



Role of Tyrosinase Extraction from Banana (*Musa acuminata*)

Titas Sarkar¹, Arijit Shil², Dipak Kumar Singha³, Manas Chakraborty⁴

¹Department of Pharmaceutical Biotechnology, Calcutta Institute of Pharmaceutical Technology & Allied Health Sciences

²Faculty, Department of Diploma in Veterinary Pharmacy, West Bengal University of Animal and Fishery Sciences, Mohanpur Campus, Nadia, West Bengal, India, Government of West Bengal.

³Associate Professor Pharmaceutical Biotechnology, Calcutta Institute of Pharmaceutical Technology & Allied Health Sciences, West Bengal, India

⁴ Professor and Head of the Department, Pharmaceutical Biotechnology, Calcutta Institute of Pharmaceutical Technology & Allied Health Sciences, West Bengal, India

***Corresponding Author** :Titas Sarkar, Department of Pharmaceutical Biotechnology, Calcutta Institute of Pharmaceutical Technology and AHS, Howrah, West Bengal, India. Email Id: titassrkr22@gmail.com

ABSTRACT :

Plants have long been a good source of antibacterial chemicals, and they are generally regarded harmless for humans. Some plant peptides limit the growth of gram-positive or gram-negative bacteria preferentially, while others inhibit the growth of fungi and viruses. Anti-tumor, antioxidant, and chitinase and proteinase inhibitory action are among the therapeutic features of plant-derived peptides. Antimicrobial peptides usually act directly on bacteria, viruses, and fungal cell membranes, for example, by destroying or disrupting them. The goal of this work was to improve the protein extraction technique from banana flowers and evaluate the isolated proteins' molecular weight distribution and antibacterial activity. Banana flowers are a by-product of banana farming and are widely consumed as a vegetable in many parts of the world. A bunch of bananas generates multi-flowered bluebells when commercially harvested. Only 4,444 of these flowers are utilized on the farmers organic raw materials and bio-fertilizers, despite their enormous nutritional value. In recent years, there has been a substantial growth in the market for products containing functional ingredients. Food waste and agricultural by-products contain phenolic compounds, antioxidants, dietary fiber, flavonoids, anthocyanins, proteins, peptides, and enzymes, among other useful and biologically active chemicals. Bioactive chemicals can be regenerated and reused in the food chain as additions, enhancers, and additives. Alternatively, as a sub-component.

Keywords: Biofertilizer, Phenolics, Antioxidants, Dietary fibers, Flavonoids, Anthocyanins, Proteins, Peptides, Dopamine, Threonine

Introduction :

Banana flower is a product of the banana plantain, and it is commonly eaten as a vegetable in many regions of the world. One bunch of bananas generates one bell, which contains numerous blossoms, when harvested commercially. Despite their high nutritional content, these blooms are only employed in plantations as organic materials and bio-fertilizer. In recent years, the demand for items containing functional components has skyrocketed. [1] Food waste and agro-food industrial byproducts contain phenolics, antioxidants, dietary fibers, flavonoids, anthocyanins, proteins, peptides, enzymes, and other functional and bioactive substances. Bioactive molecules can be recovered and repurposed in the food chain as additions, supplements, fortification, or minor ingredients. [4] Plant proteins are becoming increasingly popular in the food, feed, pharmaceutical, and cosmetics industries. Banana blossoms have been used in commercial items such as canned banana flowers in brine, dehydrated vegetables, pickles, and pharmaceuticals. Banana flower also contains a lot of dietary fiber, magnesium, iron, copper, antioxidants, flavonoids, and dopamine. Plant proteins, unlike animal proteins, lack several necessary amino acids, so it's best to eat a variety of plant proteins to suit your nutritional needs. Because of its well-balanced composition, the banana blossom includes high-quality proteins, including important amino acids leucine, lysine, and threonine in levels greater than 30 mg/g of protein. [7] Plants have long been thought to be a good source of antibacterial chemicals and safe for human consumption. Some plant-derived peptides can restrict the growth of fungi and viruses while others can prevent the growth of gram positive or negative bacteria. [9] Anticancer, anti-oxidative, chitinase, and proteinase inhibitory actions are among the therapeutic features of plant-derived peptides. Antimicrobial peptides have direct effects on microorganisms, such as destroying or disrupting the cell membrane of bacteria, viruses, and fungi. The goal of this research was to improve protein extraction from banana flowers and characterize isolated proteins for molecular weight distribution and antibacterial activity. [8,14]

