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12 Polyphenol oxidases: the future toward global sustainability

Abstract: Polyphenol oxidases (PPOs) are enzymes that catalyze the oxidation of phenolic compounds, which are abundant in many plant-based foods. PPOs are crucial in postharvest losses and food waste, especially in developing countries with underdeveloped food supply chains. There has been a growing interest in utilizing PPOs for sustainable food production and preservation, modifying phenolic compounds to develop new food products, detecting phenolic compounds in various products, and utilizing bioremediation, agriculture, biotechnology, and waste management techniques to promote global sustainability. These advances have the potential to provide effective solutions toward achieving a more sustainable future. The most promising application of PPOs for achieving global sustainability is their use as a natural preservative to prolong the shelf life of fresh produce. They can be used to produce novel food products, such as functional foods and nutraceuticals, by modifying the phenolic compounds. The approach can add value to the food industry by creating new products with health benefits and reducing waste. PPOs can be used in bioremediation processes to degrade phenolic compounds found in industrial wastewater and produce natural antioxidants from food waste, promoting circular economy principles. They can also contribute to sustainable agriculture by increasing plant resistance to pests and diseases, reducing synthetic pesticides and herbicides, and improving crop yields. Overall, PPOs have a promising role in creating a more sustainable environment. This chapter thoroughly examines the latest developments in utilizing PPOs for sustainable food production and waste management. It emphasizes the enzyme's potential in nat-

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ural preservation, novel food production, bioremediation, and sustainable agriculture. Additionally, the authors explore the wide range of applications for PPOs, such as biosensors, bioremediation, agriculture, and biotechnology.

12.1 Introduction

Polyphenol oxidases (PPOs) are a class of enzymes that are widely distributed in the plant and fungal kingdoms [1]. They play a crucial role in metabolizing phenolic compounds, which are essential antioxidants and structural components of many plant and fungal tissues. PPOs catalyze the oxidation of these phenolic compounds, forming brown pigments and other products with diverse biological and commercial implications. PPOs are also involved in enzymatic browning, a process that causes discoloration and spoilage of many fruits and vegetables. However, recent research has revealed that PPOs have potential applications in many fields, including food processing, agriculture, biotechnology, medicine, and environmental remediation. Therefore, understanding the structure, function, and regulation of PPOs is essential for developing sustainable and innovative solutions to various global challenges.

PPOs belong to the class of oxidoreductase enzymes, which catalyze the transfer of electrons between molecules during oxidation–reduction reactions. They are specifically involved in the oxidation of *o*-diphenols to *o*-quinones, which can undergo further reactions to produce a range of products, including melanins, tannins, and lignins. The chemical structure and properties of PPOs vary widely among different organisms, reflecting the diversity of their biological functions and ecological niches. However, all PPOs share a common catalytic mechanism involving using a copper cofactor to facilitate electron transfer from the substrate to molecular oxygen. In plants, PPOs are mainly located in the vacuole, which synthesizes various secondary metabolites, such as anthocyanins, flavonoids, and lignins. These metabolites are important in plant growth, reproduction, and defense against pests and diseases. For example, some plant species use PPOs to produce toxic compounds that deter herbivores and pathogens. In addition, PPOs are involved in regulating fruit ripening and senescence and the resistance to environmental stressors such as drought and UV radiation. In fungi, PPOs are mainly associated with the production of melanins, which have diverse roles in fungal physiology and pathogenicity. Melanins provide structural support to fungal cell walls, protect against oxidative stress and UV radiation, and facilitate the invasion of host tissues. In addition, some fungal PPOs have been shown to have antifungal and antibacterial activities, which could have potential applications in developing new drugs and biocides.

Enzymatic browning is a common phenomenon in fruits and vegetables, caused by the oxidation of phenolic compounds in the presence of oxygen and PPOs. This process can lead to discoloration and loss of nutritional value of many food products and the formation of off-flavors and odors. Therefore, controlling enzymatic browning is an important challenge in the food industry. One approach is to use PPO inhibitors, which are compounds that block the activity of PPOs or interfere with their synthesis. Another approach is to use PPOs as biocatalysts for producing value-added products, such as food colorants, flavors, and antioxidants. In agriculture, PPOs have potential applications in improving crop productivity and quality. For example, PPOs can be used as markers for breeding programs to develop plant varieties resistant to pests and diseases. They can also be used as biocatalysts for synthesizing phytochemicals with health-promoting properties, such as polyphenols and flavonoids. In addition, PPOs could be used to develop new technologies for producing biofuels and bioplastics, which help to reduce the dependence on fossil fuels and mitigate climate change. Moreover, PPOs can play a role in reducing food waste and enhancing food security by improving the shelf life and quality of fruits and vegetables. For instance, PPO inhibitors or RNAi-mediated suppression of PPO genes have been used to prevent enzymatic browning and maintain the freshness of various produce items, including apples, potatoes, and lettuce. In addition, PPOs have been shown to have antimicrobial activity against some plant pathogens, which could reduce the need for synthetic pesticides and fungicides.

Hence, it is crucial to comprehend the structure, function, and regulation of PPOs for creating sustainable and groundbreaking solutions to address various global challenges. This chapter aims to provide a comprehensive overview of the current understanding of PPOs, their biological significance, and their potential uses in promoting global sustainability.

12.2 Fundamentals of PPOs

PPOs use oxygen to oxidize phenols into quinones, and they are copper-containing proteins. They can either hydroxylate a monophenol or oxidize it to form *o*-diquinone (cresolase activity) or oxidize an *o*-dihydroxyphenol to form *o*-diquinone (catecholase activity). These reactions produce quinones, which are highly reactive and can chemically modify and cross-link cellular components, including protein nucleophiles such as sulfhydryl, amine, amide, indole, and imidazole groups. In food processing and postharvest physiology of plant products, PPOs are known for causing brown or black discoloration due to the formation of quinone adducts, which reduce the nutritional value of plant proteins. However, PPO-based strategies have been explored to increase the herbivore resistance of plants [2].

PPOs have been extensively researched in various plant species, and their properties differ depending on the plant. Although plant PPOs are encoded by the nucleus, they are enzymes located in the chloroplasts, and their synthesis and transport to the chloroplasts are complex processes similar to the import of nuclear-encoded proteins to their respective organelles. It is unclear where fungal PPOs are located, but some studies suggest they may be cytoplasmic or even bound to the cell wall in the extracellular matrix. Different forms of PPOs have been found in many plant species, with unique physicochemical and enzymatic properties. However, conflicting reports exist regarding the number of molecular forms of PPOs in some species/tissues due to the production of artifacts, interconversion among forms, hormonal induction, and attachment of phenolic products or carbohydrates [3].

PPOs can be distinguished based on optimal pH, temperature, and substrate specificity differences. The activity of the enzyme is strongly influenced by the pH of the assay or medium. Most enzyme activities follow a bell-shaped curve, increasing from zero in strongly acidic conditions, reaching a maximum/optimal value, and then decreasing to zero in strongly alkaline conditions. The behavior of the enzyme is affected by the protonation state of the functional groups of amino acids and cofactors involved in the enzymatic reaction, as well as the native three-dimensional structure of the enzyme. While protonation can be reversed, damaging the protein structure is irreversible and can impair enzyme activity. Furthermore, protonation can prevent substrate binding or catalysis of the substrate. pH-induced conformational changes in the enzyme can also alter the kinetic behavior of PPO [4]. PPO activity is affected by temperature, and its dependence on temperature is similar to its dependence on pH. The enzyme activity increases with increasing temperature until it reaches a maximum, after which it declines due to the destabilization of the enzyme's thermosensitive three-dimensional structure at high temperature, causing denaturation and a decrease in reaction velocity. The solubility of oxygen, which is necessary for PPO reaction, may also be affected by temperature. PPOs have different phenolic compounds as their main substrates, and the activity of PPOs varies depending on the source of the plant. PPOs show higher activity with substrates with a high affinity or preference for the enzyme. The nature of the side chain, number of hydroxyl groups, and their position on the benzene ring of the phenolic substrate significantly influence the catalytic activity of the enzyme. Some PPOs from plant species such as grape, field bean seed, sunflower seed, and strawberry have exhibited only diphenolase activity, while apple PPO lacked monophenolase activity or had very low activity compared to diphenolase activity [5].

