temperatures reduces undesirable microbial proliferation during the preparation of milk and dairy products. Patel et al. extracted an enzyme from the seeds of Putrajaya roxburghii that demonstrates β-glucosidase, β-galactosidase, and β-fucosidase activity, possessing a half-life of 40 min at an optimal temperature of 65 °C, so classifying it as a thermostable enzyme. (Kar et al., 2017; Patel et al., 2012a). Such thermostable enzyme, exhibiting triple activity and enhanced catalytic efficiency, may be utilized for multiple beneficial applications, including the synthesis of novel biofuel feedstocks and the processing of cellulose or lactose-containing by-products in the food and wine sectors. The isolation and characterization of thermostable glycosidase is particularly advantageous for the dairy, food, beverage, and bioenergy industries.

2.7. *Actinidin*

Actinidin is a cysteine protease predominantly found in kiwi fruit (*Actinidia sp*), where it plays a crucial role in the degradation of proteins, particularly during fruit ripening (Boland, 2013). This proteolytic enzyme is known for its ability to break down complex proteins and is of significant interest in both food processing and biotechnology. Actinidin is widely utilized in the meat tenderization process, as well as in the extraction of valuable bioactive peptides and proteins, thereby contributing to various industrial applications (LEWIS and LUH, 1988). To extract actinidin, the whole kiwi fruit was first processed in a food processor, along with an equal volume of ice-cold extraction buffer containing sodium metabisulfite to prevent browning reactions. The resulting mixture was then subjected to centrifugation at low temperature, and the supernatant, which contained the crude enzyme, was collected and stored at 4°C for subsequent use. The crude extract was then subjected to 90 % fractional precipitation using ammonium sulfate ($(NH_4)_2SO_4$) at a steady temperature of 4°C, with gentle stirring to ensure proper mixing. The mixture was kept under continuous stirring overnight on a magnetic stirrer. After this, the solution was centrifuged to separate the precipitated protein. The pellet was resuspended in a small amount of extraction buffer and subjected to dialysis in a cellulose membrane against the same buffer to remove any small molecular impurities and salts. The protein concentration of the dialysate was determined using the Bradford method. The dialysate was then loaded onto a DEAE Sepharose column, and the elution process was carried out by applying a NaCl gradient in sodium phosphate buffer. Active protein fractions were collected based on their elution profile, and their enzyme activity and protein content were analyzed. Fractions showing significant actinidin activity were pooled, concentrated, and stored at 4°C for further analysis. The purity of the enzyme was confirmed using Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI--TOF) mass spectrometry, which provided detailed molecular characterization of the enzyme (Dhiman et al., 2021).

Actinidin is known for its ability to degrade a wide range of proteins, making it an important tool in various biotechnological applications. One such application is its interaction with α-amylase, an essential enzyme found in saliva and pancreatic juice, which plays a key role in the breakdown of starch into simple sugar residues. Alpha-amylase cleaves the glycosidic bonds in starch, converting it into smaller saccharides such as disaccharides and trisaccharides, which are further hydrolyzed to glucose. However, actinidin is highly effective in inhibiting alpha-amylase activity, thereby impairing starch digestion and limiting glucose production. This inhibitory effect can be particularly useful in controlling the rate of carbohydrate absorption, contributing to dietary management and controlling conditions such as diabetes (Dhiman et al., 2021). Another notable application of actinidin lies in its ability to degrade gluten, a protein primarily found in cereals. Gluten is resistant to digestion by gastrointestinal tract (GIT) enzymes due to the presence of glutamine and proline residues, which protect the protein from enzymatic hydrolysis. This resistance can result in the release of gluten peptides into the small intestine, which may trigger immunogenic responses in sensitive individuals, leading to conditions such as celiac disease or gluten intolerance. Actinidin's ability to hydrolyze proline-containing peptide bonds in gluten makes it a promising candidate for gluten degradation, potentially reducing its immunogenicity and offering therapeutic benefits for individuals with gluten-related disorders (Martin et al., 2022). Additionally, actinidin has been shown to lower the antigenicity of specific milk proteins, including caseins and β-lactoglobulin, which are commonly implicated in milk allergies. These milk proteins can trigger IgE and IgG-mediated allergic reactions, particularly in infants. By degrading the immunogenic regions of these proteins, actinidin may help mitigate the allergic response, offering a potential therapeutic approach for managing milk allergies. This property of actinidin underscores its relevance in food allergy management and allergen reduction in dairy products (Kaur et al., 2022).

2.8. *Urease*

Urease, a nickel-dependent metalloenzyme, catalyzes the hydrolysis of urea 10^{14}-fold compared to standard urea hydrolysis, yielding ammonia and carbon dioxide (Singh et al., 2017). In one study, urease was successfully extracted and purified from germinating *Pisum sativum* L. seeds using a multi-step protocol that preserved its integrity and

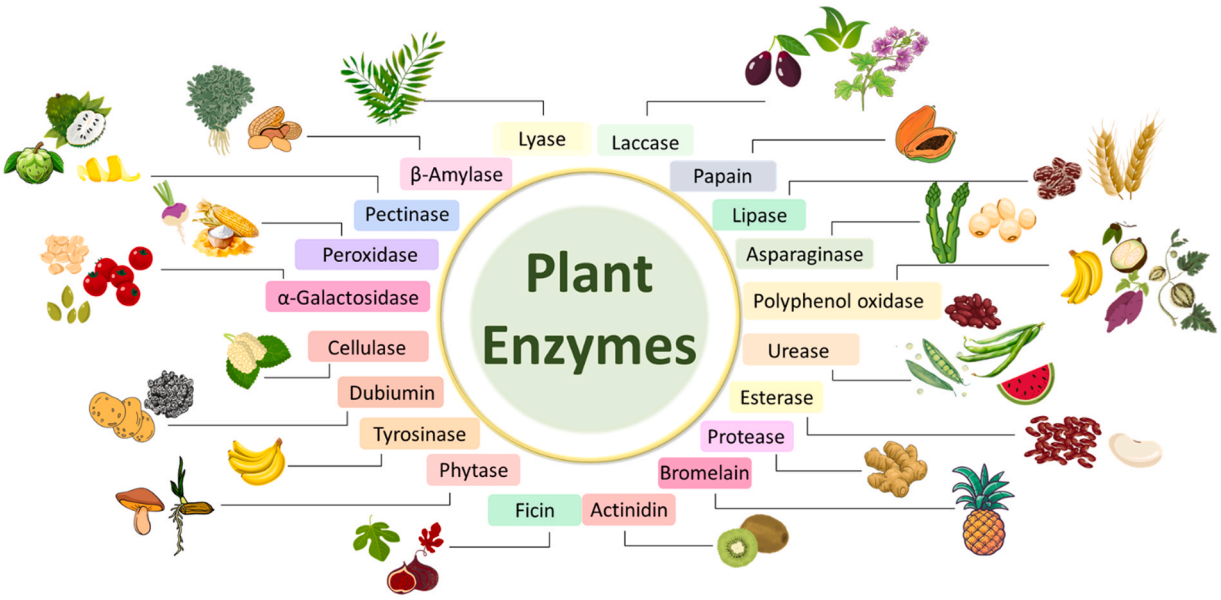

Fig. 1. Sources of the different plant derived enzymes that are used in biosensing and industrial related applications.

functional activity. The purification process involved homogenizing the seeds, followed by acetone suspension, filtration, and centrifugation to obtain a crude extract. This extract underwent acetone precipitation, ion-exchange chromatography with DEAE-cellulose, and gel filtration chromatography using a Sephacryl S-200 column, resulting in a purification fold of 12.85 and a final yield of 40 %. Ion-exchange chromatography revealed two protein peaks with urease activity, with the primary peak showing the highest activity selected for further processing. The purified urease exhibited a molecular weight of approximately 269,000 Da, confirmed through gel filtration chromatography. Optimal enzymatic activity was observed at pH 7.5 and 40°C, indicating its classification as a basic urease similar to those found in jack bean and pigeon pea. Storage stability analysis showed a decrease in activity from 80 % at 10 days to 14.1 % at 60 days when stored below 0°C, with a half-life of 22.4 days, suggesting the need for stabilizers for long-term use. Kinetic studies indicated a relatively low substrate affinity, as evidenced by the Michaelis constant (Km) and maximal velocity, pointing to an energy-efficient catalytic mechanism suitable for physiological and industrial applications. The presence of two urease activity peaks suggests multiple isoforms or conformational states, a novel finding in germinating Pisum sativum seeds. The enzyme's optimal activity at neutral to slightly basic pH supports its role in nitrogen metabolism during seed germination. Comparative analysis highlighted that urease molecular weight varies with botanical source and developmental stage, underscoring the importance of context-specific characterization (El-Hefnawy et al., 2014). These findings enhance our understanding of plant-based ureases and their potential applications in biosensing, bioremediation, and regulating urea metabolism in human health contexts. An illustrative representation of the various plant sources of enzymes is shown in Fig. 1. Table 1 provides other examples of enzymes explicitly derived from plants showing the sources, methods of extraction and purification.

