Research Article

Exploring the Production, Purification, and Multifaceted Potential of Tyrosinase: A Review

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Abstract | Tyrosinase E.C 1.14.18.1 is a copper-dependent oxidase enzyme that catalyses oxidation reactions in many biological systems and is also involved in melanin production. Due to its diverse functional properties, tyrosinase has attracted substantial attention in cosmetic products for regulating pigmentation, pharmaceutical research involving melanoma-related therapies, food industries for controlling enzymatic browning, environmental bioremediation processes, and sustainable biotechnological applications. They are widely distributed in fungi, bacteria and some marine organisms where they are secreted extracellularly. Microbial systems have become highly effective and cost-efficient sources for large-scale tyrosinase production and purification process of tyrosinase generally includes precipitation and chromatographic methods that enhance enzyme purity and improve catalytic activity. Recent scientific studies recognize tyrosinase as a highly versatile enzyme with significant industrial and biomedical potential, while continued improvements in production methods, enzyme stability, and process optimization remain necessary for wider commercial application.

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Introdction

Tyrosinase E.C 1.14.18.1 is a biologically significant enzyme involved in the production of pigments in various living organisms. It participates in several natural biochemical processes, most notably the enzymatic browning that occurs in fruits and vegetables as well as the synthesis of melanin, which is the primary pigment responsible for the coloration of human skin, hair, and eyes (Xie *et al.*, 2009). Although these functions are important for normal biological activities, an increase in tyrosinase activity can lead to negative effects. In the food sector, enzymatic browning can decrease the appearance and commercial quality of fresh products, while in the field of dermatology and cosmetics, excessive melanin production may result in skin hyperpigmentation disorders. For this reason, the search for efficient tyrosinase inhibitors has become a major focus in research related to food preservation and cosmetic skin-lightening treatments (Costin *et al.*, 2007).

The importance of tyrosinase is not limited only to pigmentation-related functions. This enzyme

facilitates the conversion of tyrosine into L-DOPA and then catalyzes the oxidation of L-DOPA into dopaquinone, both of which are essential reactions in the pathway of melanin production. Because of its key involvement in this biochemical pathway, tyrosinase has gained considerable interest in cancer-related studies, particularly melanoma research, where melanin synthesis can affect tumor growth and progression (Pillaiyar *et al.*, 2017). Furthermore, oxidation reactions caused by tyrosinase create significant challenges in the wine industry. Browning problems become more pronounced when grapes are affected by grey rot, a fungal infection that introduces laccase in addition to the naturally present grape tyrosinase. These enzymes promote the oxidation of phenolic compounds, producing o-quinones that later polymerize and form brown-colored pigments, ultimately reducing the color quality and overall value of wines (Li *et al.*, 2008).

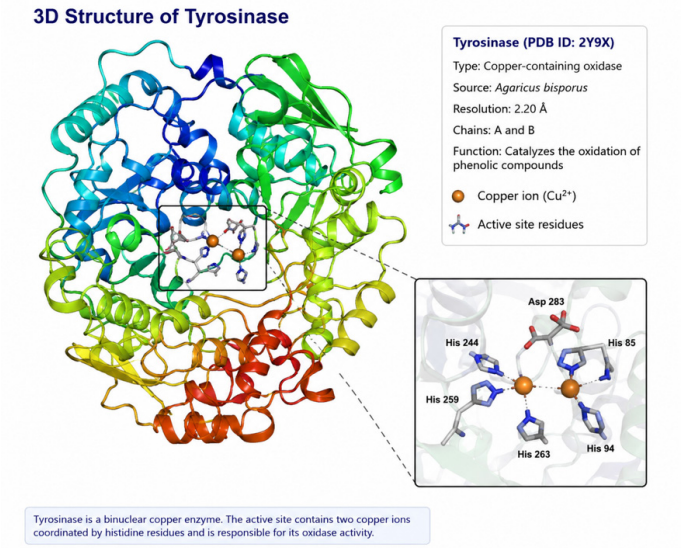

Figure 1: *3D Structure of Tyrosinase Enzyme (Lai X et al., 2023)*

Production

Tyrosinase is an essential copper-containing oxidase enzyme that is naturally present in plants, animals, and microorganisms, where it performs an important function in melanin formation and the oxidation of phenolic substances. Due to its expanding use in industries such as cosmetics, pharmaceuticals, biosensor development, and environmental bioremediation, the need for efficient tyrosinase production has grown significantly over recent years. Among different production sources, microbial systems are considered highly favorable because they offer fast growth, simple cultivation requirements, and strong potential for large-scale industrial manufacturing.