PPOs are copper-containing metalloproteins found in many plants, and various other names, including catechol oxidase, catecholase, diphenol oxidase, *o*-diphenolase, phenolase,, and tyrosinase also know them. Catechol oxidases can catalyze two reactions: the hydroxylation of monophenols to *o*-diphenols, and the oxidation of *o*-diphenols to *o*-quinones. Oxygen is the primary oxidant in PPO-catalyzed reactions. These reactions are known as cresolase/monophenolase and catecholase/diphenolase activity, and the ratio of these activities can vary from 1 to 10 or even up to 40. Laccases, another type of phenol-catalyzing enzyme, can catalyze both *o*-diphenols and *p*-diphenols. They produce their corresponding *o*- and *p*-quinones, distinguishing them from catechol oxidases. The third enzyme, E.C 1.14.18.1, is called monophenol monooxygenase (tyrosinase) and corresponds to E.C 1.10.3.1. It always catalyzes the hydroxylation of monophenols. The nomenclature of these enzymes can be confusing, but they have distinct differences in their catalytic activities [6]. *o*-Quinone is further polymerized and condensed with amino acids and proteins to produce brown substances (Fig. 12.1).

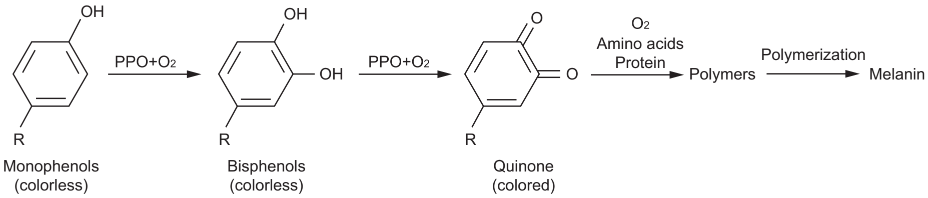

Fig. 12.1: PPO speeds up the process of converting monophenol into *o*-diphenol and facilitates the conversion of *o*-diphenol into *o*-quinone. *o*-Quinone then goes through a process of polymerization and condensation with amino acids and proteins, producing brown substances. The figure is reproduced from ref. [7] with permission. Copyright 2023 by the author. Licensee MDPI, Basel, Switzerland.

12.3 Production of PPOs

PPOs are found in various sources, including fruits, vegetables, and fungi. PPO proteins in plants and fungi are present in an inactive precursor state and an active state. In mature plant PPO, which is about 55–65 kDa, the catalytically active region (40–45 kDa) and the C-terminal structural domain (15–19 kDa) are included (Fig. 12.2). The C-terminus has been reported to be linked to the activation of PPO. It has minimal catalytic activity before activation [7]. Upon activation, PPO becomes active and gains catalytic activity (Fig. 12.2). PPOs with different molecular weights have been discovered in some other plants, such as broad bean, grape berry, sago palm, *Spinacia oleracea*, sweet potato, and potato. The variation in PPO molecular weight in the same plant may be due to C-terminal hydrolysis, which leads to the active state.

PPOs are commonly produced through fermentative processes using fungal or bacterial cultures, with yeast extract-glucose media being a common choice. Fermentation conditions, including temperature and the presence of specific salts such as manganese, copper, and iron, can affect the production of PPO. After production, PPO extraction typically involves cell disruption, which can be achieved through maceration, sonication, Hughes press, high-speed shaking with ballotini, and grinding with sand. Once extracted, PPOs can be purified using techniques such as ammonium sulfate precipitation, ion exchange chromatography, or gel filtration chromatography. However, many extracted PPOs have not been extensively studied in extraction and purification and may require further strategic study. Overall, the source, production process, extraction, and purification of PPOs involve a complex series of steps that vary depending on the source and application of the enzyme. The selection of sources for the production of

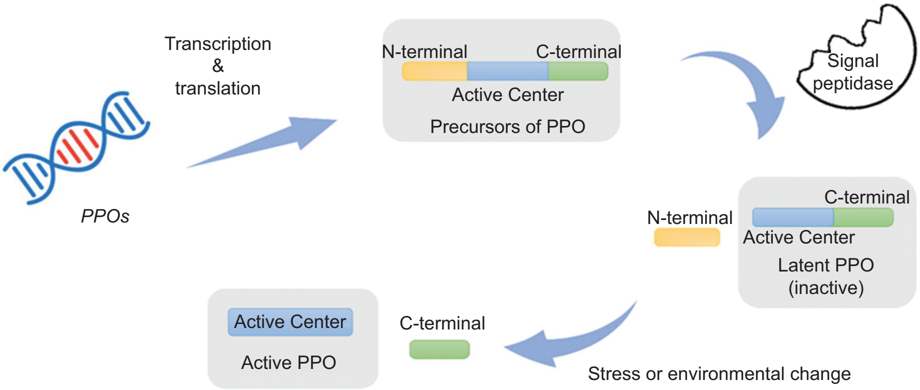

Fig. 12.2: To produce the precursor of PPO, PPO genes are first synthesized. The *N*-terminal transit peptide sequence is then eliminated, and a mature latent PPO (inactive) is formed within the plastid. When there is stress or an environmental change, a protease cleaves the C-terminal of PPO, resulting in the activation of PPO (active PPO). The figure is reproduced from ref. [7] with permission. Copyright 2023 by the author. Licensee MDPI, Basel, Switzerland.

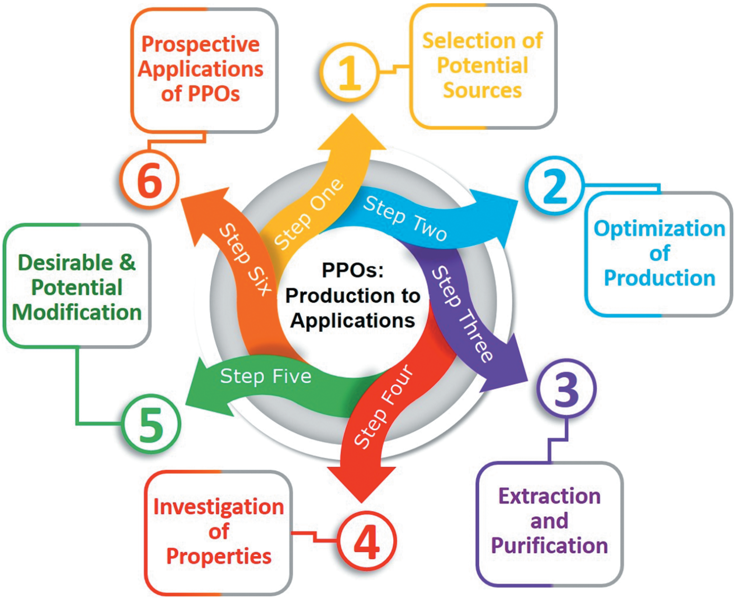

Fig. 12.3: The complex steps involved in selecting sources for producing PPOs and developing their potential multipurpose applications for achieving global sustainability.

PPOs toward the multipurpose potential applications for achieving global sustainability involves several complicated steps illustrated in Fig. 12.3.

12.3.1 Sources of PPOs

Numerous sources of PPOs have been explored by researchers, particularly fruits and leaves of various plants. Studies have shown that the PPO content in *Solanmu lycocarpum* fruit is higher in unripe fruits compared to ripe ones, which is attributed to the destruction of thylakoid membranes during the maturation process that metabolizes most of the proteins and enzymes [8]. Similar results were found in basidiocarps of *Lactarius pergamenus*, where PPO levels decrease with age. Other fungi such as *Entomocorticium* sp., *Trametes versicolor*, and *Polyporus versicolor* were also mentioned to produce PPO as an extracellular moiety. Fermentative processes associated with fungi are industrially applicable and less time-consuming than bacterial fermentation, although cell disruption is necessary for bacterial fermentation. Bacterial fermentation requires additional steps to disrupt the cell, such as maceration, sonication, Hughes press, high-speed shaking with ballotini, and grinding with sand. Yeast extract-glucose media are commonly used for fermentative PPO production, and salts of manganese, copper, and iron have been found to have a positive effect. Researchers have also investigated economic sources such as waste potato peels, apple skin, and wastewater from the sweet potato industry. There is also significant solid waste generated by the fruit, vegetable, and pulp industries that could be explored as a source of PPO to reduce raw material costs [9].

12.3.2 Production process

PPOs can be found in nearly all types of fungi and are considered a valuable resource for industrial production of PPOs. Thermophilic fungi, in particular, have an advantage in fermentation conditions. Fermentations at higher temperatures reduce the risk of contamination, which can be costly to sterilize on an industrial scale [10, 11]. Although higher temperature fermentations may increase costs, thermophilic organisms produce thermostable products [12]. There is growing interest in using thermophilic organisms as producers of desired products due to their ability to resist heat, denaturants, solvents, and proteolytic enzymes compared to their mesophilic counterparts. For example, the PPO from *Chaetomium thermophilum* was purified, and the enzyme's optimum pH and temperature values were found to be 5.0 and 55 °C, respectively. *Thermomyces lanuginosus* is also utilized to produce PPO [13, 14], which is known for its ability to produce various enzymes used in industry. For example, it is utilized to produce phytases myo-inositol hexakisphosphate phosphor hydrolyses (EC 3.1.3.8), which catalyzes di-, tri-, tetra-, and penta-phosphates of myo-inositol and inorganic phosphate, essential nutrients for animal feeds [15, 16].