3. Plant-derived bioactive enzymes and their application

3.1. Enzymatic biosensor

Conventional detection methods such as chromatography, enzyme-linked immunosorbent assays (ELISA), and capillary electrophoresis produce precise measurements; however, they typically require complex sample preparation, advanced instrumentation, and skilled operators, limiting their applicability in real-time, point-of-care analysis. Biosensors are a promising alternative in this context due to their operational simplicity, short reaction time, high sensitivity, and ability to be miniaturized (Chandra, 2016; Chandra et al., 2017; Chandra and Segal, 2016; (Biosensing and Micro-Nano Devices, 2022; Handbook of Nanobioelectrochemistry, 2023). Plant-based enzymatic biosensors combine natural biocatalysis with modern sensor technology to provide long-lasting, sensitive, and selective platforms for real-time monitoring in agriculture, biomedicine, and the environment (Araújo et al., 2022). This section discusses biosensors built with enzymatic biorecognition components extracted and purified from plant sources.

Phytic acid, or myo-inositol (1,2,3,4,5,6)-hexakisphosphate, is a naturally occurring antinutritional compound prevalent in plant-based dietary sources. It demonstrates a strong affinity for binding multivalent metal cations, including calcium (Ca^{2+}), potassium (K^{+}), magnesium (Mg^{2+}), iron (Fe^{2+}/Fe^{3+}), copper (Cu^{2+}), and zinc (Zn^{2+}) (Kumar et al., 2021). This results in the formation of insoluble chelate complexes that hinder their intestinal absorption and bioavailability, thereby affecting mineral nutrition and metabolism (Qu et al., 2018). To address the need for selective, sensitive, rapid detection of phytic acid (PA) in food systems, Mohseni *et al.*, developed an innovative electrochemical biosensor including a phytase enzyme (PHY) sourced from rice sprout extract (Rahimi-Mohseni et al., 2022). The biosensor platform employed DABCO-functionalized mesoporous silica (SBA@DABCO) as an effective nanomaterial matrix for enzyme immobilization. The selection of SBA@DABCO offered a substantial surface area, biocompatibility, and stable functional groups for both covalent and non-covalent enzyme attachment, hence improving the operational stability of the biosensor. During the synthesis procedure, SBA@DABCO nanoparticles were deposited onto filter paper, then followed by the application of phytase extract obtained from rice sprouts. The enzymatic paper was then integrated with a graphite screen-printed electrode (GSPE), creating a small and portable biosensing device. Electrochemical characterization was conducted by Differential Pulse Voltammetry (DPV). The determined limit of detection (LOD) was 0.04 μM, exhibiting enhanced sensitivity relative to current PA detection techniques. The biosensor demonstrated significant repeatability and reproducibility, maintaining around 80 % of its original activity after 40 days of refrigeration at 4 °C, underscoring the synergistic impact of the nanomaterial-enzyme interface in sustaining catalytic performance over time. The practical relevance was evidenced by the effective detection of phytic acid in

Table 1
List of enzymes extracted from plant sources with their respective extraction and purification methodologies.

Sl. No	Enzyme Name	Source	Method of Extraction	Purification Method	Distinct Characteristics	Ref.
1.	Ficin	*Fiscus carica*	Latex collected in the presence of MMTS to inhibit proteolysis and processed for protein extraction.	Cation-exchange chromatography (SP-Sepharose), S-pegylation, thiopropyl-Sepharose 6B, and Superdex 75 FPLC	pH: 6.0 It showed signs of structural relaxation at pH levels below 3.0 Temperature: 50 ℃	(Baeyens-Volant et al., 2015)
2.	Urease	*Pisum sativum*	Germinated seeds were ground and extracted using chilled 20 % acetone; crude extract was filtered and centrifuged to obtain the enzyme-rich supernatant.	Acetone precipitation, ion-exchange chromatography (DEAE-cellulose), followed by gel filtration (Sephacryl S−200)	Optimal activity at pH 7.5; activity declined outside this range Temperature Optimum at 40 ℃; stability decreased at higher temperatures Storage Enzyme activity declined gradually during storage at −4 ℃	(El-Hefnawy et al., 2014)
3.	Lipase	*Triticum aestivum (Wheat seeds)*	Seeds were ground and extracted; crude extract concentrated by ammonium sulfate precipitation	Precipitation followed by lyophilization; immobilization with sodium alginate, activated charcoal, and glutaraldehyde	The lipase exhibited maximum activity at pH 7.0. Activity decreased significantly at pH values below 6.0 and above 8.0 The enzyme showed optimal activity at 37°C. Activity declined at temperatures above 45°C, indicating sensitivity to heat.	(Pierozan et al., 2011)
4.	Urease	*Canavalia ensiformis* (Jack bean)	Jack bean meal extracted using 50 mM phosphate buffer (pH 7.5) containing β-mercaptoethanol; stepwise elution at pH 8.0	Dialysis followed by anion exchange chromatography (DEAE-Sepharose) and NaCl followed by ammonium sulfate and acetone precipitation	pH 7.0 The enzyme was stable across a pH range of 6.0–8.0, maintaining most of its activity during incubation. Extreme pH values led to denaturation and loss of activity. The enzyme showed optimum activity at 37°C. The enzyme retained significant activity up to 40°C. Beyond 40°C, the enzyme started losing activity.	(Pawar et al., 2022)
5.	Dubiumin	*Solanum dubium*	Seeds were defatted with petroleum ether, ground, and extracted in Tris–HCl buffer (pH 8.0) containing NaCl. The homogenate was stirred, filtered, and centrifuged.	Ammonium sulfate precipitation, followed by gel filtration chromatography on Sephadex G−100 and ion-exchange chromatography on DEAE-Sepharose	Optimum activity at pH 8.0. It exhibited good activity in the pH range of 7.0–9.0, losses activity under the acidic conditions. The optimum temperature for enzyme activity was 50°C. It retained substantial activity to this temperature and was thermolabile at the higher temperatures. Activity declined significantly above 60 ℃. The enzyme retained over 80 % activity at 50 ℃ for around 30 min. At temperatures above 60 ℃, rapid inactivation occurred, indicating moderate thermal stability.	(Mohamed Ahmed et al., 2009)
6.	β-Amylase	*Trigonella foenum-graecum*	Seeds were soaked overnight in extraction buffer and crushed using a blender to obtain crude extract.	Acetone fractionation (50–65 %), followed by ion-exchange chromatography and glycogen affinity precipitation.	The optimum pH was 5.5. After immobilization on GQDs, the optimum pH shifted slightly to 6.0. The optimum temperature of free β-amylase was 50 ℃. After immobilization, the optimum temperature	(Agrawal et al., 2020)

(*continued on next page*)

Table 1 (*continued*)

Sl. No	Enzyme Name	Source	Method of Extraction	Purification Method	Distinct Characteristics	Ref.
					increased to 60 ℃. The immobilized enzyme retained higher activity at elevated temperatures.	
7.	α-Galactosidase	*Tachigali multijuga* (Benth. Seeds)	Seeds germinated for 108 h after dormancy was broken with H_2SO_4. The germinated seeds were ground in sodium acetate buffer and centrifuged to obtain the crude extract.	Acid precipitation, Dialysis, Ion exchange chromatography (DEAE-Sephacel), Gel filtration (Sephadex G−150), SDS-PAGE.	The enzyme displayed maximum activity at pH 4.5. Activity dropped significantly at pH values above 6.0 and especially under neutral to alkaline conditions. The optimum temperature was found to be 50 ℃. The enzyme retained significant activity from 40 ℃ to 55 ℃.	(Fialho et al., 2008)
8	β-glucosidase, galactosidase and Fucosidase	*Putranjiva roxburghii Seeds*	Seed were soaked in water overnight. Crude extract prepared and cleared.	Enzyme was purified using the combination of CM-Sepharose and DEAE Sepharose and gel filtration chromatography	The optimum pH for β-glucosidase activity was pH 6.0. The enzyme retained good activity in a pH range of 5.0–7.0. The enzyme showed optimum activity at 70 ℃. It retained over 80 % activity after 1 h at 60 ℃ and significant activity.	(Kar et al., 2017; Patel et al., 2012a)
9	Arginase	Roots of three-year-old Panax ginseng *C.A. Meyer* plants	Ginseng root tissues were homogenized in a suitable buffer to extract the crude enzyme.	Enzyme was purified using process including ammonium sulfate fractionation, dialysis, and chromatography techniques such as ion-exchange chromatography and gel filtration.	Optimum temperature- 60 ℃ Optimum pH−9.5. Requires a manganese ion Mn^{2+} for proper function.	(Hwang et al., 2001)
10	Myrosinase	*Brassica rapa var. parachinensis* (Chinese flowering cabbage)	Homogenization of plant tissue in phosphate buffer (commonly pH 6.0–7.0), followed by centrifugation to remove debris	Ammonium sulfate precipitation (typically 40–80 % saturation), followed by dialysis and column chromatography (e.g., ion-exchange or gel filtration chromatography)	Optimum pH- 6.0–7.0 Optimum Temperature- 30–40 ℃.	(Sangkret et al., 2019)
11	Zingibain	*Zingiber officinale Roscoe (*ginger rhizomes*)*	Homogenization of fresh rhizomes in extraction buffer (e.g., phosphate buffer, pH ~7.0) followed by filtration and centrifugation	Three-Phase Partitioning (TPP) using ammonium sulfate and t-butanol	Optimum pH- around 7.0. Optimum temperature- around 60 ℃. Highly stable when stored at −20 ℃ for 30 days.	(Gagaoua et al., 2015)
12	Alliinase	*Allium cepa var. aggregatum (*Shallots*)*	Homogenized in phosphate buffer and filtered 3 times by 3-layers of cheesecloth, then centrifuged.	Ammonium sulfate precipitation, dialysis, and gel filtration chromatography (e.g., Sephadex G−100)	Optimum pH−8.0 Optimum Temperature−40 ℃.	(Zeng et al., 2017)
13	Oxalate oxidase	Germinated barley	Barley or malt was ground and sieved. The flour was stirred on ice with NaCl then filtered and centrifuged.	Germinated barley was ground and mixed with citrate-phosphate buffer containing EDTA and NaCl. After centrifugation, the supernatant was treated ammonium sulfate then resuspended and dialyzed overnight. The dialyzed solution was purified using ion exchange chromatography, Second ammonium sulfate precipitation and size-exclusion chromatography.	Optimum pH - 4.0 Enzyme retains about 40 % of its activity even at pH 8.0. Highly heat resistant, maintain 25 % of its activity at 80 ℃ when exposed for two hours.	(Kanauchi et al., 2009)