Different microbial production systems have been studied for the synthesis of tyrosinase, with each system presenting its own benefits and challenges. Bacterial organisms including *Streptomyces*, *Bacillus*, and *Pseudomonas* are commonly used because of their rapid multiplication and comparatively high enzyme productivity. On the other hand, fungal organisms such as *Aspergillus* and *Neurospora* are known to release tyrosinase extracellularly, making the recovery and purification process easier. However, fungal production systems usually require extended fermentation time and are often more vulnerable to contamination issues. Among bacterial sources, *Streptomyces* is considered one of the most effective producers because of its natural involvement in melanin biosynthetic pathways and its capacity to generate high amounts of functional enzyme (Kumar *et al.*, 2022).

The production efficiency of tyrosinase largely depends on maintaining suitable culture conditions. Proper carbon and nitrogen sources are necessary to ensure microbial growth and promote enzyme biosynthesis, while copper supplementation is especially critical since copper ions are an essential part of the enzyme's catalytic active site. Research findings have shown that adjusting copper concentration under optimized conditions can greatly improve enzyme production yield (Zhang *et al.*, 2023). Furthermore, environmental conditions including pH, temperature, aeration rate, and dissolved oxygen concentration need careful control to achieve maximum productivity and preserve enzyme activity.

For industrial-scale manufacturing, both submerged fermentation (SmF) and solid-state fermentation (SSF) methods have been widely studied. SmF continues to be the most commonly preferred technique because it allows improved control of operational parameters, supports easy scale-up, and ensures uniform product quality. In comparison, SSF has gained attention as an economical alternative, especially when low-cost agricultural waste materials are utilized as substrates. Although SSF may lower production costs and sometimes improve enzyme stability, difficulties associated with process monitoring and large-scale operation continue to restrict its wider industrial use (Singh *et al.*, 2024). As

Table 1: *Purification of Tyrosinase*

Step	Process/Method	Description	Purification fold	Yield (%)	Reference
1	Crude extraction	Culture centrifuged at 4°C to obtain supernatant containing crude tyrosinase. This extract contains highest amount of enzyme activity because no purification loses have occurred;however it also contains large number of unwanted proteins and other impurities resulting in low specific activity.	1.0	100%	Ajeel *et al.*, 2025
2	Ammonium sulphate precipitation	80% saturation to concentrate enzyme based on their solubility and removes non target proteins and increase enzyme purity.	~2.5-fold	~75%	El-Shora *et al.*, 2020
3	Dialysis	Removal of salts using buffer (pH 6.5) to remove excess small unwanted molecules and also provides slight increase in purity	~3.2-fold	~65%	Marcial -Quino *et al.*, 2023.
4	Ion exchange chromatography (DEAE-cellulose)	Separation based on charge using NaCl gradient is used to elute the bound tyrosinase and it produces major increase in enzyme purity by removing retaining contaiminants and enzyme loss occur because of incomplete binding.	~7.8-fold	~40%	Ajeel *et al.*, 2025
5	Gel filtration chromatography (Sephadex G-200)	Separation based on molecular size allowing removal of residual impurities, it provides highest purification level and final enzyme yield decreases	10.6-fold	28–30%	El-Shora., 2020

a result, selecting either SmF or SSF mainly depends on production goals, economic feasibility, and specific process demands.

Purification

There are many methods to purify the tyrosinase enzyme while preserving the catalytic activity.