12.3.3 Extraction and purification of PPO

The extraction and purification methods for PPOs vary based on the enzyme source and the end-use application. When selecting methods, researchers consider factors such as enzyme activity, purification fold, protein concentration, and contaminant presence. The downstream process can significantly impact the production cost, so understanding specific enzyme requirements is crucial. A combination of salt precipitation, temperature-induced phase separation, and chromatography are commonly used for PPO extraction and purification. However, more recent techniques like three-phase partitioning and two-phase aqueous extraction have also been explored. Both traditional methods, such as solvent precipitation and pI-based precipitation, and newer methods like disk electrophoresis are used to achieve better results. In most cases, the fresh source of PPO is homogenized with buffers such as phosphate and acetate within a pH range of 5–7 before applying different extraction methods. Potential techniques for extracting and purifying PPOs have been listed in Fig. 12.4.

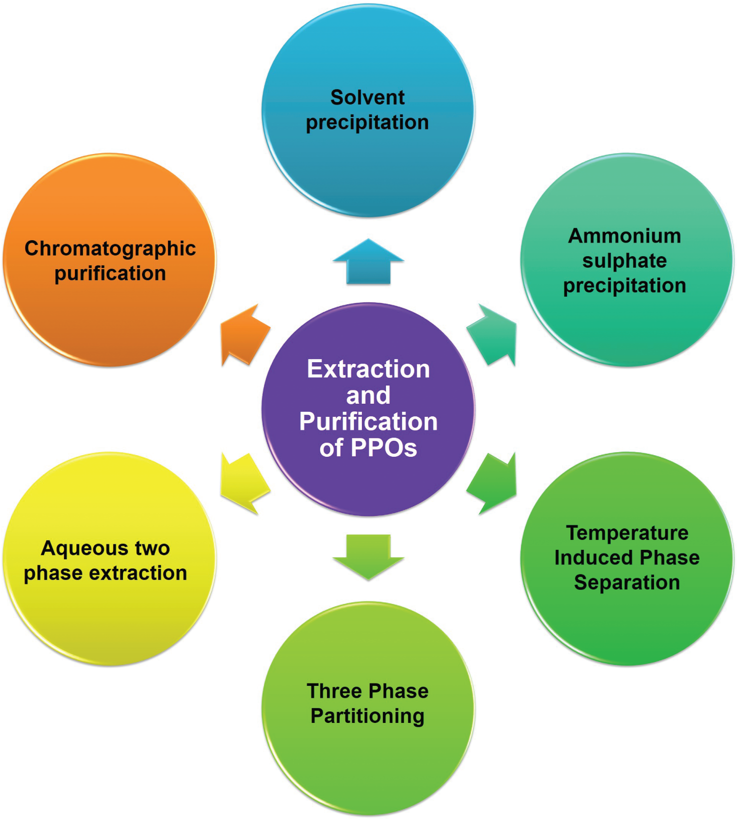

Fig. 12.4: Various methods for the extraction and purification of PPOs.

12.3.4 Characterization of PPO

Researchers include a characterization study, which involves analyzing molecular weight, pH, temperature optima, substrate specificity, effects of inhibitors, and kinetic and thermodynamic properties. Molecular weight can be determined through SDS-PAGE electrophoresis using a known molecular weight protein ladder, although, unlike most enzymes, PPO activity is not hindered by SDS. Therefore, a bioautographic assay or activity staining of PPO during SDS-PAGE using substrates such as catechol or L-DOPA can be performed to infer a distinct color band. The molecular weight of PPO varies from 27 to 144 kDa depending on its source [8]. However, an exception was observed in the case of *Lactarius pergamenus* PPO, where an isoform with different mobility in an alkaline medium exhibited the same molecular weight thereafter [17].

The stability under different pH and temperature range is also investigated. The enzyme's solubility, ability to bind with different substrates or inhibitors, and conformation can be affected by changes in the pH of the surrounding media [18]. Researchers have experimented with specific substrates to determine the optimum pH for the enzyme, but this value can vary depending on the substrate used [19]. In some cases, multiple pH optima may indicate the presence of different enzyme isoforms. Temperature is important to study because it can control the enzyme's kinetics and affect oxygen solubility in the solution. The optimum temperature for the enzyme can range from 10 to 60 °C depending on the source and substrate used. However, the enzyme PPO was observed to degrade at temperatures beyond 80 °C. Researchers also used the RESATO FPU food processing equipment to study the effects of pressure on plum PPO at different temperatures and found that degradation occurred at pressures beyond 700 MPa within a temperature range of 30–50 °C [20].

12.3.5 Selective modification for desirable applications of PPOs

Several techniques can be used for the modification of PPOs for various applications. One such technique is genetic engineering, which involves altering the genetic makeup of PPO to improve its properties. For example, PPO genes have been modified to increase their expression levels or alter substrate specificity [21–23]. Another technique is chemical modification, which involves changing PPO's structure using chemical agents [24]. This technique has been used to modify PPO's activity, stability, and specificity. A third technique is an immobilization, where PPO is attached to a solid support, such as a resin or a membrane [25–27]. This immobilization can improve PPO's stability and allow it to be reused for multiple reactions. Finally, PPO can be modified by the addition of specific inhibitors, which can control its activity and prevent unwanted reactions. These techniques, alone or in combination, can be used to modify PPO for various applications, including food preservation, biotechnology, and environmental monitoring [28, 29].

12.3.6 Potential applications of PPOs

PPOs have attracted the scientific community and industry stakeholders due to their potential applications in diverse sectors, including food industries such as food production, preservation, and modification; environmental sustainability through pollution control, waste management, and bioremediation; agriculture by helping plants their defense against pests and pathogens; forestry through the involvement in the breakdown of lignin; and terrestrial ecosystems. In addition to the typical applications, PPOs have shown their promising applications in biotechnology, nanotechnology, pharmaceutical cosmetic industry, and medical research.

12.4 Food industry and PPOs

PPOs are crucial but complex in food production, preservation, and nutritional quality. In food production, PPOs can be utilized to improve the sensory properties of foods, such as enhancing the flavor and color of fruits and vegetables. However, PPOs can also cause negative effects on food quality, such as the degradation of nutritional components like vitamins and antioxidants. In terms of food preservation, PPOs can inhibit microbial growth through the generation of toxic compounds that are harmful to microorganisms. The browning reaction that PPOs catalyze can also prevent enzymatic browning. However, the effectiveness of PPOs in food preservation can depend on factors such as pH, temperature, and storage conditions. Therefore, it is important to understand the role of PPOs in food systems and to manage their effects on food quality and preservation carefully. Proper utilization of PPOs can enhance the sensory properties of foods and improve their shelf-life while minimizing the degradation of nutritional components.

12.4.1 Food production

PPOs are involved in the enzymatic browning reaction that occurs in fruits and vegetables, which can negatively impact the appearance, texture, and flavor of these foods [22]. PPOs catalyze this oxidation reaction, leading to the formation of quinones and, ultimately, the production of brown pigments called melanins. This reaction can have several negative effects on the quality of fruits and vegetables, including changes in texture, flavor, and color, as well as reduced nutrient content and increased susceptibility to spoilage. Various methods (listed in Fig. 12.5) have been developed to control PPO activity, including chemical inhibitors, such as ascorbic acid or citric acid, which can prevent PPOs from catalyzing the oxidation of phenolic compounds. Other methods include blanching or cooking the produce, which can denature or inactivate PPOs.

More recently, genetic engineering and biotechnology have been used to develop crops with reduced PPO activity or to modify PPOs to improve their stability and activity.

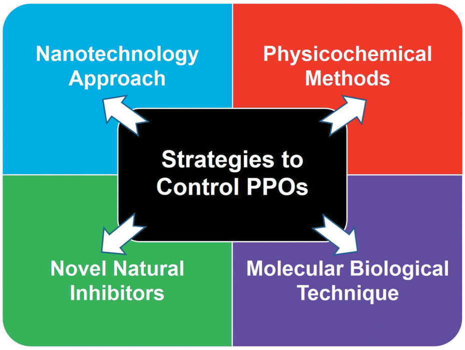

Fig. 12.5: Strategies to control polyphenol oxidase.

Several ways to inhibit PPO activity include physical, chemical, and biological methods (Fig. 12.6). Physical methods involve temperature control, controlled atmosphere, high pressure, ultrasound, and so on. High temperatures can quickly deactivate PPO, but can also have adverse effects on the quality, texture, and nutritional value of plant-based products [7]. A controlled atmosphere is a commonly used method in fruit and vegetable production to inhibit enzymatic browning by increasing CO_2 and decreasing O_2 concentration to a certain extent. This method is currently used in various fruits and vegetables such as litchi, potatoes, lotus roots, apples, and even graham flour. High-pressure treatment in water is also used to deactivate PPO with minimal damage to the raw material and has been used in fruits like blueberries and avocados. Ultrasound deactivates PPO by forming tiny bubbles and cavities through self-explosion, a physicochemical effect.