commercial food samples, such as digestive biscuits and whole-wheat flour. This research demonstrates the successful amalgamation of a plant-sourced enzyme with functional nanomaterials to create a high-efficiency biosensor. Rice sprout phytase has benefits including biocompatibility, simplicity of extraction, cheap manufacturing costs, and environmental sustainability. These advances underscore the increasing importance of plant-based enzymes in the advancement of biosensors for food safety, nutritional analysis, and potentially for biomedical diagnostics.

Polyphenol oxidase (PPO), commonly referred to as tyrosinase, is a copper-containing enzyme present in the majority of eukaryotic and prokaryotic species. It facilitates the oxidation of phenol to quinone, a crucial process in the synthesis of melanin and the browning of fruits and vegetables (Cesko et al., 2024). In a work reported by Aliabadi *et.al.,* (Aliabadi et al., 2017), banana tissue was pulverized and combined with graphite powder, hydrogel, and paraffin oil to create BH-CPE for the

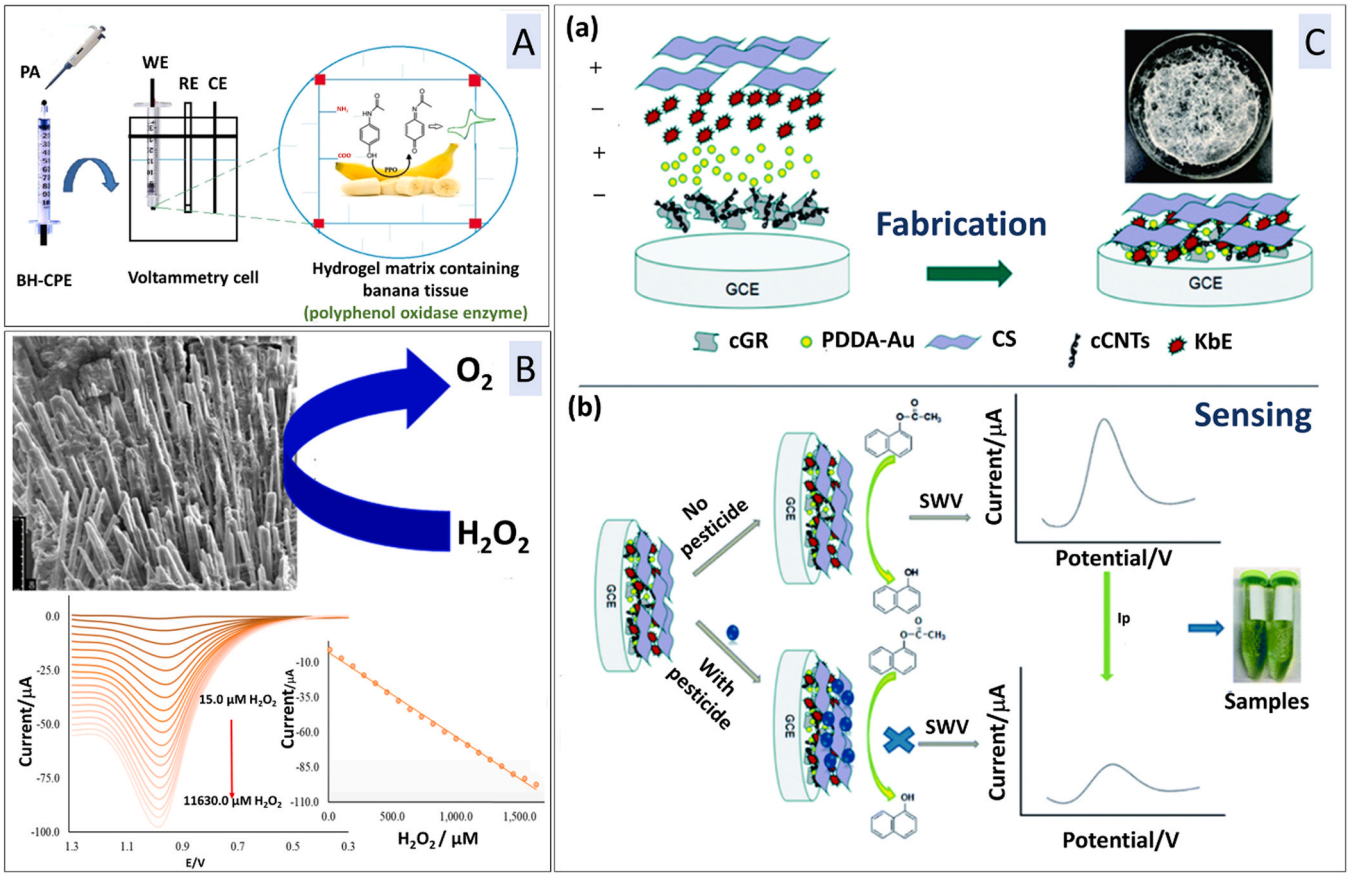

Fig. 2.
A. Schematic depiction of the detection methodology for acetaminophen analysis, Reproduced with permission from (Aliabadi et al., 2017). **B.** SEM images of the PPMP enzyme that facilitates the detection of H_2O_2 and LSV responses of PPMP/Au electrode in 10 % orange juice, Reproduced with permission from (Izadyar et al., 2019). **C.** Schematic representation of the biosensor construction (a) and the methodology for pesticide detection (b) Inset in (a) is an image of the lyophilized KbE, Reproduced with permission from (Tao et al., 2022).

construction of a biosensor to detect acetaminophen **(**Fig. 2**A)**. Acetaminophen, commonly referred to as paracetamol, is an analgesic and antipyretic medication utilized as a first treatment for fever and acute pain, accessible over-the-counter globally (Ayoub, 2021). Following the ingestion of paracetamol, the liver metabolizes it into non-toxic metabolites by mechanisms such as sulfation and glucuronidation, which are then excreted in urine together with glutathione. While the quantification of paracetamol in pharmaceutical samples is extensively reported, most methods are costly and involve complex procedures. In this direction, the group created a droplet-based banana-hydrogel carbon paste electrode (BH-CPE) for the detection of paracetamol, which exhibited benefits such as great selectivity, exceptional stability, and minimum sample requirements. The hydrogel was synthesized using free radical polymerization employing acrylamide (AM) and neutralized acrylic acid (AA) as monomers, N, N′-methylenebisacrylamide (MBA) as a crosslinker, and ammonium persulfate (APS) as the polymerization initiator. Banana tissue was pulverized and combined with graphite powder, hydrogel, and paraffin oil to create BH-CPE. Polyphenol oxidase on the BH-CPE oxidizes paracetamol (PA) to NAPQI, which is then electrochemically reduced back to PA. In the study, BH-CPE had the greatest oxidation peak current, signifying outstanding electrochemical performance attributed to the trapping of paracetamol molecules inside a biocompatible porous hydrogel and effective enzyme immobilization. The pH-responsive hydrogel contains both acidic (COOH) and basic (NH_2) groups, which convert to NH^{3+} and COO^- with pH changes, while the enzymes maintain stability throughout a broad pH range. SWV was conducted to ascertain the concentration of paracetamol utilizing just 1 µL of sample. The sample included with BH-CPE and chitosan was utilized as an adhesive. This approach demonstrated exceptional sensitivity, a low detection limit of 1.6 µM, and a linear detection range of 10–250 µM. The electrode demonstrated consistent performance over 20 days of storage at 4 °C. The sole disadvantage was the drying duration for each measurement. When known amounts of standard paracetamol were introduced to human urine samples, voltametric findings indicated good recovery rates, validating the efficacy of the BH-CPE electrode for detecting paracetamol in urine samples and highlighting its potential for practical biological analysis.