Browning is primarily caused by polyphenol oxidases oxidizing phenolic substances (PPO). These enzymes accelerate the conversion of various phenols to quinines, which are extremely reactive chemicals that eventually polymerize to form melanins. The link between phenolic content, PPO activity, pH, temperature, oxygen availability within the tissue, and browning rate has been investigated for a variety of fruits, with evidence indicating

that banana fruit browning is caused by enzymatic oxidation of 3,4-dihydroxyphenylethylamine (dopamine). [9]

Tyrosinase has a wide range of functions including :

- wound healing,
- sclerotization,
- melanin synthesis
- parasite encapsulation.

Tyrosinase is an oxidase that regulates the formation of melanin by being the rate-limiting enzyme. The enzyme is primarily involved in the Raper Mason pathway, which consists of two separate melanin production processes. The hydroxylation of a monophenol and the conversion of an o-diphenol to the equivalent o-quinone are the first two steps. To become melanin, o-Quinone passes through a series of processes. Tyrosinase is a copper-containing enzyme found in plant and animal tissues that catalyzes the oxidation of tyrosine to produce melanin and other colors. It's located inside melanosomes, which are made by melanocytes in the skin. The TYR gene encodes the tyrosinase enzyme in humans.

Tyrosinase is a single membrane-spanning transmembrane protein found in mammals. Tyrosinase is segregated into melanosomes in humans, and the catalytically active domain of the protein is found within them. Only a little portion of the protein, which is enzymatically unimportant, reaches into the cytoplasm of the melanocyte.

Despite some important studies on the characteristics and functions of the banana, there are still questions about its identification and, more importantly, its physiological purpose (both pulp and peel tissues). A better understanding of banana PPO not only allows for better management of the enzyme, but it also elucidates its physiological importance. The ambiguity surrounding the identity of the banana pulp PPO (BP-PPO) is reflected in the literature, since both the enzyme numbers (EC 1.10.3.2) for catechol oxidase and (EC 1.14.18.1) for phenolases are used (Yang et al., 2004; Wuyts et al., 2006; Unal, 2007). To resolve this debate, the BP-PPO was purified to a single isozyme level, after which the enzyme's activities and some key biochemical features were studied. The assay techniques and results are compared to prior publications, and the physiological roles of the BP-PPO are finally examined in terms of assumptions.

General Overview :

Bananas are a healthy fruit with a delicious taste that is frequently consumed around the world. It is a highly profitable fruit crop in the international market. Bananas account for more than 25% of overall exports in nations like Costa Rica and Honduras (Ortiz et al., 1998). During handling, peeling, slicing, and even storing, bananas are prone to quick browning. One of the most essential variables in the creation of brownish pigment spots on the peel is Tyrosinase - Michaelis Constants, Optimal Temperature, and pH-assisted formation of brownish pigment spots. By creating melanin, it also has a direct influence on lowering the quality of fruits and vegetables. Tyrosinase is a metalloenzyme that is employed in food, pharmaceutical, and cosmetic research. For the time being, edible mushrooms are the only supply of tyrosinase for industrial and research units that require this enzyme.

Role of Tyrosinase Extraction from Banana

The goal of this study is to see if tyrosinase from a banana peel can be used in the pharmaceutical, food, cosmetic, and research industries instead of tyrosinase from mushrooms. In practice, the enzyme recovered from banana peel was purified, and some of its features were identified and compared to tyrosinase from other proteins, including biological activity, pH, and optimal temperature. The results showed that the banana peel tyrosinase enzyme contains two isoforms with a higher molecular weight than mushroom tyrosinase. The temperature and pH of the enzyme, on the other hand, were 50 and 6.1 C, respectively. The maximal rate of velocity (V_{max}) and Michaelis-Menten kinetics were likewise calculated to be 263.3 mM (m/mol) and 0.00401 mmol/min, respectively. Tyrosinase differs from one species to the next in terms of cellular placements, tissue allocations, size, glycosylation model, and so on, but it shares common features such as a binuclear copper III center with two atoms of copper, each atom surrounded by three histidine residues inside the active site. Everything about tyrosinase is unknown due to the difficulties in acquiring it in its purest form. [10] Scientists have been able to detect amino acid sequences from numerous species using technological DNA approaches, but not pure tyrosinase. A Tyrosinase, on the other hand, has Mr's ranging from 29,000 to 200,000. Tyrosinase is glycosylated and contains a lot of cysteine. Five of the seventeen residues are present in the center, whereas the other ten are concentrated in the first 100 amino acids. These residues are disulfide in nature. [12]