However, enzymatic browning can also positively affect food quality, particularly in producing certain food products such as tea and wine, which contribute to the final product's flavor and color. In these cases, the enzymatic browning reaction is controlled by adjusting the temperature, pH, or other conditions to optimize PPO activity [30–32]. Controlling the PPO activity is important in food production for several reasons. First, it can help prevent or reduce enzymatic browning, improving the appearance, texture, and flavor of fruits and vegetables. This is particularly important for fresh produce sold in supermarkets or used in food processing, as the quality of these products can significantly impact consumer acceptance and marketability. Second, controlling the PPO activity can help increase the shelf life of fruits and vegetables by reducing the spoilage rate [33–35]. Enzymatic browning can accelerate the breakdown of plant tissues and increase the growth of microorganisms, leading to decay and spoilage. By reducing the activity of PPOs, producers can slow down the enzymatic browning reaction and extend the shelf life of fruits and vegetables. Third, controlling

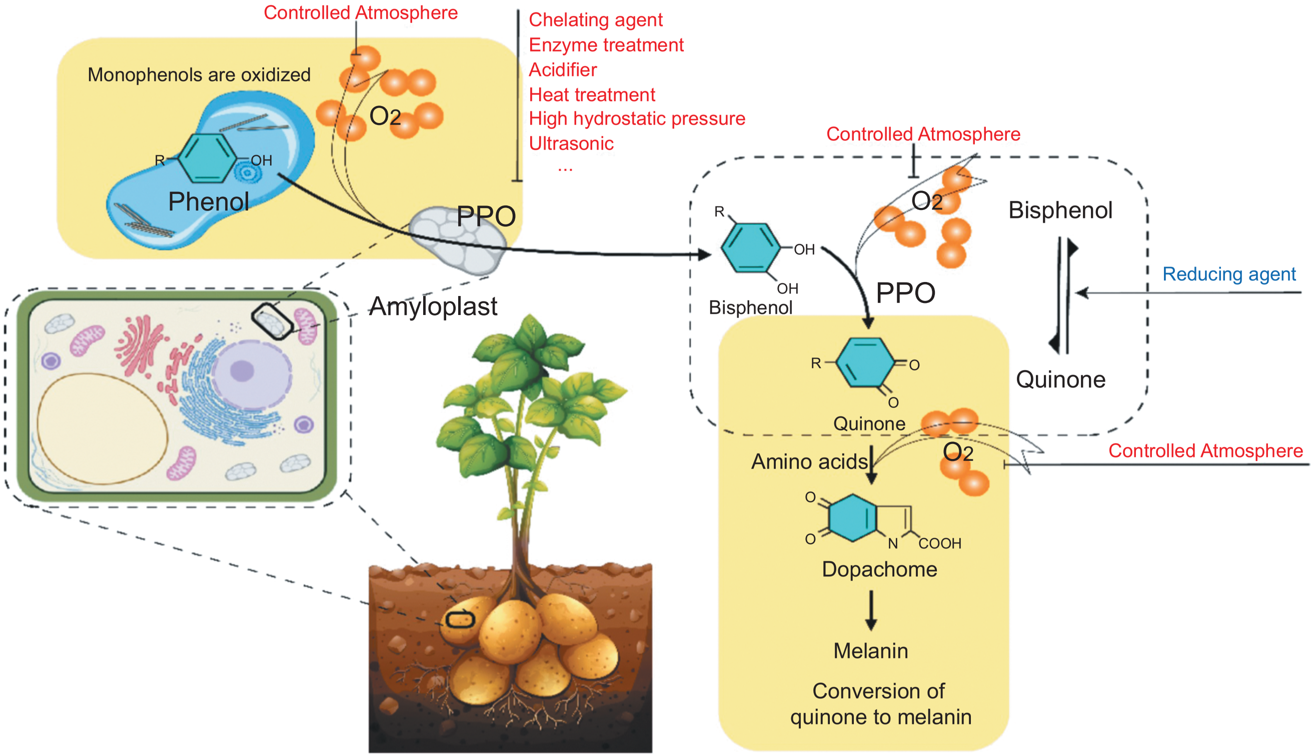

Fig. 12.6: In potato tubers, PPO is located in amyloplasts, while phenols are present in vesicles. In the presence of oxygen, PPO is activated and oxidizes monophenols to bisphenols, which are further oxidized to quinones. These quinones then react with amino acids and oxygen to form dopachrome, which ultimately leads to the production of melanin. The enzymatic browning process can be controlled by regulating the oxygen levels in a controlled atmosphere. Moreover, heat, enzymes, chemical treatment, and other methods can reduce the biological activity of PPO, thereby inhibiting enzymatic browning. The figure is reproduced from ref. [7] with permission. Copyright 2023 by the author. Licensee MDPI, Basel, Switzerland.

the PPO activity can help reduce food waste by minimizing the amount of discarded produce due to browning or spoilage [36–38]. This can have significant economic and environmental benefits, as it can reduce the amount of resources used to produce and transport food and the amount of waste generated.

Despite the importance of PPOs in food production, several challenges and opportunities are associated with their research and application. The first challenge is the complexity of the enzymatic browning reaction, which involves multiple enzymes and pathways and other factors such as temperature, pH, and substrate availability. This complexity makes it difficult to predict and control the reaction, particularly in different types of fruits and vegetables or under different processing conditions. The second challenge is the variability in PPO activity among different cultivars or varieties of fruits and vegetables, as well as within the same crop, depending on factors such as maturity, storage, and handling. This variability can make it challenging to develop consistent and effective strategies for PPO control. In addition, the complexity of enzymatic browning reactions and the numerous factors that can influence them, such as pH, temperature, and substrate concentration, further complicate PPO research and application.

One potential solution to these challenges is genetic engineering to modify crop PPO activity [39–42]. This approach has been explored in several crops, including apples, potatoes, and tomatoes, with promising results. For example, researchers have successfully downregulated PPO expression in apples using RNA interference, resulting in reduced enzymatic browning and improved fruit quality. However, genetic engineering also raises ethical and regulatory concerns, particularly regarding the potential risks and unintended consequences of manipulating the genetic makeup of crops. Conducting thorough risk assessments and engaging in transparent and inclusive dialogue with stakeholders before implementing genetic engineering approaches in food production is important. Overall, while the PPO research and application present challenges and opportunities, it is clear that this enzyme plays a crucial role in the quality, safety, and sustainability of food production. By understanding the mechanisms of enzymatic browning and developing effective strategies for PPO control, we can improve the efficiency and sustainability of food production and support global efforts toward a more sustainable and equitable food system.

The third challenge is the potential for unintended consequences of reducing or modifying the PPO activity. For example, reducing PPO activity in crops may also affect other physiological processes, such as resistance to pests and diseases, or nutrient uptake and transport. It is important to carefully evaluate the potential risks and benefits of these approaches before implementing them on a large scale. Despite these challenges, PPO research and application are also associated with opportunities. One opportunity is the development of new methods to control enzymatic browning that are more efficient, sustainable, and cost-effective. For example, nanotechnology and biopolymers have been investigated as potential alternatives to chemical inhibitors, as they can provide targeted and controlled release of active compounds. Another opportunity is the development of new products and markets that leverage the beneficial effects of enzymatic browning. For example, using PPOs to produce tea and wine can create unique flavor profiles and increase consumer demand. Similarly, using enzymatic browning to produce dried fruits and vegetables can enhance their flavor and nutritional content.

Finally, PPO research and application can contribute to global sustainability by reducing food waste and improving the efficiency and sustainability of food production. By reducing the amount of produce discarded due to browning or spoilage, PPO control can help reduce the environmental impact of food production, including using resources such as water, energy, and fertilizers. In addition, by improving the efficiency and sustainability of food production, PPO research can contribute to global food security and support the achievement of the United Nations Sustainable Development Goals.

In conclusion, PPOs are vital in food production, particularly in controlling enzymatic browning in fruits and vegetables. By controlling the PPO activity, producers can improve the appearance, texture, and flavor of fruits and vegetables, increase their shelf life, and reduce food waste. While there are challenges associated with PPO research and application, such as the complexity and variability of enzymatic browning and the potential for unintended consequences, there are also opportunities to develop new methods and products that leverage the beneficial effects of PPOs. Ultimately, PPO research and application can contribute to global sustainability by improving the efficiency and sustainability of food production and reducing food waste.