Manganese peroxidase (PPMP), a plant-derived enzyme extracted from corn (*Zea mays*), presents a sustainable and effective alternative to the traditionally used horseradish peroxidase (HRP) in biosensing applications. In a work demonstrated by Izadyar *et. al.,* (Izadyar et al., 2019) the extraction process for PPMP involved preparing a slurry of corn flour with sodium tartrate and diatomaceous earth, followed by vacuum filtration. The resulting filtrate was concentrated to half its original volume using Pellicon-2 tangential flow filtration (TFF), and ammonium sulfate was added to achieve 95 % saturation. After filtration, the precipitate was resuspended in 50 mM sodium tartrate and mixed thoroughly, followed by additional filtration in which the precipitate was discarded. The filtrate was then desalted using TFF (10 kDa cutoff), and residual corn proteins were removed by passing the solution through a Giga Cap S-650 M column. The purified MnP fractions were concentrated, lyophilized, and used to prepare a biosensing solution containing PPMP, glucose oxidase (GOx), bovine serum albumin (BSA), and glutaraldehyde in phosphate-buffered saline (PBS). This enzyme solution was deposited onto a gold electrode via spin coating, and a Nafion polymer membrane was subsequently layered to enhance

Table 2
List of enzymes extracted from plant sources in biosensor applications.

Sl. No	Enzyme	Plant Source	Distinct Characteristics	Biosensor Composition	Target Analyte	Detection Method	Detection Range	Limit of Detection (LOD)	Ref
1	Manganese Peroxidase (PPMP)	*Zea mays* (Corn)	pH: 7.0 Room temperature: 20 ℃	PANI–GNPs–GOx–PPMP modified gold microelectrode (GME)	Glucose	LSV, CV, CA	0.001–16.0 mM	0.3 – 0.6 μM (depending on technique)	(Izadyar et al., 2022)
2	Polyphenol oxidase (PPO)	*Ipomoea batatas* (Sweet potato)	pH: 7.0 Room temperature: 25 ℃	Paraffin/graphite electrode	Hydrazine	CV	7.0×10^{-6} - 1.2×10^{-4} Mol/L 2.9×10^{-5} - 2.0×10^{-4} Mol/L 2.9×10^{-5} - 2.9×10^{-4} Mol/L	5.1×10^{-7} Mol/L 8.4×10^{-7} Mol/L 1.1×10^{-6} Mol/L	(Cruz Vieira et al., 2002)
3	Peroxidase	Corn	pH: 7.0 Room temperature: 20 ℃	Nafion/PPMP–GOx-BSA/Au biosensor	Glucose	LSV	20.0 μM–15.0 mM 0.02–14.0 mM	2.9 μM 3.1 μM	(Izadyar et al., 2021)
4	Urease	*Citrullus lanatus* (Watermelon seeds)	pH: 8.5 Optimum temperature: 85 ℃ Vmax: 3225.8 μmol/min/mg Km: 0.24 mM	Chitosan-UZMWCNTs nanobiocomposite	Urea	Optical	10–400 mg/dL	3.17 ± 0.158 mg/dL	(Kumar et al., 2025)
5	Tyrosinase	Potato, Banana	For banana: pH: 6.8 Optimum temperature: 25 ℃ Specific Activity (units/μg): 23.351 Vmax (μmol/min): 0.006 For potato: pH: 6.8 Optimum temperature: 25 ℃ Specific Activity (units/μg): 14.253 Vmax (μmol/min): 0.141	Platinum electrode	Tea polyphenol	DPV	100 −500 mg/L	0.579–0.635 mg/l	(Chattopadhyay et al., 2015)
6	Tyrosinase	*Musa acuminata*	pH: 7.0 Optimum temperature: 25 ℃ Enzymatic Activity (U/mL): 3 ± 1	Graphite/PVC-COOH	Phenol	Potentiometry	9.3×10^{-7} -8.3×10^{-2} M	7.3×10^{-7} M	(Draghi and Fernandes, 2017)
7	Polyphenol Oxidase	Banana	pH: 7.0 Optimum temperature: 25 ± 0.5 ℃ Short-term stability:No change in signal for first 2 weeks 24-day stability: Signal dropped to 85 % 40-day stability:Signal dropped to 75 %	Carbon paste electrode modified with banana tissue	Catechol	SWV	1.4–15.7 mg/L	0.1 mg/L	(Broli et al., 2019)
8	Polyphenol Oxidase	Banana	pH: 7.4 Optimum temperature: 25 ℃	Carbon paste electrode modified with banana tissue	Atenolol	CV, SWV	0.7–99 μM	0.23 μM	(Broli et al., 2022)
9	Tyrosinase	Banana	pH: 7.0 Optimum temperature: 25 ℃ Enzyme activity: 0.187 U/mL Specific activity: 0.166 U/mg protein Protein content:1.12 mg/mL (by Lowry's method) Km: 4.11 mM (for catechol)	Multiwall carbon nanotubes-titanium oxide nanocomposite (MWCNTs-TiO2)	Catechol	CV	0.2–2.7 mM	0.014 μM	(Fathy et al., 2018)
10	Peroxidase	Turnip	pH: 7.0 Optimum temperature: 25 ° C	Screen-printed carbon electrode/ peroxidase	H_2O_2	Amperometry	0.02–0.50 mM	4.1 μM	(Sekar et al., 2015)

(*continued on next page*)

Table 2 (*continued*)

Sl. No	Enzyme	Plant Source	Distinct Characteristics	Biosensor Composition	Target Analyte	Detection Method	Detection Range	Limit of Detection (LOD)	Ref
			Storage Stability: ~70 % activity retained after 25 days at 4°C Repeatability: RSD = 3.3 % at 2 mM H_2O_2 (n = 6) Reproducibility:RSD = 7.3 % across biosensors (n = 6)						
11	Polyphenol oxidases	*Solanum lycocarpum*	pH: 7.0 Optimum temperature: 20 ± 2 °C (room temp, test conditions) Fruit Stage: immature: PPO Activity (U/mg protein): 612 Mature-ripe: 297 (U/mg protein)	Carbon paste electrode modified with enzyme	Paracetamol	CV, DPV	50–300 µM	3 µM	(Antunes et al., 2021)
12	Polyphenol oxidases	*Genipa americana* (Jenipapo fruit)	pH: 7.0 Optimum temperature: 20 ± 2 °C Storage Stability: After 15 days at 4 °C, the biosensor retained 68.4 % of its initial signal.	Carbon paste electrode modified with enzyme	Methyldopa Paracetamol	CV, DPV	10–310 µmol/L	8 µmol/L 5 µmol/L	(Antunes et al., 2019)
13.	Esterase	Red Kidney bean	pH: 7.0 Optimum temperature: 21 ± 2 °C	RKBE enzyme + chromogenic substrate (1-NA) + Solid Blue B dye system	Methamidophos, Methomyl, Dichlorvos, and Carbaryl	Optical	2.5×10^{-6} to 2.5×10^{-1} g/kg	0.0031, 0.0045, 0.001, and 0.0065 mg/kg	(Xiang et al., 2024)

stability and selectivity. The resulting PPMP/Au biosensor was employed for the detection of hydrogen peroxide (H_2O_2), a key reactive oxygen species and an important biomarker in biological systems, as well as an antimicrobial and bleaching agent in industrial processes (Fig. 2**B**). The biosensor exhibited high electrocatalytic activity towards H_2O_2, as evidenced by cyclic voltammetry CV. Upon incremental addition of H_2O_2 in the range of 0.01–1.91 mM, both the oxidation current and peak potential increased, indicating elevated oxygen generation at the electrode surface and confirming the enzyme's catalytic efficacy. Calibration curves demonstrated excellent linearity with a limit of detection (LOD) of 7.0 µM. Linear sweep voltammetry (LSV) confirmed a similar linear trend with an LOD of 8.2 µM. Amperometric measurements further supported the biosensor's sensitivity, with a wide detection range of 0.005–2.5 mM and an even lower LOD of 1.3 µM. At concentrations beyond 2.5 mM, a saturation effect was observed, likely due to excessive H_2O_2 oxidation at the electrode surface. Real-sample analysis was conducted using freshly squeezed orange juice, a common application matrix due to the routine use of H_2O_2 in juice sterilization. The biosensor successfully quantified H_2O_2 in orange juice with a linear range of 0.015–1.63 mM and a detection limit of 7.5 µM. Further amperometric studies using 58 sequential injections of varying H_2O_2 concentrations demonstrated high reproducibility and a detection limit of 2.6 µM in a deoxygenated PBS solution containing 10 % orange juice. Importantly, the PPMP/Au biosensor retained its enzymatic activity and operational stability for up to 21 days, indicating its suitability for long-term use in practical applications. Together, these findings underscore the utility of corn-derived PPMP as a reliable, biocompatible, and cost-effective alternative to traditional peroxidases in electrochemical biosensing. Its robust catalytic performance, broad detection range, and stability in real-sample matrices establish it as a promising candidate for future applications in food safety monitoring, clinical diagnostics, and environmental sensing.