Each purification stage involves a trade-off between purity and enzyme recovery. As shown in Table 1, where the purification fold gradually increases from the crude extract to the gel filtration step ,while the percentage of recovered enzyme yield decreases significantly.This highlights the challenge of obtaining highly purified tyrosinase without substantial loses in the total enzyme activity.

The crude extract obtained after centrifugation contains the highest amount of enzyme activity because no purification loses have occurred at this stage. However, it also contains many unwanted proteins and other cellular components, which results in relatively low specific activity. Ammonium sulphate is generally employed as the first purification step because it concentrates the enzyme and removes a portion of contaminating proteins .This step improves the impurity and still retaines nearly three quarters of the original enzyme activity making it a simple and economical approach . Nevertheless, some enzyme activity may be lost due to incomplete precipitation or the simultaneous precipitation of other proteins, (Ajeel *et al.*, 2025).

Further precipitation, dialysis is used to remove excess salts before chromatography separation. According to Table 1, This step improves purification modestly, while yield decreases. Although dialysis contributes little to overall purification, it is essential for preparing the enzyme for chromatography. The main limitations are long processing times and the possibility of enzyme loss due to dilution or instability during prolonged incubation.

The largest increase in impurity is achieved during ion-exchange chromatography (DEAE-cellulose). As shown in Table 1, purification rises sharply, confirming the charge-based separation is highly effective for removing contaminating proteins. This improvement comes at the expense of recovery, with yield decreasing. This finding suggests that ion-exchange chromatography is the most influential purification step but also one of the most major source of enzyme loss due to incomplete binding, elusion, inefficiencies and sample dilution. (El-Shora., 2020).

A final polishing step is provided by gel filtration chromatography such as Sephadex, which separate proteins according to molecular size.This step increases

Table 2: *Of applications*

Role of tyrosinase	Key applications	Examples	References
Cosmetic industry	Controls melanin to reduce dark spots, melasma, and uneven skin tone	Kojic acid, arbutin, flavonoids, plant-based inhibitors	Hassan *et al.*, 2023
Medical & Therapeutic	Used as a marker and target in the study and treatment of melanoma (skin cancer)	Melanoma therapy, antioxidant use, support during chemotherapy	Baber *et al.*, 2023
Food Industry	Causes browning but also helps in managing and maintaining food quality	Food preservation, shelf-life enhancement, anti-browning techniques	Beltrán *et al.*, 2022
Biotechnology	Acts as a catalyst to speed up important biochemical reactions	Bioactive compound production, protein cross-linking, biomaterials	Ghasemi *et al.*, 2023
Environmental Applications	Helps break down harmful pollutants in water and soil	Bioremediation, wastewater treatment	Selin *et al.*, 2022
Research & Industrial	Used to study enzyme activity and develop useful industrial materials	Drug design, enzyme research, polymer and coating production	Noori *et al.*, 2023

purification, representing the highest purity achieved among the reported studies. The final yield declines, indicating the substantial enzyme loses occur during the pursuit of maximum purity. Although the gain in purification is smaller than achieved by ion-exchange chromatography, gel filtration is removing residual contaminants and resolving tyrosinase isoforms.

Overall, analysis of data demonstrates a clear inverse relationship between purification fold and enzyme yield. Early purification steps such as precipitation provide moderate improvements in purity while maintaining relatively high recovery, whereas chromatographic techniques deliver the greatest increase in purity but account most of the activity loss. Across the literature, multistep purification strategies consistently achieve purification but only one-third or less of the original enzyme activity is typically retained. The optical purification strategy depends on whether the intended application prioritizes maximum purity or maximum enzyme recovery. Final enzyme quality is commonly verified by SDS-PAGE, which confirms molecular weight and assesses the homogeneity of the purified preparation. (Marcial - Quino *et al.*, 2025).

Applications of tyrosinase enzyme

Numerous scientific and industrial fields have paid close attention to it because of its central role in oxidation and pigmentation reactions. Its diverse functionality makes it possible to use it in biotechnology, environmental management, cosmetics, medicine, food processing, and other fields.

