International Journal of Nutrition and Food Sciences
2015; 4(6): 656-659
Published online November 2, 2015 (http://www.sciencepublishinggroup.com/j/ijnfs)
doi: 10.11648/j.ijnfs.20150406.19
ISSN: 2327-2694 (Print); ISSN: 2327-2716 (Online)

Review Article

Efficient Methods for Polyphenol Oxidase Production

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To cite this article:
Kamal-Alahmad, Mohammed Abdalbasit A. Gasmalla, Hala Alyousef. Efficient Methods for Polyphenol Oxidase Production. *International Journal of Nutrition and Food Sciences*. Vol. 4, No. 6, 2015, pp. 656-659. doi: 10.11648/j.ijnfs.20150406.19

Abstract: Polyphenol oxidases are enzymes that catalyze the oxidation of certain phenolic substrates to quinones in the presence of molecular oxygen. Polyphenol oxidases are widely used in several applications. In food industry, they are used for enhancement of flavor in coffee, tea and cocoa production, and determination of food quality. In medicine, they have several uses in treatments of Parkinson's disease, phenlyketonurea and leukemia. In wastewater treatment, they are used for the removal of phenolic pollutants from wastewaters. In pharmaceutical industry differentiation of morphine from codeine is possible by means of polyphenol oxidase immobilized electrodes. Although many details about structure and probably function of PPO have been revealed in this review.

Keywords: PPO, Quinine, Applications, Enzymatic Browning, Mechanisms and Functions

1. Introduction

The first step in producing enzymes is to identify the optimal organism or host. The most common approach is to investigate plants and microorganisms found in nature [2], where enzymes may already be doing what is desired for an industrial application. There is a rich and broad variety of life on earth, particularly involving microorganisms.

Polyphenol oxidizes (PPOs) are enzymes, belonging to a group of copper containing metalloproteinase and are members of oxidoreductases, that catalyze the oxidation of a wide range of phenolic compounds by utilizing molecular oxygen. There are mainly three types of polyphenol oxidases classified according to their substrate specificities and mechanism of actions. These are; tyrosinase, catechol oxidase and laccase [1][2].

Polyphenol oxidase (monophenol, dihidroxyphenylalanine, oxygen oxidoreductases, E.C. 1.14.18.1) catalyzes two distinct reactions involving molecular oxygen, namely 1) the o-hydroxylation of monophenols to odiphenols, or cresolase activity; and 2) the subsequent oxidation of o-diphenols to o-quinones, or catecholase activity [5]. The catalytic action of PPO is connected to undesirable browning and flavor generation in stored and processed foods of plant origin. The processing of artichoke tubers for the production of inulin hydrolyzates has been linked to the presence of a highly active PPO system.

The familiar brown color of tea, coffee and cocoa [4] is developed by enzymatic browning by PPO Conventionally; PPOs are classified into monophenol oxidases (tyrosinase) and o-diphenol: oxygen oxidoreductases (catechol oxidases).

2. Polyphenol Oxidases in Nature

In literature three types of polyphenol oxidases which were classified according to their ability to oxidase different types of phenolic compounds Tyrosinase (monophenol mono oxygenase E.C. 1.14.18.1) oxidizes monophenols such as tyrosine, p-cresol and p-coumaric acid but not diphenols and triphenols (Figure 1)[3][5]. Tyrosinase is most found in animals including humans and it is important for hair, skin and eye pigmentation and a number of diseases result from insufficient or too much activity.

Second type of polyphenol oxidase (1,2- benzenediol: oxygen oxidoreductases; E.C 1.10.3.1) is also known as polyphenolase, phenolase atechol oxidase, cresolase, or catecholase mostly found in higher plants especially mushroom, apple, peach, tobacco and tea leaves[6][7].

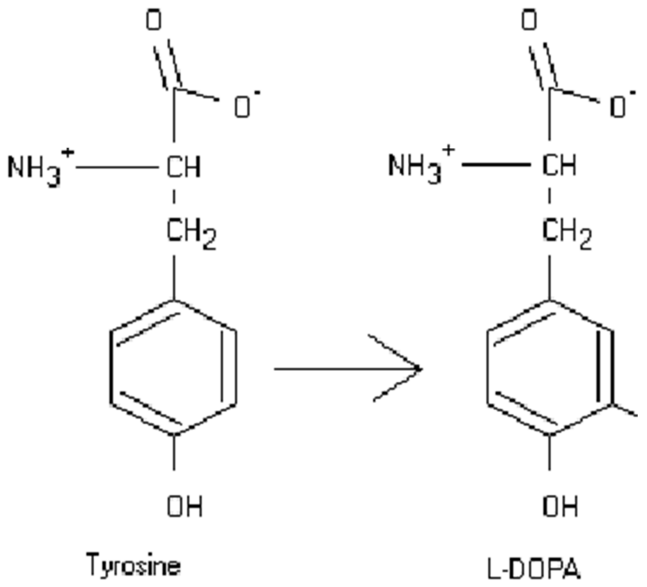

Figure 1. *Tyrosinase (monophenol mono oxygenase E.C. 1.14.18.1) oxidizes monophenols.*

These enzymes in higher plants and fungi oxidize a great variety of monophenolic and o-diphenolic compounds and catalyze two types of reactions. First reaction involves the hydroxylation of a monophenol to give a diphenol (Fig. 2) and second reaction involves the removal of hydrogen's from diphenol to give quinine[10].