Urease is a multi-subunit metalloenzyme that depends on nickel and is present in a wide range of organisms, including plants, fungi, bacteria, and algae. The hydrolysis of urea by urease produces ammonia and carbon dioxide, which subsequently undergo hydrolysis to form carbonic acid and ammonia. In a work demonstrated by Kumar *et.al.,* (Kumar et al., 2024b) urease was extracted and purified from watermelon (*Citrullus lanatus*) seeds. These were subjected to soaking in extraction buffer, followed by centrifugation. The resulting extract underwent acetone fractionation, anion exchange chromatography, and size-exclusion chromatography. The purified enzyme was subsequently immobilized on a glassy carbon electrode. Urea is typically present in raw milk; however, it is sometimes added as an adulterant to increase the non-protein nitrogen content. The FSSAI has established a maximum limit of 70 mg/dL for milk. Studies indicate that the concentrations in cow and buffalo milk can vary between 18 and 100 mg/dL. Consumption of milk containing excessive urea may lead to the development of ulcers, cancers, and kidney failure in humans. Additionally, the physicochemical properties, including the overall solid and moisture content of milk, may be influenced by the incorporation of urea. The group developed a GCE/AuNPs/GO/Urs biosensor for the detection of urea in milk samples. The immobilization of urease on the AuNPs/GO film was confirmed through XPS, FTIR, and AFM techniques. Differential Pulse Voltammetry (DPV) analysis was conducted to perform the analytical studies. The biosensor's responses for urea detection were assessed within the range of 5–90 mg/dL. LOD was determined to be 0.037 (±0.012) mg/dL, while the Limit of Quantification (LOQ) was established at 0.125 (±0.001) mg/dL. The biosensor was exposed to various interfering substances, and the absence of a peak near ammonia's oxidation potential confirmed its excellent specificity for urea. Urea detection in milk samples was conducted using DPV. The limit of detection (LOD) and quantification (LOQ) in milk were determined to be 0.049 (±0.011) mg/dL and 0.166 (±0.002) mg/dL, respectively. The urease-modified biosensor maintained its signal for up to four months at 4 °C, indicating a superior shelf life. The watermelon urease

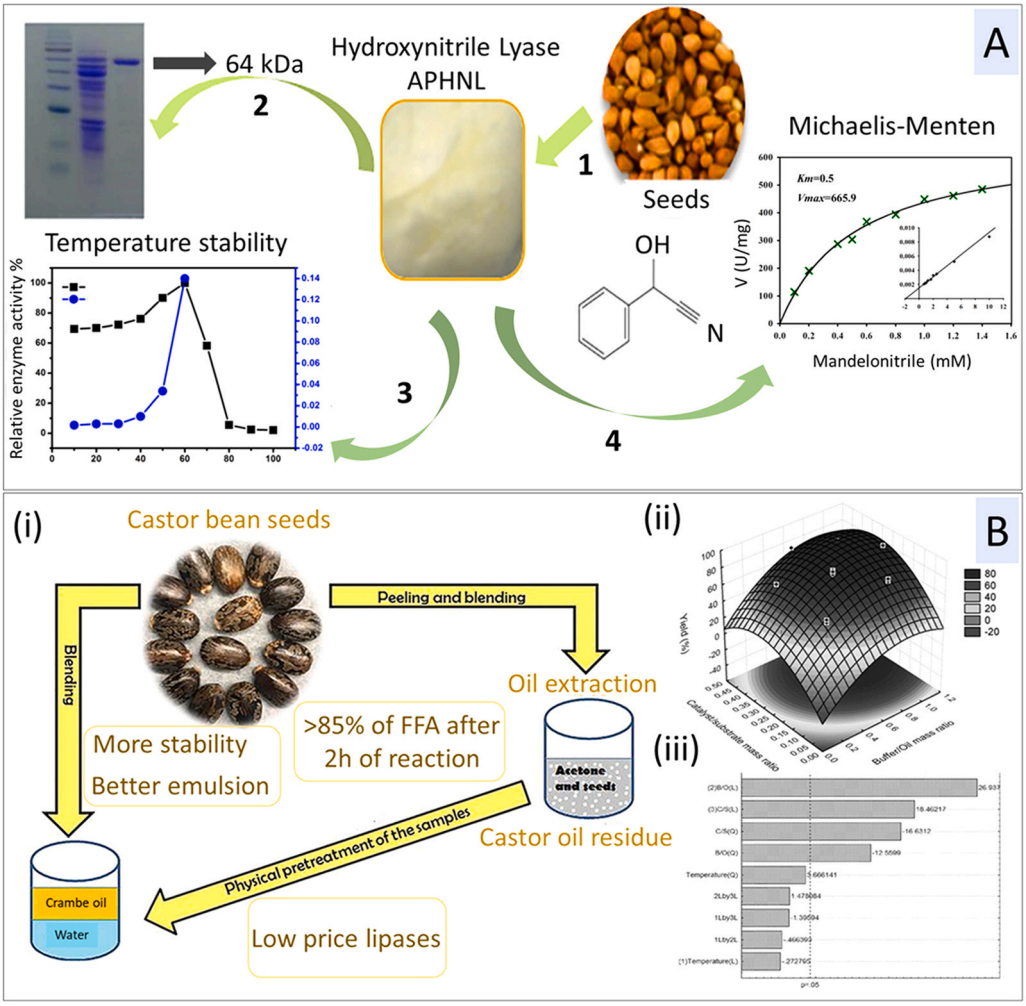

Fig. 3. ***: A.*** *Schematic representation showing the (1) extraction of hydroxynitrile lyase from Seeds of A. pedunculata Pall seeds, (2) SDS page showing molecular weight, (3) kinetic studies and (4) effect of temperature on the enzyme activity, Reproduced with permission from* (Yao et al., 2018)***. B.*** *(i) Pictorial representation of the extraction and properties of the purified lipase (ii) Response surface showing the effect of catalyst-to-substrate and buffer-to-oil mass ratios on crambe oil hydrolysis yield (iii) application in the hydrolysis of crambe oil, Reproduced with permission from* (Tavares et al., 2018)*.*

immobilized sensor generated a higher DPV signal compared to the commercial jack bean urease at identical urea concentrations. This indicates that watermelon urease serves as a more sensitive and efficient biocatalyst, positioning it as a preferable alternative for urea biosensing applications. The urea measurement obtained via the developed biosensor (GCE/AuNPs/GO/Urs) was also compared to the gold standard analytical technique, the DMAB method, in milk samples. Both methods produced similar results, thereby validating the accuracy, sensitivity, and reliability of the developed biosensor.

The widespread use of chemical pesticides in agriculture, while crucial for enhancing crop yield and pest management, has resulted in significant environmental and health issues (Zhou et al., 2025). Fruits and vegetables are particularly susceptible to pesticide contamination due to their frequent and intense chemical applications intended to optimize market production (Tambo et al., 2024). Among these pollutants, organophosphorus pesticides (OPPs) are deemed especially perilous due to their elevated toxicity and environmental durability. These substances infiltrate the human body via the food chain and obstruct essential enzymes like acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), resulting in the buildup of acetylcholine in the nervous system and precipitating severe neurotoxic consequences and neurological diseases (Čadež et al., 2021). A significant accomplishment in this domain is the creation of an electrochemical biosensor utilizing esterase derived from white kidney beans (*Phaseolus vulgaris*) as the biorecognition component for organophosphate pesticide detection by Tao et. al., (Tao et al., 2022) (Fig. 2**C**). The plant-derived esterase (KbE) demonstrates enzymatic activity on α-naphthyl acetate (α-NA), facilitating its hydrolysis to α-naphthalenol. The resultant α-naphthalenol experiences electrochemical oxidation, generating a detectable current signal. The presence of organophosphorus pesticides like trichlorfon inhibits esterase activity, resulting in a corresponding decline in α-naphthalenol synthesis and a subsequent drop in the oxidation peak current. This inhibition-based mechanism underpins quantitative pesticide detection. To construct the biosensor, KbE was immobilized onto a gold-modified electrode (Au/cCNTs–cGR/GCE) augmented with carboxylated carbon nanotubes and chemically reduced graphene. A chitosan (CS) matrix was utilized as a biocompatible film to sustain the enzyme, culminating in the final sensor configuration: CS/KbE/Au/cCNTs–cGR/GCE. Pesticide detection was conducted using square wave voltammetry (SWV). Exposure to trichlorfon resulted in a concentration-dependent reduction in the current responses, signifying an increasing inhibition of KbE. The sensor demonstrated two linear response ranges: 5–150 ng/L and 150–700 ng/L with an exceptionally low detection limit of 3 ng/L. At high pesticide concentrations, a saturation effect was seen due to significant enzyme inhibition, diminished active sites, and possible structural alterations, results that align with existing enzyme-inhibition-based biosensor systems. This study highlights the promise of esterase from kidney beans, as sustainable and

economical substitutes in biosensor development. Their biocompatibility, diminished immunogenicity, and capacity to maintain activity in diverse matrices provide them interesting candidates for real-time monitoring of environmental contaminants, especially in food safety applications. The incorporation of these enzymes with nanostructured materials improves the operational efficacy of biosensors, facilitating their use in decentralized and field-based diagnostic systems. Additional examples of enzymes sourced from plants and utilized in fabrication of biosensors are detailed in Table 2.