12.4.2 Food preservation

Enzymatic browning is critical in preserving plant-based foods such as fruits, vegetables, and nuts. One of the primary ways that PPOs contribute to food preservation is by inhibiting microbial growth. Enzymatic browning creates an acidic environment that is hostile to many microorganisms, making it more difficult for them to proliferate and spoil the food [43–45]. This can help to extend the shelf life of fruits and vegetables, reduce food waste, and increase the availability of nutritious foods. Another way that PPOs contribute to food preservation is by forming protective coatings around the surface of fruits and vegetables [46, 47]. The brown pigments that result from enzymatic browning can act as a barrier to oxygen, preventing the food from undergoing oxidative degradation and spoilage. This can be particularly important in the preservation of cut fruits and vegetables, which are more susceptible to spoilage due to increased surface area and exposure to oxygen. In addition to inhibiting microbial growth and forming protective coatings, PPOs can contribute to plant-based foods' nutritional quality. The quinones and melanins produced by PPOs have been shown to have antioxidant and anti-inflammatory properties, which can help to reduce the risk of chronic diseases such as cancer, diabetes, and cardiovascular disease.

By preserving the nutritional quality of fruits and vegetables, PPOs can help to support healthy diets and improve overall health outcomes.

However, there are also challenges associated with PPOs in food preservation. One of the main challenges is the variability in PPO activity among different cultivars or varieties of fruits and vegetables and within the same crop depending on factors such as maturity, storage, and handling. This variability can make it difficult to develop consistent and effective strategies for PPO control. Next challenge is the complexity of enzymatic browning reactions and the numerous factors that can influence them, such as pH, temperature, and substrate concentration. This complexity can make it challenging to predict and control the rate and extent of enzymatic browning, which can affect both food preservation and aesthetic appeal.

One potential solution to these challenges is the use of natural inhibitors of PPOs, such as ascorbic acid and citric acid [48–50]. These compounds can help to reduce PPO activity and enzymatic browning, and are commonly used in the food industry to preserve the color and quality of fruits and vegetables. Other potential strategies for PPO control include using modified atmosphere packaging, which can limit the exposure of fruits and vegetables to oxygen, and using heat treatments or irradiation, which can denature or destroy PPOs. This covers the importance of PPOs in food preservation. However, it is worth noting that PPOs also have other important roles in the food industry, such as in producing fermented foods and processing certain crops like cocoa and tea. PPOs can contribute to flavor development, color, and other desirable characteristics in these applications. However, they can also pose challenges, such as producing off-flavors or degrading certain compounds. PPOs are a complex and multifaceted group of enzymes that play important roles in food production and preservation.

In fact, PPOs play a critical role in food preservation by inhibiting microbial growth, forming protective coatings, and preserving nutritional quality. However, PPOs also have challenges, including variability in PPO activity and the complexity of enzymatic browning reactions. By developing effective strategies for PPO control, such as using natural inhibitors and modified atmosphere packaging, we can improve the efficiency and sustainability of food preservation and support global efforts toward a more sustainable and equitable food system.

12.4.3 Food nutritional quality

PPOs play important roles in the production of pigments and the preservation of quality. In addition to these roles, PPOs also significantly impact the nutritional quality of fruits and vegetables. This chapter explores the importance of PPOs to the nutritional quality of fruits and vegetables. One of the key ways that PPOs impact the nutritional quality of fruits and vegetables is through their influence on the bioavailability of nutrients. Many of the key nutrients found in fruits and vegetables, such as vitamin C, folate, and carotenoids, are sensitive to oxidation and can be easily degraded during storage and processing. PPOs can play a critical role in preserving the bioavailability of these nutrients by controlling the oxidative reactions that can lead to their degradation [51]. For example, vitamin C is a key antioxidant in many fruits and vegetables. This nutrient plays an essential role in maintaining immune function, protecting against oxidative stress, and supporting collagen production. However, vitamin C is also highly susceptible to oxidation and can be easily degraded during storage and processing. PPOs can help to prevent the oxidation of vitamin C by catalyzing the conversion of polyphenols to quinones, which can act as antioxidants and help to preserve the bioavailability of vitamin C [52].

Similarly, folate is another important nutrient found in fruits and vegetables. This nutrient is essential for a variety of biological processes, including DNA synthesis and repair, red blood cell formation, and nerve function. However, folate is also sensitive to oxidation and can be easily degraded during storage and processing. PPOs can help preserve the bioavailability of folate by controlling the oxidative reactions that can lead to its degradation [53]. In addition to their impact on the bioavailability of nutrients, PPOs can also contribute to the nutritional quality of fruits and vegetables by influencing their phytochemical content [54–56]. Phytochemicals are compounds found in plants and have been shown to have various health benefits. Many of these compounds, including flavonoids and anthocyanins, are synthesized through the action of PPOs. For example, flavonoids are a group of phytochemicals found in many fruits and vegetables. These compounds have been shown to have various health benefits, including reducing inflammation, improving cardiovascular health, and protecting against cancer. PPOs play a critical role in synthesizing flavonoids by catalyzing the conversion of phenols to *o*-diphenols, which can be further oxidized to form flavonoids.

Anthocyanins are another group of phytochemicals that are synthesized through the action of PPOs. These compounds are responsible for the vibrant colors of many fruits and vegetables, including berries, grapes, and red cabbage. In addition to their role in color development, anthocyanins have been shown to have various health benefits, including reducing inflammation and protecting against cancer. PPOs play a critical role in synthesizing anthocyanins by catalyzing the conversion of phenols to *o*-diphenols, which can then be further oxidized to form anthocyanins [57, 58]. Overall, PPOs play important roles in the nutritional quality of fruits and vegetables by influencing the bioavailability of key nutrients and contributing to the synthesis of important phytochemicals. Understanding the role of PPOs in fruit and vegetable nutrition can help inform strategies for preserving their nutritional value during storage and processing and maximizing the health benefits associated with their consumption.

12.5 Environment and PPOs

PPOs are not only important in food industries but also play a crucial role in environmental sustainability. In this context, PPOs are involved in various biological and ecological processes that help maintain and promote environmental health. One important way that PPOs contribute to environmental sustainability is their involvement in plant defense mechanisms [59–61]. PPOs protect plants against herbivores and pathogens by oxidizing phenolic compounds to form toxic quinones that deter feeding or act as antimicrobial agents. This reduces the need for chemical pesticides, which can negatively impact the environment, including water and soil contamination. PPOs promote natural plant defense mechanisms and promote a healthier and more sustainable ecosystem.

12.5.1 Biodegradation of plant material

PPOs play a role in the biodegradation of plant material [62, 63]. When plants die or shed leaves, PPOs help break down phenolic compounds, critical structural components of plant cells. This degradation process helps to recycle nutrients and maintain healthy soil ecosystems. PPOs are also involved in the degradation of lignin, a complex organic compound that is a major component of wood and other plant tissues. This process contributes to the carbon cycle by releasing stored carbon back into the atmosphere as carbon dioxide. In this way, PPOs play an essential role in maintaining the carbon balance in the environment.

12.5.2 Removal of phenolic contaminants

The effectiveness of PPO enzymes in converting a wide range of phenolic and polyphenolic compounds into harmless quinones has led to their use in eliminating dangerous phenolic pollutants from wastewater and effluent [64]. Polyphenols are a type of organic compound that includes monocyclic compounds such as phenols and phenolic acids, phenylpropanoids, and polymeric compounds like tannins, lignins, and melanins. These compounds are responsible for phytotoxicity in agro-food wastewater, and conventional microbial biodegradation methods are impractical due to bacterial activity inhibition. When wastewater containing these compounds is discharged onto soil, it can cause changes in biogeochemical cycles, such as inhibiting nitrification and nitrogen-fixing and nitrifying bacteria growth, leading to phytotoxicity. In addition, these compounds can induce anoxic phenomena in rivers, lakes, or seas.

Another important function of PPOs in environmental sustainability is their role in degrading xenobiotic compounds such as polycyclic aromatic hydrocarbons (PAHs) and phenols commonly found in industrial waste and pollution [65–67]. PPOs are involved in the oxidative metabolism of these compounds, which can reduce their toxicity and facilitate their degradation. This process helps reduce the environmental impact of industrial pollution and improve the quality of air, water, and soil quality.

12.5.3 PPOs for healthy ecosystems

PPOs are also involved in the nitrogen cycle, a critical process in maintaining healthy ecosystems [68]. They help oxidize phenolic compounds released by plants and contribute to the formation of soil organic matter. This organic matter is an important nitrogen source for plants, which is essential for their growth and survival. PPOs also play a role in converting nitrogen in the soil to a form that plants use. This process, called nitrogen fixation, is critical for maintaining healthy soil ecosystems and promoting sustainable agriculture.