A Bananas are elongated, edible berries produced by several species of big herbaceous flowering plants of the genus *Musa*. Cooking bananas are sometimes referred to as "plantains" in some countries, to distinguish them from dessert bananas. The size, color, and firmness of the fruit varies, but it is normally elongated and curved, with soft, starchy flesh covered by a rind that is green, yellow, red, purple, or brown when mature. The fruits form clusters towards the top of the plant and rise upward. ***Musa acuminata* and *Musa balbisiana*** are two wild species that produce almost all modern edible seedless (parthenocarp) bananas. Most cultivated bananas have the scientific names ***Musa acuminata* or *Musa balbisiana*** for the hybrid *Musa acuminata* M. *balbisiana*, depending on their genomic makeup. *Musa sapientum*, the former scientific name for this hybrid, is no longer used. [9,13]

Musa species are native to tropical Asia and Australia, and they were probably domesticated in Papua New Guinea first. They are produced in 135 countries, largely for their fruit, but also for fiber, banana wine, and banana beer, as well as for decorative purposes. In 2017, India and China were the world's leading banana producers, accounting for over 38% of total production. [7]

There is no clear differentiation between "bananas" and "plantains" anywhere in the world. "Banana" usually refers to soft, sweet, dessert bananas, particularly those of the Cavendish variety, which are the principal exports from banana-growing countries, especially in the Americas and Europe. *Musa* cultivars with firmer, starchier fruit are referred to as "plantains." Many more types of banana are grown and eaten in other countries, such as Southeast Asia, thus the binary distinction isn't as useful and isn't made in local languages. [15] The banana peel, sometimes known as banana skin in British English, is the fruit's outer layer. Banana peels are employed as animal food, a cooking element, in water purification, in the production of a variety of biochemical products, as well as in jokes and comedic situations. [7,16] Cutting or snapping the stem and dividing the peel into portions

while drawing them away from the bared fruit are two methods for peeling bananas. Another method of peeling a banana is to start at the end with the brownish flower residue and peel in the opposite direction, which is commonly referred to as "upside down." This method is also known as the "monkey method," because it is claimed to be how monkeys peel bananas.

When you pinch the tip of a banana with two fingers, it splits and the peel separates into two clean portions. The inner fibers, or "strings," between the fruit and the peel will stay attached to the peel, and the banana's stem can be used as a handle when eating it. Banana peels are thought to contain a psychoactive chemical, and that smoking them can result in a "high" or a feeling of relaxation.

Banana peels are occasionally used as a feedstock for cattle, goats, pigs, monkeys, chickens, rabbits, fish, zebras, and other animals on small farms in banana-growing regions.

[3] There are also concerns about the tannins in the peels having an effect on animals that eat them.

[4]

[5] The nutritional value of banana peels varies depending on their maturation level and cultivar; for example, plantain peels have less fiber than dessert banana peels, and lignin concentration rises as the banana ripens (from 7 to 15 percent dry matter). [15] Banana peels contain 6-9 percent protein dry matter and 20-30 percent fiber on average (measured as NDF). Green plantain peels contain 40% starch, which is converted to sugars when it ripens. Green banana peels have less starch (approximately 15%) than plantain peels when they are green, whereas mature banana peels contain up to 30% free sugars. [4] Banana peels are also used to purify water,[6] as well as to make ethanol and cellulase for composting.