3.2. Industrial application

Plant-derived enzymes are a sustainable and cost-effective alternative to traditional microbial or chemical production methods in industrial biotechnology. Plant systems can produce complex proteins with proper folding and post-translational modifications, making them ideal for agricultural expansion (Park and Wi, 2016). Enzymes like laccase, trypsin, avidin, and β-glucuronidase have been successfully produced in maize, demonstrating the efficiency of plant-based platforms. This approach also offers environmental benefits by replacing polluting chemical treatments in sectors like textile, paper, and wood processing. Thus, plant-derived enzymes represent a scalable, safe, and eco-friendly solution for modern industrial demands. This section summarizes a few examples of the reported works that have shown strategic importance and benefits of plant-based enzyme systems in industrial biotechnology.

Hydroxynitrile lyase (APHNL), an enzyme of significant interest for asymmetric synthesis, was successfully extracted and purified from the seeds of *Amygdalus pedunculata* Pall, a hardy plant known for its ecological resilience in arid regions (Krammer et al., 2007; Yao et al., 2018). In a study conducted by Yao *et.al.,* (Yao et al., 2018) the extraction process began with the de-shelling and crushing of seeds in warm water at 40 °C, followed by homogenization in cold 20 mM phosphate-buffered saline (PBS, pH 7.4) for 12 h (Fig. 3**A**). Subsequent centrifugation at 12,000 rpm removed residual solids and lipids, yielding a crude protein extract rich in enzymatic activity. The initial purification step employed ammonium sulfate precipitation, a common protein fractionation technique, which was carried out incrementally across saturation ranges from 0–100 %. The most enzymatically active precipitate was obtained in the 40–60 % saturation range, showing a significant increase in specific activity from 4.8 U/mg (crude) to 38.8 U/mg. The precipitate was redissolved in PBS and dialyzed to remove residual salts, confirming complete sulfate removal by the addition of 1 % $BaCl_2$. For chromatographic purification, 87 mg of the partially purified protein was subjected to ion-exchange chromatography using a HiTrap DEAE-Sepharose Fast Flow column. Proteins were eluted with 0.8 M NaCl in PBS (pH 7.4), and active fractions were collected for further enrichment. This was followed by hydrophobic interaction chromatography using a Phenyl Sepharose Fast Flow column under high-salt conditions (2 M ammonium sulfate). Proteins were eluted using decreasing salt concentration in PBS, and HNL-active fractions were pooled. This dual-column protocol led to a substantial increase in purity, yielding an enzyme preparation with a specific activity of 661 U/mg and a purification fold of 138.The entire purification process, requiring only two chromatographic steps, was markedly more efficient than methods previously reported for other plant HNLs. For instance, the HNL from *Prunus mume* required four chromatographic steps to reach a specific activity of 220 U/mg, while *Passiflora edulis* HNL purification involved five columns to achieve 136 U/mg. In contrast, the current protocol not only reduced time and reagent consumption but also delivered a significantly more active enzyme, underscoring the efficiency and practical value of the approach. Molecular characterization via SDS-PAGE revealed that the purified APHNL appeared as a single protein band, indicating high purity and confirming its monomeric nature. Further mass spectrometric analysis (MALDI-TOF MS) determined the molecular weight of the enzyme to be approximately 61 kDa, aligning it within the broad molecular weight range observed for plant-derived HNLs (15–180 kDa). N-terminal sequencing by Edman degradation showed the first 12 residues to be highly homologous to known (R)-hydroxynitrile lyases from *Prunus* species, thereby affirming the identity of the enzyme. Amino acid composition analysis revealed that acidic residues, particularly glutamic acid (22.9 %) and aspartic acid (10.3 %), constituted a significant portion of the protein, contributing to its overall acidic nature and likely enhancing solubility. The high hydrophilic amino acid content (53.9 %) supports the enzyme's solubility profile observed during extraction and purification. Hence, the study successfully established an effective, streamlined protocol for the extraction and purification of APHNL from *Amygdalus pedunculata* Pall seeds. The process yielded a highly active, monomeric enzyme with excellent purity and superior catalytic potential, demonstrating the plant's viability as a novel and sustainable source of hydroxynitrile lyase. The potential of this enzyme to synthesize cyanohydrins was studied. 10 mmol of benzaldehyde, phenoxy benzaldehyde and excessive sodium cyanide were used in the reaction. Molar conversion of (R)-mandelonitrile (tR = 8.1 min) and (R)-2-Hydroxy-2-(3-phenoxyphenyl)-acetonitrile (tR = 24.8 min) were 90 % and 98 % with 94 % and 93 %, respectively.

Phytase is an enzyme that hydrolyzes phytic acid in order to release the inorganic phosphate and the myo inositol which was efficiently extracted and then purified from the *Lactarius volemus* (Tirmit mushroom) which is a non-toxic and an edible fungal source (Collopy and Royse, 2004). In a work reported by Onem *et.al.,* (Onem and Nadaroglu, 2018) the enzyme was then obtained using a three-step purification protocol: ammonium sulfate precipitation, DEAE-Sephadex ion--exchange chromatography and the gel filtration chromatography. This procedure resulted in the final purification fold of 120.2 and a yield of 11.6 %, with a specific activity of 690.1 EU/mg protein [34]. SDS-PAGE analysis then confirmed the molecular weight of the purified phytase at 45 kDa, consistent with the phytases from the microbial sources. In order to enhance the industrial utility, particularly in the cereal processing, the purified enzyme was then immobilized onto the magnetized chitosan nanoparticles via the covalent bonding using the glutaraldehyde and the Fe_3O_4. Immobilization conditions were then optimized at pH 5.5 and 20°C, where the binding efficiency of 87.2 % was achieved then within the 45 min. Biochemical evaluation revealed that both free and immobilized phytase had optimal activity at pH 5.5 and 50°C, albeit the immobilized form was substantially more stable. The immobilized enzyme retained more than 80 % activity after 2 h at 90°C, but the free enzyme lost the majority of its activity during that time. Similarly, pH stability studies revealed that immobilized phytase retained activity more consistently throughout pH 3.0–9.0 after a 7-day incubation than the free form. The enzyme's half-life rose from 5 to 31.64 h after immobilization, indicating improved conformational stability due to nanoparticle binding. Metal ion impacts demonstrated that immobilized phytase was far more resistant to inhibition than its free equivalent. Ions such as Cu^{2+}, Fe^{2+}, Ca^{2+}, and Hg^{2+} increased the activity of immobilized phytase, but only Mg^{2+} inhibited it. This tolerance to metal inhibition suggests appropriateness for use in complex food matrices that may contain such ions. Critically, the enzyme's functioning was tested in real food systems to determine its viability in cereal and legume processing. Phytase, both free and immobilized, was used to hydrolyze phytic acid in popular food staples such lentils, beans, wheat, chickpeas, corn, oats, and peanuts. The immobilized version consistently outperformed the free enzyme, with 4-hour hydrolysis efficiency of 77.8 % in wheat, 75.2 % in beans, and 76.7 % in peanuts. This better performance is critical for increasing mineral bioavailability in plant-based meals by counteracting the anti-nutritional effects of phytic acid, which normally chelates key micronutrients such as iron, calcium, and zinc. The enzyme's robustness, improved temperature and pH stability, and tolerance to inhibitory ions make it an ideal biocatalyst for industrial applications. Its application in bread making, cereal processing, and plant protein hydrolysate production can increase product nutritional profiles while retaining process efficiency. The immobilized system also

has the potential for reusability and integration into continuous processing systems, which supports its cost-effectiveness and sustainability in the food industry. Finally, phytase from *Lactarius volemus* was successfully purified and immobilized on magnetic chitosan nanoparticles, yielding a stable and efficient enzymatic system. This phytase's demonstrated capacity to effectively digest phytates in cereals and legumes under industrial circumstances, combined with increased resistance to denaturation and inhibition, makes it a promising candidate for use in food, feed, and functional ingredient production.