12.5.4 Biosynthesis of pigments by PPOs

PPOs are involved in the biosynthesis of plant pigments, such as anthocyanins and melanins for plant growth, reproduction, and defense [69, 70]. These pigments also have important environmental functions, including protecting plants against UV radiation and attracting pollinators. By promoting the biosynthesis of these pigments, PPOs contribute to the health and sustainability of plant populations and ecosystems. Overall, PPOs play a vital role in environmental sustainability by promoting natural plant defense mechanisms, contributing to the biodegradation of plant material, degrading xenobiotic compounds, contributing to the nitrogen cycle, and promoting the biosynthesis of plant pigments. By understanding and harnessing the functions of PPOs, it is possible to promote healthier and more sustainable ecosystems, reduce the environmental impact of industrial pollution, and support sustainable agriculture.

12.6 Bioremediation

PPOs are particularly effective in the degradation of various types of pollutants, including phenolic compounds, which are common environmental contaminants. One of the key aspects of PPOs in bioremediation is their ability to oxidize phenolic compounds. Phenolic compounds are organic molecules widely used in industry and can be found in various environmental settings, including soil and water. These compounds can be toxic to plants and animals and persist in the environment for long periods. PPOs can oxidize phenolic compounds to produce quinones, which are less harmful and more easily degraded by microorganisms. They have two functions: catechol oxidase and tyrosinase. The mechanisms and structures that differentiate these two functions are not completely understood. TtPPO is a PPO from the thermophilic fungus *Thermothelomyces thermophila* that can break down chlorophenols, harmful by-products of certain pesticides. Valmas et al. [71] have reported a study to clarify the structural factors determining TtPPO's function by conducting protein–ligand docking experiments using YASARA software. The results of the docking experiments are compared to biochemical data, and the role of specific amino acids in TtPPO's function is examined (illustrated in Fig. 12.7). Identifying the amino acids involved in binding different substrates to the enzyme's active site could lead to developing a more effective biocatalyst for wastewater treatment based on the enzyme's structure.

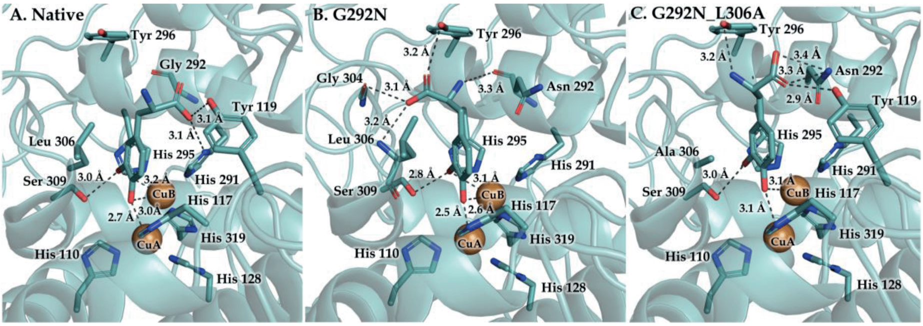

Fig. 12.7: Docking poses of L-DOPA in (A) native TtPPO, (B) variants G292N, and (C) variant G292N_L306A. The figure is reproduced with permission from ref. [71]. Copyright 2020 by the authors. Licensee MDPI, Basel, Switzerland.

Muhamad et al. [72] have investigated the potential for both enzymatic and adsorbent banana peel extracts to be utilized as a bioremediation agent for removing reactive dyes commonly used in the textile industry. The study investigates the effects of PPO and peroxidase enzymes, pH, temperature, and dye concentration on the adsorption capacity of banana peel extract. Results showed that the PPO enzyme isolated from banana peel extracted with buffer pH 6.5 and 7.5 demonstrated the highest decolorization of methylene blue and basic fuchsin, respectively. Complete adsorption was observed after 24 h of incubation for all concentrations of banana peel extract. The adsorption capacity of banana peel was highest at pH 7–11, with a peak of 96% after 24 h. Furthermore, an increase in temperature increased adsorption capacity, with 98% reaching after 24 h at temperatures above 50 °C. In conclusion, banana peel extract shows promising potential as an effective bioremediation agent due to its high adsorption capacity for selected textile dyes.

In addition to their role in oxidizing phenolic compounds, PPOs can also play a role in the degradation of other environmental pollutants. For example, PPOs have been effective in breaking down PAHs, a group of toxic organic compounds commonly found in soil and water. PAHs are known to be carcinogenic and mutagenic, and they can have a significant impact on the health of both humans and wildlife. By degrading

PAHs, PPOs can help reduce their environmental impact and protect human and animal health. Lu and group selected composting as a remediation method to address PAH pollution [73]. This method utilized the dissipation of PAHs using the resource utilization of organic solid waste and its degradation by bacteria. To evaluate the effectiveness of this approach, a study was conducted involving the co-composting of contaminated soil and cow manure. The results demonstrated that during the composting process, the degradation rates of naphthalene (Nap), phenanthrene (Phe), and benzo[α]pyrene (BaP) could reach up to 82.2%, 79.4%, and 59.6%, respectively. Cluster analysis indicated that PPO, laccase, and protease were essential drivers of PAH transformation. In addition, the content of humic substances (HSs) in the PAH treatment was significantly higher than that in the control group after 65 days, measuring 106.67 g/kg. To determine the degradation mechanism of PAHs by microorganisms, phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) and network analysis were employed. It was discovered that the degradation of PAHs by PPO contributed significantly to the formation of HS. Furthermore, PAHs and metabolic intermediates were oxidized and decomposed by PPO to form quinone, which subsequently condensed with amino acids to form HS. PAH degradation and compost quality were improved through composting, achieving a beneficial outcome.

Another vital aspect of PPOs in bioremediation is their ability to be produced by various organisms, including plants, bacteria, and fungi. This makes them a versatile tool for bioremediation efforts, as they can be used in various environmental settings. For example, PPOs produced by plants can be used to remove pollutants from soil, while PPOs produced by bacteria can be used to degrade pollutants in water. The use of PPOs in bioremediation can also have important economic benefits. Many contaminants that PPOs are effective at degrading are also common by-products of industrial processes. By using PPOs to remove these pollutants from the environment, it is possible to reduce the environmental impact of these industries and improve their sustainability. In addition, using PPOs in bioremediation can often be less expensive than other remediation methods, such as excavation or chemical treatment.

PPOs can degrade various pollutants, including phenolic compounds commonly found in industrial effluents, pesticides, and herbicides [62]. Phenolic compounds are considered toxic and persistent pollutants and can negatively impact the environment and human health. PPOs can oxidize phenolic compounds and convert them into less harmful compounds or break them down into smaller molecules, which other microorganisms can further degrade. This process is known as enzymatic detoxification, and PPOs effectively degrade a wide range of phenolic compounds. Furthermore, PPOs have also been found to play a crucial role in the degradation of lignocellulosic biomass, one of Earth's most abundant renewable resources [74]. Lignocellulosic biomass mainly comprises lignin, cellulose, and hemicellulose, which are difficult to degrade due to their complex structures. However, PPOs can degrade lignin and other aromatic compounds found in lignocellulosic biomass, making them an important enzyme for producing biofuels and other high-value products from biomass.

In addition to the above, PPOs can also be used to remove heavy metals from contaminated soils and waters [75]. Heavy metals such as lead, cadmium, and mercury are toxic and can cause severe environmental pollution. PPOs can bind to these heavy metals and convert them into less toxic forms, making them easier to remove from the environment. Furthermore, PPOs can also increase the mobility of heavy metals in soils, making it easier for other microorganisms to remove them from the environment.

However, there are also some challenges associated with the use of PPOs in bioremediation. One of the main challenges is the variability in PPO activity among different organisms and within the same organism depending on factors such as growth conditions and the presence of other pollutants. This variability can make it difficult to predict the effectiveness of PPOs in a given environmental setting, and it may also limit their use in certain situations. Another challenge is the potential for unintended consequences when using PPOs in bioremediation. For example, some studies have suggested that using PPOs to degrade certain pollutants can release toxic intermediates, which can have their own environmental impact. Therefore, it is important to carefully consider the potential risks and benefits of using PPOs in bioremediation, and to monitor the outcomes of these efforts carefully.

Despite these challenges, the importance of PPOs in bioremediation is evident. These enzymes can be a powerful tool for removing environmental pollutants and protecting the health of humans and wildlife. As our understanding of PPOs and their role in bioremediation continues to grow, we will likely see increased use of these enzymes in environmental cleanup efforts. Overall, the importance of PPOs in bioremediation lies in their ability to degrade various pollutants and toxic compounds, which can negatively impact the environment and human health. PPOs can also contribute to producing biofuels and high-value products from lignocellulosic biomass, which can help reduce dependence on fossil fuels and promote a sustainable economy. Using PPOs in bioremediation can help mitigate environmental pollution and provide a cost-effective and eco-friendly solution to various environmental problems.