Cosmetic applications

Cosmetics greatly benefit from the presence of tyrosinase.This is because tyrosinase directly controls how much melanin our skin produces which in turn affects our skin color. Skin conditions like melasma, dark spots, and uneven skin tone can be treated if we can control tyrosinase. Tyrosinase inhibition is a design feature of many skin care products. Arbutin and kojic acid are two common ingredients that are used to reduce the amount of melanin that forms on the skin, giving it a more even and bright appearance. However, people are concerned about how safe these ingredients are and whether or not they are stable.So researchers are looking for options.Recently scientists found some things that work really well.These include carbothioamidopyrazole derivatives, which bind to tyrosinase and stop it from working effectively.Also people are paying attention to things that come from plants like flavonoids and phenolic acids. This is because they are safer for us they have properties and they are good, for the environment. Tyrosinase and melanin production are still the focus and finding natural ways to regulate tyrosinase is a big deal. Long-term safety issues, formulation stability challenges, and the potential risk of skin irritation have restricted the broader application of some inhibitors; therefore, future studies should concentrate on identifying safer natural compounds with greater effectiveness (Hassan *et al.*, 2023; Zolghadri *et al.*, 2019).

Medical and therapeutic aspects

Tyrosinase is regarded as a target in the medical field when it comes to the treatment of melanoma, a very dangerous type of skin cancer that begins in pigment-producing cells.This enzyme is useful for diagnosing and treating cancer because these cancer cells typically produce more of it. If we stop tyrosinase from working it does not just reduce the amount of

pigment it can also slow down the growth of tumors. Some things that stop tyrosinase have been shown to have than one effect they can make the skin lighter and also fight cancer, which makes them more useful for treating people. Also stopping this enzyme can make cancer cells respond better to drugs, which means we can use amounts and have fewer bad side effects. Apart from helping with cancer controlling tyrosinase is also helpful for managing problems with pigmentation and reducing stress caused by oxygen because the things tyrosinase does can make things that have oxygen in them.It may also help us make treatments that are more targeted and have fewer bad effects. Researchers are still looking for things that can stop tyrosinase in a more selective way. Overall tyrosinase is still an important thing to study in medical research because it has a big effect, on living things. However, achieving selective targeting continues to be a major challenge, as non-specific inhibition can also impact healthy cells; therefore, future therapeutic approaches should focus on increasing specificity while minimizing unwanted side effects (Baber *et al.*, 2023; Chen *et al.*, 2021; Claus *et al.*, 2006).

Food industry applications

Tyrosinase causes fruits and vegetables to turn brown when they are cut, damaged or exposed to air. This happens because the enzyme helps turn some compounds into colored pigments.It does this by turning compounds into quinones, which then turn brown.The browning of fruits and vegetables is usually not good because it affects how they look, taste and their nutritional value.However now that scientists know how tyrosinase works they have found ways to control it.Some of these ways include using inhibitors keeping the produce cold or reducing its contact with oxygen.Tyrosinase can also be helpful in making some food products look and taste better if it is used carefully and under controlled conditions. So tyrosinase can play both a negative and a positive role in the food industry.This is especially true when it comes to keeping food fresh and making it last longer.The role of tyrosinase, in food quality and shelf life is very important.Tyrosinase affects food in ways and its effects can be good or bad. Although these methods provide important advantages, certain conventional control techniques can raise processing expenses or negatively influence food quality, which is why current research is increasingly focusing on safer bio-based preservation strategies (Beltrán *et al.*, 2022; Yoruk and *et al.*, 2003; Duran *et al.*, 2002).