Lipase, also known as the triacylglycerol acylhydrolases, are the essential biocatalysts that can catalyze the hydrolysis, esterification and the transesterification processes in the mild circumstances (usually below 65 °C) and without the need for the any cofactors (Abigor et al., 2002). Their regioselectivity and the substrate adaptability makes them useful in the variety of the industrial fields, such as the synthesis of the surfactants, biodiesel and the medicinal intermediates (Beisson et al., 2000). The vegetable lipases are gaining the popularity among the various lipase sources, including the microbial, animal and the plant derived ones due to their low extraction costs, accessibility and the specificity. Unlike the many other oilseeds that only activate the lipase during the germination, the castor bean seeds (*Ricinus communis)* are noteworthy because they show the lipase activity even while they are in the dormant condition. The castor bean seeds are investigated by Tavares *et. al.,* (Tavares et al., 2018) as a powerful source of the vegetal lipase for the hydrolysis of the crambe oil **(**Fig. 3**B).** The seeds underwent different preparation procedures for the in natura and the oil free processing. The seeds were then pulverized in a home blender for in the natura extracts and the particles less than the 4.8 mm were then used straight away. To eliminate the endogenous oils, the seeds were treated with acetone in order to defatten the oil free extracts. Following the incubation, the mixture was then air dried and then vacuum filtered whereby the residue was then sieved to gather the particles smaller than 1 mm. In addition to the improving enzyme accessibility, this extraction technique even gave the researchers more control over the substrate emulsification, which is an essential component of lipid-based catalysis. The olive oil hydrolysis was used to measure the enzymatic activity, where the in natura extracts showed an activity of 45.1 U/g while the oil free extracts reached 71.2 U/g. The in natura enzyme showed the greater stability, hence maintaining about the same activity even after the 30 days of the storage despite the oil free extract's higher beginning activity. In contrast, the oil free version exhibited the notable drop to 64.8 U/g. The unprocessed seeds naturally occurring emulsifiers which are thought to improve the substrate interaction and the lipase stability, are responsible for this robustness. Also, the study focused on the process simplicity by directly using the seed powders, in contrast to the conventional purification procedures that include the chromatographic techniques, hence enhancing the economic feasibility of the plant-based enzymes. In order to assess the effects of the temperature, the buffer-to-oil ratio (B/O), and the catalyst-to-substrate ratio (C/S), Response Surface Methodology (RSM) with the Central Composite Rotatable Design (CCRD) was used in order to optimize the enzymatic hydrolysis of the crambe oil, a non-edible oil rich in the erucic acid, for the application phase. After the two hours, the in natura extract achieved 87.6 % conversion, which demonstrated the optimal performance at 33°C, 0.87 B/O, and 0.31 C/S. In contrast, the oil free extract produced 85.4 % in the same amount of time when it was subjected to the ideal conditions of 43.8 °C, 1.8 B/O, and the 0.07 C/S. Notably, both extracts showed noticeably faster reaction rates than the traditional commercial lipases which frequently takes a long time usually between the 10 and the 48 h to achieve the similar conversions. The simplicity of preparation and the lack of the expensive solvents like the acetone highlighted the in natura preparation is economic and the practical viability. The study places its findings in the perspective of the wider industrial ramifications, going beyond the laboratory optimization. For the synthesis of the fatty acids from the inedible oils, castor seed lipase, especially in its raw form emerges as the low cost and the sustainable substitute. It is effective incorporation into the environmentally friendly processing routes, particularly to produce the biodiesel feedstock, is consistent with the continuous initiatives to the lessen dependency on the fossil fuels and increases the value of the agro industrial waste. The study has provided the strong proof that the castor bean seeds, particularly when they are in their natural state, can be used as the reliable, affordable and the effective source of the lipase for the hydrolysis of the oil. The promise for the scalable industrial applications of the plant derived enzymes in the sustainable bioprocesses is highlighted by the straightforward extraction procedure, strong kinetic modeling and high reaction yields in the moderate circumstances.

Laccase is a blue multicopper oxidase that uses the molecular oxygen as the electron acceptor in the single electron transfer mechanism in order to oxidize a variety of the phenolic and the aromatic substances (Cañas and Camarero, 2010). In a study reported by Aziz *et. al.,* (Aziz et al., 2023) *Malva parviflora* which is one of the plant sources from which the laccase enzyme was extracted in the study showed the best specific activity of all the other plant sources when extracted using the phosphate buffer. *Malva parviflora* was chosen for the additional extraction optimization and the application analysis because to its exceptional activity. The fresh plant material was first homogenized in the phosphate buffer, and then the crude enzyme solution was then obtained by the centrifugation. Response Surface Methodology (RSM), which models the effects of the buffer concentration, extraction time and the extraction ratio using the Design Expert Software, was used in order to optimize the laccase extraction process. Using Sephadex G-150 and the gel filtration chromatography, the crude enzyme was then purified further. The gel was then pre saturated with the distilled water and then equilibrated with the phosphate buffer before being eluted and the fractions were collected. With a total yield of 94.4 %, the active laccase fractions were found between the elution stages of the 14 and the 33. They showed an impressive specific activity of 47,830 U/mg protein, which is fifteen times purer than the crude extract. The purification procedure successfully removed the unnecessary proteins and then improved the catalytic activity of the enzyme which proved that the Sephadex G-150 is a useful tool for purifying the enzymes generated from the plants. The enzyme's ability to degrade the different textile dyes, such as the reactive red, reactive blue, congo red, phenol red and bromophenol blue, was used in order to assess its functional use after the extraction and the purification. In these tests, the dye solutions were mixed with the enzyme solution, and the mixture was then incubated for three hours at 37 °C. The significant decolorization was then observed in the results, and the crude laccase frequently outperformed the purified version. The crude enzyme showed the greater operational stability and the economic viability for the large-scale applications by removing up to 97.5 % of the dye. The crude laccase was more effective overall, especially in degrading the dyes like the bromophenol blue (43.8 %), textile red (20.8 %) and the phenol red (17.3 %), whereas the purified enzyme was excellent at breaking down the Congo red (69 % removal). The textile blue, on the other hand, only showed a slight reduction (0.6 %). The synergistic effects of the other biomolecules in the unrefined solution, which may stabilize or increase the laccase activity, are probably the cause of the crude extract's higher effectiveness. Even though the crude enzymes contain some of the contaminants, their cost-effectiveness and the ease of the production makes them more appealing for the industrial use. The study emphasizes the great potential of the plant derived laccase as an efficient and the environmentally friendly dye decolorization agent. Laccase offers a low carbon, economical and the biodegradable substitute for the conventional dye removal techniques, which frequently calls for the hazardous chemicals or the significant energy expenditures. Hence, the laccase enzyme found in the *Malva parviflora* is a strong contender in the field of the environmental biotechnology, particularly due to its exceptional ability to break down the intricate and the frequently resistant dye complexes in the aqueous solutions. To increase the variety and the effectiveness of the dye degradation processes employing this biocatalyst, the future

Table 3
List of enzymes extracted from plant sources for industrial related applications.

Sl. No	Enzyme name	Source	Distinct characteristics	Application	Methodology	Distinct Characteristics	Ref.
1.	Papain	Latex of *Carica papaya* (unripe fruit)	pH: 6.0 It showed signs of structural relaxation at pH levels below 3.0 Temperature: 50 ℃	Used for tenderizing Chilean abalone	Three methods of impregnation: injection, immersion, and high hydrostatic pressure (HHP).	pH: 5.0–7.0; Temperature: 60–80 ℃	(Pizarro-Oteíza et al., 2020)
2.	Ginger Protease	*Zingiber officinale* (Zingibain)	pHOptimal activity at pH 7.5; activity declined outside this range Temperature Optimum at 40 ℃; stability decreased at higher temperatures StorageEnzyme activity declined gradually during storage at –4 ℃	Tenderization of chicken breast (Pectoralis major). Resulted in 37.7 % reduction in shear force, significant increase in myofibrillar fragmentation, no yield loss after cooking	5 % (w/v) crude ginger extract injected into chicken breast, stored at 4 ± 1 °C for 24 h. Cuts then analyzed for shear force, myofibrillar fragmentation, and water holding capacity.	Specific activity of 0.120 ± 0.04 U/mg of protein at 60 ℃ and pH 5.5	(Cruz et al., 2020)
3.	Laccase	*Populus trichocarpa*	The lipase exhibited maximum activity at pH 7.0. Activity decreased significantly at pH values below 6.0 and above 8.0 The enzyme showed optimal activity at 37°C. Activity declined at temperatures above 45°C, indicating sensitivity to heat.	Demonstrated direct polymerization of sinapyl alcohol (syringyl lignin). Inhibition reduced lignin content and S/G ratio in Populus.	Expressed in tobacco, purified, and used to catalyze lignin monomer polymerization in vitro. Antisense lines developed in Populus to analyze lignin synthesis.	pH: 4.5–6.0; Optimum temperature: 40–60 ℃	(Liu et al., 2021)
4.	Urease	*Citrullus lanatus* (Watermelon seeds)	pH 7.0 The enzyme was stable across a pH range of 6.0–8.0, maintaining most of its activity during incubation. Extreme pH values led to denaturation and loss of activity. The enzyme showed optimum activity at 37°C. The enzyme retained significant activity up to 40°C. Beyond 40°C, the enzyme started losing activity.	Detection and removal of heavy metals from aqueous solutions	Urease catalyzes the hydrolysis of urea to produce ammonia and carbonate ions, resulting in an increase in pH. The rise in pH facilitates the precipitation of heavy metals (such as Pb^{2+}, Cu^{2+}, Hg^{2+}, Mn^{2+}, Co^{2+}) as insoluble carbonates, enabling their detection and removal from contaminated water.	pH: 7.3; Optimum temperature: 37 ℃ Vmax: 3571 μmol/min/mg Km:0.16 mM	(Kumar et al., 2024a)
5.	Actinidin Bromelain	Kiwi Pineapple	Optimum activity at pH 8.0. It exhibited good activity in the pH range of 7.0–9.0, losses activity under the acidic conditions. The optimum temperature for enzyme activity was 50 ℃. It retained substantial activity to this temperature and was thermolabile at the higher temperatures. Activity declined significantly above 60 ℃. The enzyme retained over 80 % activity at 50 ℃ for around 30 min.	Tenderization and microbial safety.	Injected into pork fore shank and sous-vide treated (45–100 °C, up to 8 h).	pH:8.5 Optimum temperature:58–62 ℃	(Chang and Han, 2020)