12.7 PPOs in agriculture

PPOs impact agriculture from various aspects, including plant defense against herbivores and pathogens, postharvest preservation of fruits and vegetables, forming plant–microbe symbioses of agriculture, the potential application in biotechnology, and breeding and selection of crops. They are found in high concentrations in the cells of damaged tissues, where they react with oxygen and phenolic compounds to produce a range of secondary metabolites that have antimicrobial and antifungal properties [76]. These compounds can inhibit the growth of pathogens and herbivores and prevent further damage to the plant. PPOs also contribute to forming physical barriers in the form of lignin and suberin, which protect the plant tissues from further damage.

Moreover, PPOs have been found to play an important role in the postharvest preservation of fruits and vegetables [77]. After harvest, fruits and vegetables undergo metabolic processes that can cause spoilage and reduce their shelf life. They can catalyze the oxidation of phenolic compounds found in fruits and vegetables, forming brown pigments and off-flavors that are not appealing to consumers. However, the activity of PPOs can be controlled by adjusting factors such as temperature, humidity, and oxygen levels to prevent oxidation and improve the shelf life of fruits and vegetables. In addition to their role in plant defense and postharvest preservation, PPOs also have implications in the breeding and selection of crops [78]. The activity of PPOs can vary widely among different cultivars or varieties of plants, which can affect the crop's appearance, taste, and nutritional quality. For instance, PPO activity can influence the color of fruits and vegetables, an essential factor in consumer preference. PPO activity can also affect the nutritional quality of crops by controlling the content of phenolic compounds, which are known to have antioxidant and anti-inflammatory properties.

Furthermore, PPOs have been found to play a role in forming plant–microbe symbioses, such as the rhizobia–legume symbiosis [79]. In this symbiosis, rhizobia bacteria infect the root hairs of legumes and form nodules, converting atmospheric nitrogen into ammonia, which the plant can use as a nutrient. PPOs are involved in developing these nodules by forming lignin barriers around the infected cells, which protect the plant from pathogenic microorganisms. They have been used to develop transgenic crops with enhanced resistance to pests and diseases [80–83]. For instance, PPOs from potatoes have been used to develop transgenic tomatoes with increased resistance to the fungal pathogen *Botrytis cinerea*. PPOs have also been used to produce biofuels from lignocellulosic biomass, which can reduce dependence on fossil fuels and promote a sustainable economy. They are also involved in the plant's response to environmental stress factors such as drought, heat, and salinity [84]. Studies have shown that the activity of PPOs increases in response to these stressors, suggesting that they may play a role in protecting the plant from damage caused by oxidative stress.

Overall, the importance of PPOs in agriculture lies in their role in plant defense against herbivores and pathogens, postharvest preservation of fruits and vegetables, breeding and selection of crops, formation of plant–microbe symbioses, and potential applications in biotechnology. The study of PPOs in agriculture can provide insights into the mechanisms of plant–microbe interactions, plant defense mechanisms, and metabolic pathways in plants. Applying PPOs in agriculture can help improve crop yield, quality, and sustainability and promote a more resilient agricultural system.

12.8 Role of PPOs in nanotechnology

Nanotechnology is the science and technology of developing and manipulating materials at the nanoscale, which is typically between 1 and 100 nm in size. It involves designing, producing, and applying structures, devices, and systems with novel and useful properties due to their small size. Nanomaterials have unique and desirable properties, such as high surface area, increased reactivity, and improved mechanical and electrical properties, not exhibited by their bulk counterparts. Nanotechnology relies on the properties of nanomaterials to create innovative technologies and devices. The emergence of engineered nanomaterials such as nanocomposite gels [85–88], nanocomposite films [89–91], structural colored nanomaterials [92–94], organometallic nanomaterials [95], molecular machines [96], nanomaterial-based biosensors [97], and others has replaced conventional bulk materials in various industrial sectors [98–101] and theoretical investigations [102]. PPOs, in cooperation with nanomaterials, serve as the building blocks of nanotechnology, enabling researchers to create novel materials with tailored unique properties and excellent functionalities. While PPO has been extensively studied for its role in food science and biotechnology, its applications in nanotechnology are still being explored.

12.8.1 Synthesis of metal nanoparticles

The field of nanoparticle production via nanobiotechnological route has seen significant growth in recent years. However, many studies rely on bacteria and microscopic fungi, which may be pathogenic. A more promising approach involves using xylotrophic cultivated edible and medicinal basidiomycetes for nanoparticle biosynthesis. These organisms are introduced into an artificial culture and are commonly used in food, food additives, and pharmaceuticals. They are nontoxic and nonpathogenic, possess strong enzyme systems, and can produce large amounts of biomass. Cultures of xylotrophic basidiomycetes such as *Lentinus edodes*, *Ganoderma lucidum*, *Pleurotus ostreatus*, and *Grifola frondosa* are easy and safe to cultivate. They are also highly valued as edible fungi, as producers of enzymes, and as a unique complex of biologically active substances and drugs. These properties make them widely used in the food and pharmaceutical industries. One potential application of PPO in nanotechnology is in the synthesis of metal nanoparticles. PPO can act as a reducing agent and stabilizer for synthesizing metal nanoparticles, including gold, silver, selenium, silicon, and silica nanoparticles [103–105]. PPO can also be used to synthesize metal oxide nanoparticles, such as iron oxide and zinc oxide nanoparticles.

Vetchinkina et al. [103] have demonstrated the capability of various taxonomic groups of cultured basidiomycetes, namely *Lentinus edodes*, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Grifola frondosa*, to recover gold, silver, selenium, and silicon from their ionic state and form nanoparticles. The study investigates the impact of these metal and metalloid compounds on the growth and biomass accumulation parameters and identifies optimal cultivation conditions and concentrations of the tested ion-containing compounds for nanoparticle recovery. The research employs techniques such as transmission electron microscopy, dynamic light scattering, X-ray fluorescence, and X-ray phase analysis to determine the degree of oxidation of the bioreduced elements, the ζ-potential of colloidal solutions, uniformity, size, shape, and location of nanoparticles in the culture fluid, as well as on the surface and the inside of filamentous hyphae. The study highlights the role of fungal phenol-oxidizing enzymes (such as laccases, tyrosinases, and Mn-peroxidases) in the nanoparticle recovery mechanism, leading to the formation of electrostatically stabilized colloidal solutions. The research proposes a hypothetical mechanism for reducing gold(III) from $HAuCl_4$ to gold(0) by phenol oxidases, forming gold nanoparticles of different shapes and sizes.

12.8.2 Fabrication of nanocomposites

Another potential application of PPO in nanotechnology is in the fabrication of nanocomposites [25, 106]. PPO can oxidize phenolic compounds, which can then be used as a matrix material to fabricate nanocomposites. For example, PPO can be used to oxidize tannic acid to form a matrix material for synthesizing iron oxide nanoparticles. Almulaiky and Almaghrabi [25] have fabricated PPO-based calcium alginate and zinc oxide nanoparticles (Ca-ALG-ZnO NPs) and nanocomposites. In this study, researchers isolated PPO from *Coleus forskohlii* using a three-step process involving $(NH_4)_2SO_4$ precipitation, ion exchange chromatography, and gel filtration. They purified the enzyme 15-fold with a yield of 31% and a specific activity of 3,168 U/mg, and found that it had a molecular weight of 42 kDa. To increase the stability and reusability of the enzyme, they successfully prepared and characterized a support material made of Ca-ALG-ZnO NPs for immobilizing the PPO. The immobilized PPO had an enzyme activity of 3,950 U/g support and an immobilization yield of 83%, and could be reused up to 10 times while retaining 69% of its original activity. The researchers found that the optimal pH for the purified and immobilized PPO was 7.0–7.5, and the optimal temperature was 40–50 °C. They also calculated $V_{\max}$ values of 255.75 and 251.89 Units/mL and K_m values of 4.99 and 3.12 mM for the purified and immobilized PPO, respectively. Overall, this study provides insight into the immobilization of PPO from *Coleus forskohlii* for industrial applications and highlights the positive impact of Ca-ALG-ZnO NPs on enzyme activity and stability.

12.8.3 PPO-based nanobiosensors

PPO has been utilized in developing nanobiosensors due to its ability to catalyze the oxidation of a wide range of polyphenolic compounds. Nanobiosensors fabricated with PPO have been used for the detection of various analytes, including glucose, phenolic compounds, and pesticides [107–115]. The PPO-based nanobiosensors are usually constructed by immobilizing the enzyme onto a nanomaterial surface, such as gold nanoparticles or carbon nanotubes. The enzyme-based biosensor has several advantages, such as high sensitivity, specificity, and stability. One of the most significant advantages of using PPO for nanobiosensor fabrication is its ability to function under mild experimental conditions. The enzyme can operate at neutral pH and moderate temperature, making it suitable for various biosensing applications. Moreover, PPO-based nanobiosensors are highly selective and can detect target analytes with high sensitivity, even at low concentrations. This property makes PPO-based nanobiosensors ideal for medical diagnostics, environmental monitoring, and food quality control applications.