Discussion:

The enzyme was extracted from three distinct bananas as a source. The results demonstrated that for tyrosinase isolation, the Tris-HCl buffer approach is more effective than the phosphate buffer method. Two methods were used to isolate tyrosinase from bananas. The findings revealed that *Agaricus bisporus* mushrooms are the most effective source of tyrosinase. The isolation of tyrosinase was more efficient with Tris-HCl buffer than with phosphate buffer. The optimal pH and temperature profiles of industrial purified and laboratory isolated tyrosinase were identical

References:

1. Brisolari A. and Gonçalves D., 2014, Immobilization of tyrosinase from avocado crude extract in polypyrrole films for inhibitive detection of benzoic acid, *Chemosensors*, 2(3), 182-192.
2. Carvalho da Silva S., Wisniewski C., Luccas P. O., Schmidt de Magalhães C., 2013, Enzyme from banana (*Musa sp.*) extraction procedures for sensitive adrenaline biosensor construction, *American Journal of Analytical Chemistry*, 4, 293-300.
3. Decker A. L., 1977, *Worthington Enzyme Manual*, Polyphenol oxidase, Worthington Biochemical Corporation, 39-40.
4. Duarte A. C. P., Coelho M. A. Z. and Leite S. G. F., 2002, Identification of peroxidase and tyrosinase in green coconut water, *Ciencia y Tecnologia Alimentaria*, 3, (5), 266-270.
5. Galeazzi M. M., Sgarbieri V. C. and Constantinides S. M., 1981, Isolation, purification and physicochemical characterization of polyphenol oxidases (PPO) from a dwarf variety of banana (*Musa cavendishii*, L), *Journal of Food Science*, 46, (1), 150-155.
6. Haghbeen K., Jazii F. R., Karkhane A. A. and Borojerdi Sh. Sh., 2004, Purification of tyrosinase from edible mushroom, *Iranian journal of biotechnology*, 2, (3), 189-194.
7. Halaoui S., Asther M., Sigoillot J. C., Hamdi M., Lomascolo A., 2006, Fungal tyrosinases: new prospects in molecular characteristics, bioengineering and biotechnological applications, *Journal of Applied Microbiology*, 100, 219-232.
8. Kim Y. J., Uyama H., 2005, Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective, *Cell. Mol. Life Sci.*, 62, 1707-1723.
9. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., 1951 *J. Biol. Chem.*, 193, 265-75.
10. Matoba Y., Kumagai T., Yamamoto A., Yoshitsu H., Sugiyama M., 2006 Crystallographic evidence that the dinuclear copper center of tyrosinase is flexible during catalysis, *J. Biol. Chem.*, 281, 8981-8986.
11. Otavio de Faria R., Moure V. R., Lopes de Almeida Amazonas M. A., 2007, Krieger N. and Mitchell D. A., The biotechnological potential of mushroom tyrosinases –review, *Food Technol. Biotechnol.*, 45 (3), 287-294.
12. Pérez-Gilabert M. and Carmona F. G., 2000, Characterization of catecholase and cresolase activities of eggplant polyphenol oxidase, *J. Agric. Food Chem.*, 48 (3), 695-700.
13. Sambasiva Rao K. R. S., Tripathy N. K., Srinivasa Rao D., Prakasham R.S., 2013, Production, characterization, catalytic and inhibitory activities of tyrosinase, *Research, Journal of Biotechnology*, 8 187-99.
14. Selinheimo E., Ni Eidhin D., Steffensen C., Nielsen J., Lomascolo A., Halaoui S., Record E., O'Beirne D., Buchert J., Kruus K., 2007, Comparison of the characteristics of fungal and plant tyrosinases, *Journal of Biotechnology*, 130, 4471-480.
15. Spagna G., Barbagallo R. N., Chisari M. and Branca F., 2005, Characterization of tomato polyphenol oxidase and its role in browning and lycopene content, *J. Agric. Food Chem.*, 53 (6) 2032-2038.
16. Todaro A., Cavallaro R., Argento S., Branca F. and Spagna G., 2011, Study and characterization of polyphenol oxidase from eggplant (*Solanum melongena* L.), *J. Agric. Food Chem.*, 59 (20), 11244-11248.
17. Vieira N. C. S., Ferreira R. A., Valquiria da Cruz R., Guimarães F. E. G., Alvaro de Queiroz A. A., 2013, Self-assembled films containing crude extract of avocado as a source of tyrosinase for monophenol detection, *Materials Science and Engineering: C*, 33, (7), 3899-3902.

18. Yang Zh. and Wu F., 2006, Catalytic properties of tyrosinase from potato and ediblefungi, *Biotechnology*, 5 (3), 344-348.

19. Yuan H., Ke-wu L., Dong Y., Xin L., Xin-he H., Cheng-kang T., Hua J., Wen-juan J., 2005, Some properties of potato tyrosinase, *Chemical Research and Application*, 1, 22-27.