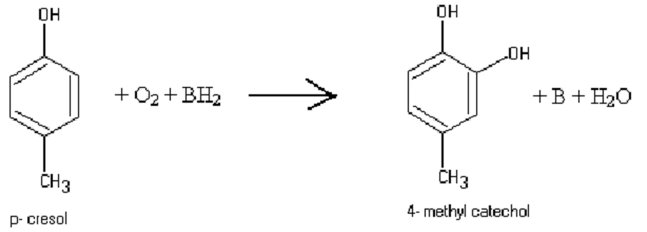

Figure 2. *Hydroxylation reaction by catechol oxidase.*

Polyphenol oxidases are found in almost all living organisms including plants, animals, bacteria and fungi.

In plants, polyphenol oxidase is involved in defense mechanism. When a plant gets a bruise or cut, certain phenolic compounds are oxidized in the presence of oxygen to forma polymer structure in case of oxygen penetration or microbial contamination.

In terms of molecular weight of polyphenol oxidases, little is known about the overall diversity. Molecular weights of different polyphenol oxidases vary according to the source of the enzyme [9]. Molecular weights of plant polyphenol oxidases are approximately 144,000. The generally accepted molecular weight of mushroom polyphenol oxidase is 128,000Da whereas other fungi polyphenol oxidases have molecular weights varying from46, 000 to 88,000 Da [11].

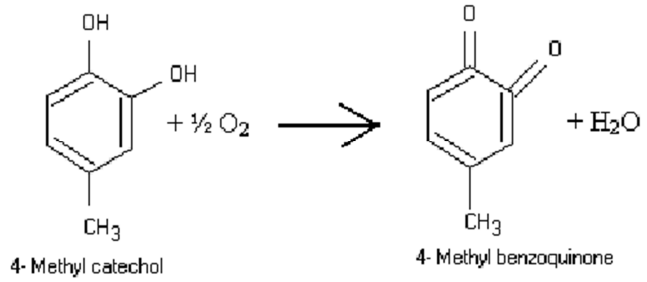

Figure 3. *Dehydrogenation reaction by catechol oxidase.*

3. Production of Polyphenol Oxidase

3.1. Use of Thermophilic Fungi

Polyphenol oxidases are found in almost all fungal strains and they are considered to be excellent sources for industrial polyphenol oxidase production. Thermophilic fungi have an advantage particularly in fermentation conditions. Higher temperatures used in fermentations reduce the risk of contamination [9][8]. Especially in industrial scale production sterilization is an expensive parameter and fermentation at higher temperatures prevents the growth of undesired contaminants. From another point of view, fermentations performed at higher temperatures increase the cost, however, thermophilic organisms produce thermostable products [12].

Today, thermophilic organisms as producers of certain desired products are under interest due to their resistance to heat, denaturants solvents and proteolytic enzymes with respect to their counterparts from mesophiles (Fontana, 1988). For instance Chaetomium thermophile polyphenol oxidase was purified and optimum pH and temperature value for the enzyme was found to be 5.0 and 55°C, respectively.

3.2. Thermomyces Lanuginosus

In this study polyphenol oxidase production was performed by using T. lanuginosus and the enzyme was characterized to determine possible application areas [14] [16].

Thermomyces lanuginosusis considered to be producer of several enzymes in industry. For instance it is used for the production of Phytases myo-inositol hexa kisphosphate phosphor hydrolyses; (EC 3.1.3.8) catalyzing di-, tri-, tetra-, and penta phosphates of myo-inositol and inorganic phosphate and these phosphates are essential nutrients of animal feeds[13] [15].

4. Bacterial Polyphenol Oxidases

With the discovery of polyphenol oxidases in bacterial species, the studies of them from these new sources have picked up in order to find more species that exhibit polyphenol oxidase activity [20].

The first bacterial laccase was reported from Azospirillum lipoferum Diamantidis, as a multimeric enzyme [20]. Laccase was also found in bacterial species living in extreme environments e.g. Aquifex aeolicus Bacterial tyrosinase were first purified from Streptomyces species Streptomyces

tyrosinase are well characterized and studied [19] [18]. Besides, polyphenol oxidases are found in a number of Bacillus species. Some biochemical characteristics and activity of polyphenol oxidases from different bacteria [17] [18].