PPOs are found in many plants and animals and are crucial in various biological processes. In medical research, PPOs have been found to be important in several ways, such as in diagnosing and treating diseases and as a potential therapeutic target. One of the critical roles of PPOs in medical research is their involvement in diagnosing diseases. PPOs have been used as a marker to diagnose several diseases, including cancer. For example, PPOs are highly expressed in melanoma cells, which have been exploited to develop diagnostic tools for the early detection of melanoma. Similarly, they have also been used as a biomarker for diagnosing other types of cancer, such as prostate and breast cancer. These diagnostic tools have helped in the early detection and treatment of cancer, leading to better patient outcomes.

Another important aspect of PPOs in medical research is their potential role as a therapeutic target. They have been found to be overexpressed in several diseases, including cancer, inflammation, and neurodegenerative disorders. As a result, PPOs have been explored as potential targets for developing new therapies. For example, several compounds that can inhibit PPO activity have been developed. These compounds have been found to have anti-inflammatory and anticancer effects in vitro and in vivo. These findings suggest that PPO inhibitors could be potential therapeutic agents for treating various diseases.

PPOs are involved in the formation of melanin, which is a pigment that protects the skin from UV radiation and also helps in the healing of wounds. Melanin is synthesized from tyrosine, and PPOs are involved in converting tyrosine to melanin. Therefore, PPOs have been studied for their potential role in improving wound healing. In one study, PPO inhibitors were found to enhance wound healing in mice, suggesting that PPO inhibitors could be used as a therapeutic agent for treating injuries.

Furthermore, PPOs have also been implicated in the development of age-related diseases. As people age, PPOs' activity increases, accumulating oxidized proteins and other harmful molecules in the body. This accumulation has been linked to the development of age-related diseases such as Alzheimer's and Parkinson's [117, 118]. Therefore, PPOs have been explored as potential targets for treating these diseases. In one study, PPO inhibitors reduced the accumulation of oxidized proteins in the brain and improved cognitive function in mice with Alzheimer's disease. These findings suggest that PPO inhibitors could be a potential therapeutic agent for treating age-related diseases. In addition to the potential therapeutic applications of PPOs, they are also being investigated for diagnostic purposes. For example, some studies have explored using PPOs as biomarkers for certain diseases. In one study, researchers found that the PPO activity was significantly higher in the saliva of patients with oral cancer than healthy individuals, suggesting that PPOs could be used as a diagnostic tool for this disease. Another study found that the PPO activity was elevated in the urine of patients with bladder cancer compared to healthy individuals, indicating that PPOs could be used as a noninvasive biomarker for this type of cancer. Furthermore, PPOs are being studied for their potential use in drug delivery systems. They are known to be sensitive to changes in pH and temperature. This property can be exploited to develop PPO-based drug delivery systems that can release drugs in response to specific stimuli. For example, PPOs could be used to trigger the release of drugs from liposomes in response to changes in pH, which could potentially improve the efficacy of certain drugs.

In conclusion, PPOs play an essential role in medical research, including their involvement in diagnosing and treating diseases, wound healing, and developing age-related diseases. They have been used as biomarkers for diagnosing several types of cancer and as a potential therapeutic target for treating various diseases. PPO inhibitors have shown promise as a therapeutic agent for treating inflammation, cancer, and age-related diseases. Therefore, further research on PPOs in medical research could lead to the developing of new diagnostic tools and therapies for various diseases.

12.9 Biomedical applications of PPOs

12.9.1 Therapeutic application

PPOs have proven versatility in medical research and biomedical applications such as cancer. The fight against cancer has always been challenging, and current treatments are still unsatisfactory. Therefore, there is a need to discover new drugs for cancer treatment. Traditional Chinese medicine has been effective in treating cancer, and PPO extracted from edible mushroom has been reported to have several characteristics. However, its role in cancer treatment is still unclear. Yuan et al. [116] have investigated the potential of PPO from edible mushroom in inhibiting cancer cell proliferation, migration, invasion, and promoting apoptosis in vitro, as well as exploring its therapeutic effects on tumors in vivo. The cell counting kit-8 assay was used to measure the impact of PPO on cancer cell proliferation, while the scratch test and transwell assay were used to determine its effect on migration and invasion, respectively. The flow cytometry technique was used to measure the effect of PPO on cancer cell apoptosis. In vivo experiments were carried out using female BALB/c mice (18–25 g, 6–8 weeks) divided into a control group, model group, low-dose group (25 mg/kg), and high-dose group (50 mg/kg). The results showed that PPO from edible mushrooms significantly inhibited the proliferation, migration, and invasion of breast cancer cell 4T1, lung cancer cell A549, and prostate cancer cell C4-2, while substantially promoting their apoptosis. Furthermore, in vivo experiments showed that PPO had an inhibitory effect on tumor growth. In summary, PPO from edible mushrooms has the potential as an effective agent for treating various cancers, and it may become a promising anticancer drug in the future.

12.9.2 Drug delivery application

PPOs have shown potential as a drug delivery system due to its ability to bind to various substrates and catalyze the formation of covalent bonds between them. They are known to be sensitive to changes in pH and temperature. This property can be exploited to develop PPO-based drug delivery systems that can release drugs in response to specific stimuli. They can be used to immobilize drugs or proteins onto surfaces, such as nanoparticles, microbeads, or fabrics, by simply mixing the substrate with the enzyme. The immobilized drugs or proteins can then be released from the surface by enzymatic degradation of the PPO substrate, allowing controlled drug release. PPO has also been used to coat nanoparticles with a biocompatible polymer, enhancing the stability and circulation time of the nanoparticles in the body. PPO-based drug delivery systems can potentially improve drug efficacy, reduce toxicity and side effects, and increase patient compliance [119]. However, further research is needed to optimize the performance of PPO-based drug delivery systems and evaluate their safety and efficacy in vivo.

12.9.3 Antimicrobial coatings

PPOs can be used to synthesize antimicrobial coatings. They can oxidize phenolic compounds, which can then be used to form coatings with antimicrobial properties. These coatings have potential applications in medical devices and food packaging. The demand for biomedical-grade titanium (Ti) alloys is increasing due to the advancements in orthodontic and orthopedic technologies. However, Ti-based implants are prone to bacterial infections, leading to implant failure or repeated surgeries. Silk sericin (SS) is a hydrophilic, biocompatible, and biodegradable material with low immunological response. Qiang et al. [120] developed PPO-based antimicrobial coating through the modification of the surface of Ti-based implants, PPO (tyrosinase) was used to oxidize the tyrosine moiety in SS to generate SSC, which contains catechol groups. The SSC coatings were deposited onto Ti surfaces to create active surfaces with catechol groups, which were hybridized with silver nanoparticles (Ag NPs) via in situ silver ion reduction. The Ag NPs/SSC-coated Ti surfaces showed antibacterial properties and could prevent bacterial cell adhesion and early-stage biofilm formation. Moreover, these surfaces exhibited negligible cytotoxicity in L929 mouse fibroblast cells.

12.10 Conclusion

PPOs are a versatile group of enzymes that play a vital role in various fields, including food industry, environment, agriculture, and biomedicine. They are involved in various processes, such as food production, preservation, nutritional quality, biodegradation of plant material, removal of phenolic contaminants, and biosynthesis of pigments. They have also shown potential in bioremediation, nanotechnology, and drug delivery applications. The importance of PPOs in the food industry is immense, as they can improve food quality, prevent spoilage, and enhance nutritional value. Additionally, PPOs can help reduce the use of harmful chemicals in food production, leading to a healthier and more sustainable food system. In the environment, PPOs play a crucial role in maintaining healthy ecosystems by breaking down plant material and removing phenolic pollutants. The emerging applications of PPOs in nanotechnology, biomedical science, and drug delivery systems are exciting and promising. PPOs have shown potential in synthesizing metal nanoparticles, fabricating nanocomposites, and developing nanobiosensors. In addition, they have been explored as a drug delivery system due to their biocompatibility, biodegradability, and low immunogenicity. They have also shown antimicrobial properties, making them a potential candidate for developing antimicrobial coatings in biomedical devices.

Overall, PPOs' research and development hold significant global sustainability potential. The versatility of PPOs in various fields makes them an attractive enzyme for further research and exploration. Future studies could focus on understanding the structure and function of PPOs and identifying new applications for them. Furthermore, efforts could be made to explore their commercial potential and to promote the sustainable use of PPOs in different fields. Ultimately, PPOs offer a promising future for various industries and contribute toward a more sustainable and healthier planet.

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