Biotechnological applications

In biotechnology tyrosinase is really useful because it has some properties that help with different things. Tyrosinase is mainly used for reactions that help form compounds. This is why tyrosinase is used a lot to make medicines and special molecules that're good for us. Tyrosinase also helps make kinds of materials. One good thing about tyrosinase is that it helps make these processes more efficient and better for the environment than ways of doing things. Tyrosinase helps change proteins by making them stick together. This makes the proteins stronger. Work better. Because of this tyrosinase is useful in things like biomaterials and medicine. Tyrosinase can also help make products that last a time and work well. Overall tyrosinase is a choice for new and sustainable biotechnology because it is natural.Tyrosinase can also be used to make materials for medical and industrial uses. Another good thing about tyrosinase is that it works under gentle conditions so it does not hurt sensitive compounds. Because of these things tyrosinase is becoming more important in new and sustainable biotechnology. Tyrosinase is really useful, in biotechnology. It is used with tyrosinase to make new things. However, large-scale industrial applications are still limited by enzyme instability and a decline in efficiency after repeated use, highlighting the need for advanced enzyme engineering and improved immobilization strategies (Ghasemi *et al.*, 2023; Ramesh *et al.*, 2022; Pilaiyar *et al.*, 2017).

Environmental applications

Tyrosinase works in a way. It does not make pollution when it is used to clean up waste. We do not need to use chemicals that are bad for us and cost a lot of money. Another good thing about tyrosinase is that it can work in conditions. This makes it easier to use in the world.More and more people are using tyrosinase because it is better for the environment. It helps make the water we drink cleaner. Because of all these things tyrosinase is a good choice for taking care of waste in a way that is good for the earth. Tyrosinase is an option, for sustainable waste management because tyrosinase helps us take care of the environment. Large-scale implementation still presents significant challenges because enzyme performance can decline when exposed to harsh environmental conditions; therefore, current research is focused on developing more stable and scalable remediation systems (Selin *et al.*, 2022; Santos *et al.*, 2021).

Scientific and industrial applications

In research, tyrosinase is frequently used as a model enzyme. Understanding the conditions under which tyrosinase behaves is made easier for scientists by this. By binding to the copper site of tyrosinase, many inhibitors prevent the substrate from binding to tyrosinase and reduce its activity. Some substances can also change the structure of tyrosinase, which affects stability and overall tyrosinase function. Tyrosinase helps in forming bonds, between molecules, which improves the quality of the final material. This makes the materials more durable and useful for purposes. Using tyrosinase is also an option compared to harsh chemicals as tyrosinase works in a safer and more environmentally friendly way. Despite these advantages, limitations related to production cost, substrate specificity, and enzyme stability still hinder wider industrial application, emphasizing the importance of developing more advanced engineering strategies (Noori *et al*., 2023; Park *et al*., 2022; Solano 2014).

Conclusions and Recommendations

Tyrosinase is a copper-containing enzyme of major biological importance that plays an essential role in melanin formation and oxidation processes in different living organisms. The increasing scientific attention toward this enzyme is mainly due to its wide range of applications in cosmetics, medicine, food processing, biotechnology, and environmental management. Microbial production systems have become promising alternatives for large-scale enzyme production because of their efficiency, cost-effectiveness, and simple cultivation requirements. Although obtaining high enzyme recovery during purification remains an important challenge, several purification techniques have proven effective in improving enzyme purity. Recent studies have further demonstrated that tyrosinase possesses significant potential in therapeutic development, industrial biocatalysis, and environmentally sustainable applications. Despite these benefits, challenges associated with enzyme stability, selectivity, and large-scale industrial application still require additional research and improvement. Future progress in enzyme engineering and process optimization may further establish tyrosinase as an increasingly valuable enzyme in modern biotechnology and applied scientific research (Pretzler and Rompel, 2024; Pisano *et al*., 2024).

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Novelty Statement

This article provides a comprehensive analysis of the latest advancements in microbial-derived tyrosinase, highlighting novel strategies for high-yield production and cost-effective purification that bridge the gap between laboratory synthesis and large-scale industrial implementation.

Author's Contribution

Zainab Mehmood: Conceptualization, Methodology, Writing Original. Draft, Administration, and Supervision.
Laiba Javed: Data Curation, Writing and Visualization.
Minahil Razzaq: Data Curation, Formal Analysis, Writing.
Muskaan Azeem: Literature Search, Writing Original Draft.
Zobia Mubeen: Conceptualization, Supervision, Writing.
Sameeha Shakeel: Data Curation, Writing Review & Editing.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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