5. Measurement of Polyphenol Oxidase Activity Using Optical Waveguide Light Mode Spectroscopy-Based Immunosensor

Polyphenol oxidase (PPO, EC 1.14.18.1) is a tetramer that contains four atoms of copper per molecule, and binding sites for two aromatic compounds and oxygen. The enzyme catalyses the-hydroxylation of mono phenols to o-diphenols. It can also further catalyze the oxidation of o-diphenols to produce o-quinones [16].

Browning of fruits and vegetables, and processed products including juices is an important indicator for quality deterioration and is caused by rapid poly merisation of o-quinones into black, brown or red pigments called polyphenol. However, PPO-induced browning is not always an undesirable reaction [22]; the familiar brown color of tea, coffee and cocoa is developed by enzymatic browning by PPO Conventionally, PPOs are classified into monophenol oxidases (tyrosinase) and o-diphenol: oxygen oxidoreductases (catechol oxidases). A mixture of these enzymes is present in nearly all plant tissues, and can also be found in bacteria, fungi, and insects[12] [22].

6. Industrial Applications of Polyphenol Oxidases

In recent years polyphenol oxidases have garnered significant interest because of their high capacity for oxidizing aromatic compounds [21] [18]. This feature makes the use of polyphenol oxidases very suitable for some biotechnological applications in food industry, pulp and paper industry, textile industry, medicine and environmental technology [23].

Applications of polphenoloxidases in different aspects of food industry includes color formation and flavor enhancement of tea, cocoa and coffee ascorbic acid determination, sugar beet pectin gelation and as a biosensor[24] [25].

In environmental technology, the presence of hazardous phenolic compounds and their derivatives in industrial wastewaters from coal conversion, petroleum refining wood preservation, textile, paper, food and chemical industries constitutes a big problem [22] [26]. Government legislation is becoming more stringent in developed countries for the removal of the toxic compounds from wastewaters before they are discharged into the environment. Recent interest has focused on the use of peroxidases and polyphenol oxidases as an enzymatic approach for the removal of phenolics from industrial effluents [27].

In medical area, according to a recent research, polyphenol oxidases were found to inhibit the adhesion of Streptococcus sobrinus, bacteria responsible from oral cavity formation, on tooth surface. Moreover, polyphenol oxidases can be used for the treatment of Parkinson's disease [27] [28]. By the action of polyphenol oxidase, L tyrosine is converted to L-DOPA that is used to supplement the insufficient amount of dopamine in Parkinson's disease Polyphenol oxidases are also of interest in clinical applications as a marker of vitiligo which is an autoimmune disease, as a prodrug therapy agent and as a tumor-suppressing.

Polyphenol oxidases find additional applications in other fields of industry. They can be used in the development of biosensors for immunoassays, for the detection of phenols and phenolic compounds in wastewaters, food and beverage phenols and phenolics compounds. In cosmetics, some hair dyes and dermatological skin lightning preparations are based on laccase [25]. In textile industry, they are used for the purposes of denim bleaching and dye decolorization.

On the other hand, in some food processes, polyphenol oxidase activity is undesirable and plays an important role indetermination of food quality. Many fruits including peaches, apricots, apples, grapes, bananas strawberries, and the vegetables potatoes, lettuce, mushrooms and eggplants are lost due to the action of polyphenol oxidase activity because activity in mechanically damaged foods leads to a browning reaction in the presence of O2, and this causes a nutritional loss and being unacceptable for the consumer. In this respect, the controlled PPO activity is supposed to be important in control of the quality [29] [17] [5].

In medicine, polyphenol oxidases are used for prevention of bacterial adhesion, treatment of Parkinson's disease and control of melanin synthesis. According to the recent studies, attachment of the Streptococcus to the tooth surface was inhibited by the water extract of potato polyphenol oxidase [30].

Recent interest in the enzymatic methods for phenol removal has focused primarily on polyphenol oxidase and peroxidase to treat low concentration phenol containing waste waters. The use of peroxidase has the disadvantage that stoichiometric amounts of H2O2are the oxidant whereas polyphenol oxidase requires O2.

7. Conclusion

The function of PPO in plant, Bactria and fungal metabolism was generally relegated in many places in our discussions. Probably advance since then can best be judged by comparing the discussions in this review, the major problems to be addressed by future research on PPO are the same as in the past, but a change of emphasis is needed.

More attention should be given to mechanisms and function, and less to surveys of the presence of the enzyme. Despite the importance of PPO in food processing and browning reactions, there has been limited progress in this respect, and perhaps a new approach is needed to ask more questions in this review.

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