(*continued on next page*)

Table 3 (*continued*)

Sl. No	Enzyme name	Source	Distinct characteristics	Application	Methodology	Distinct Characteristics	Ref.
			At temperatures above 60 ℃, rapid inactivation occurred, indicating moderate thermal stability.				
6.	Urease	*Vigna umbellata* (Rice bean seeds)		Investigated for ureolytic activity and antifungal potential. Showed no antifungal activity, but confirmed high urease activity, especially in seed coat-free samples (13 μg NH_3/μL/15 min). Potential for agricultural nitrogen cycling.	Isolated from germinated seeds (with and without seed coat); homogenized in 0.2 M phosphate buffer (pH 7), followed by centrifugation and protein concentration. Purified via gel filtration using Sephadex G−150. Activity assayed by UV–vis spectrophotometry using phenol-hypochlorite reaction.	pH:4.6–7.0 Optimum temperature: 45–70 ℃	(Siregar et al., 2024)
7.	Lipase	*Ricinus communis* (Castor bean)		Bioremediation of used lubricating oil-contaminated soils	Seeds were collected and endosperms were separated and defatted with chilled acetone. After filtration and drying, a lipase extract powder was obtained. The enzymatic activity was tested under various soil conditions (pH, temperature).	pH:7.0 Optimum temperature: 35–40 ℃ Achieving 94.26 % removal of lubricating oil contaminations from the soil under the ideal conditions.	(Aguilera Flores et al., 2022)
8.	Ficin	*Ficus carica* (Fig leaves)		Extraction of type I collagen from tannery-trimming wastes	Pretreated lambskin trimming waste was subjected to enzymatic hydrolysis using ficin under optimized conditions (5.54 % enzyme dose, 39.27 h reaction time). Ultrasound assistance was applied to improve hydrolysis efficiency and collagen yield. Collagen was then recovered, purified and characterized.	pH:5.0–8.0 Optimum temperature: 45–55 ℃	(Chen et al., 2025)
9.	β-Amylase	*Arachis hypogaea* (Peanut)		Antioxidant enhancement of food products	Purified β-amylase was incorporated into wheat flour to evaluate its ability to enhance antioxidant activity. The treated flour was analyzed using the DPPH free radical scavenging assay, which demonstrated improved antioxidant capacity due to phenolic compound release during enzymatic starch hydrolysis.	pH:5.0 Optimum temperature:60 ℃ Specific activity:361 μmol/min/mg protein	(Das and Kayastha, 2018)

research may also concentrate on the immobilization techniques, reactor design and on the mediator assisted enhancement. Other examples of enzymes that have been extracted indigenously from plants and used for food processing, bioremediation, and other industry related applications are enlisted in Table 3.

3.3. Challenges and future prospective

Despite the significant potential of plant-based enzymes, various challenges need be solved to fully harness their capabilities. The low extraction and yields remain a significant limitation, necessitating the development of genetic engineering approaches to boost enzyme expression in plant systems. Stability issues under harsh industrial conditions can be mitigated through innovative solutions like nano-encapsulation, which enhances enzyme durability. The high costs associated with purification processes could be reduced through affinity tag-based chromatography and other advanced separation techniques. Looking forward, several promising research directions could overcome these limitations. CRISPR-based genome editing offers exciting possibilities for developing enzyme-enriched crops with higher yields of desired enzymes. Hybrid plant-microbial fermentation systems could combine the advantages of both production platforms, while AI-driven enzyme optimization could accelerate the development of plant enzymes tailored for specific industrial applications. Despite their promising potential, challenges remain in the large-scale production, consistent activity, and structural stability of plant-based enzymes. Factors such as seasonal variability, plant growth conditions, and extraction efficiencies can affect enzyme yield and functionality. However, the ongoing research in transgenic plant systems, enzyme stabilization techniques, and green extraction methods offers viable solutions to overcome these limitations. Plant cell culture and bioreactor-based production are being explored to ensure sustainable and standardized enzyme manufacturing.

The expanding field of biosensors demands recognition of innovative, eco-friendly biocatalysts that can support high sensitivity, specificity, and device longevity. Among various bio-recognition elements, enzymes derived from plants are emerging as sustainable alternatives to their microbial or animal-derived counterparts. In the context of point-of-care diagnostics, environmental monitoring, and clinical therapeutics, plant-based enzymes hold immense potential to shape the next generation of biosensors. Plant enzymes offer inherent advantages such as cost-effective production, high substrate specificity, better thermal

stability (in some species), and reduced immunogenicity. Furthermore, the ease of extraction from agricultural waste or food processing by-products positions them as environmentally and economically viable candidates for scalable biosensor development. These aspects are particularly beneficial in resource-limited settings or in the development of disposable biosensor devices where enzyme reusability or long-term storage stability is less critical but cost and biocompatibility are. From a sustainability standpoint, plant enzymes reduce reliance on animal sources, aligning with ethical and regulatory demands for cruelty-free and biodegradable diagnostic solutions. This feature also supports green chemistry principles and circular bioeconomy strategies. As synthetic biology and plant-based bioprocessing techniques continue to evolve, tailored expression systems in plants can be developed to yield high-purity enzymes optimized for specific biosensor configurations, including amperometric, impedimetric, and optical formats. In terms of sensitivity and performance, the compatibility of plant enzymes with functional nanomaterials such as carbon nanotubes, graphene derivatives, gold nanoparticles, and metal–organic frameworks can be harnessed to enhance signal transduction mechanisms. The immobilization of these enzymes on advanced transducer surfaces has already demonstrated promising electrochemical responses, enabling the detection of ultra-trace levels of analytes including glucose, urea, organophosphates, and exosomal biomarkers. Looking forward, the integration of plant-based enzymes in wearable and smartphone-integrated biosensing platforms could democratize access to real-time health monitoring tools. Their non-toxic nature and potential for oral or dermal biocompatibility make them suitable candidates for non-invasive sensors. Additionally, their robustness in fluctuating environmental conditions expands their usability in field-deployable environmental biosensors for detecting heavy metals, pesticides, or industrial pollutants. Overall, the convergence of plant biotechnology, materials science, and sensor engineering paves a transformative path for biosensors powered by plant-derived enzymes. Continued efforts in enzyme characterization, stabilization, and surface engineering, combined with multi-omics tools for plant screening and metabolic optimization, will be essential in realizing their full potential. In this trajectory, plant-based enzymes stand to become vital components in the next wave of sustainable, affordable, and high-performance biosensing devices.

4. Conclusion

In summary, plant-derived enzymes constitute a flexible and sustainable category of biocatalysts with extensive applications in biosensing, biomedical, therapeutic, and industrial domains. Their distinct benefits compared to animal-derived enzymes, such as enhanced biocompatibility, reduced immunogenicity, and conformity with ethical and environmental considerations, establish them as essential elements of the future bioeconomy. The advancement of scientific knowledge and technological innovations is set to transform the incorporation of plant enzymes across various fields, resulting in more sustainable, accessible, and effective solutions in health, industry, and environmental management. However, extraction of enzymes from plant tissues often faces issues like poor yields, reduced activity, and interference from endogenous compounds. Industrial conditions also limit enzyme stability, impacting their practical application. Advances in enzyme engineering and synthetic biology are enabling the development of more efficient plant enzymes for industrial or diagnostic applications. Green extraction methodologies, enzyme immobilization strategies, and integration of omics data with machine learning can enhance enzyme efficacy and process scalability. Therefore, overcoming the existing challenges via transdisciplinary innovations may be essential for converting laboratory-scale achievements of plant derived enzymes for varied applications into commercial feasibility.

CRediT authorship contribution statement

Rashmita Priyadarshini Swain: Writing – review & editing, Writing – original draft. **Daphika S Dkhar:** Writing – review & editing, Writing – original draft, Conceptualization. **Pranjal Chandra:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Girijesh Kumar Patel:** Writing – review & editing, Writing – original draft, Supervision. **Riddhi Dubey:** Writing – review & editing, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The authors do not have permission to share data.

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