Original Article

Insight On Polyphenol Oxidase Enzyme And Its Chemical And Natural Inhibitors

[*]Ibtesam Yaseen Alja'afreh

[*]Department of Chemistry, Tafila Technical University, Tafila, P.O. Box 179, Tafila 66110, Jordan; ibtesam_aljaafreh@ttu.edu.jo
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Abstract

A significant cause of food and beverage quality loss is enzymatic browning. Polyphenol oxidase (PPO) catalyzed undesirable browning process has decreased the nutritional value and consumer acceptability of the items. A copper-containing metalloprotein called polyphenol oxidase catalyses the conversion of phenolic chemicals into quinones, which result in the production of brown pigments in damaged tissues. Post-harvest losses are caused by this enzymatic process, which mostly impacts tropical fruits. This page reviews some of the properties of polyphenol oxidase from various plants and provides details on both traditional and unconventional ways to inactivate this enzyme. The polyphenol oxidase's characteristics may be used to design or select better strategies for preventing the browning of goods and vegetables.

Keywords: Polyphenol oxidase (PPO); enzymatic browning; oxidative reactions; chemical inhibitor;

Introduction:

Enzyme Polyphenol oxidase (PPO, EC 1.10.3.1) which is widely dispersed in animals, plants and microorganisms[1]. PPO have group of copper-proteins. PPO has play majority role in the oxidation of phenolic compounds like oxide-reductase enzymes[2]**.** For higher plants, vertebrate animals, fungi, and bacteria, PPO clump together. Homologies inside these clusters are far greater than those between them, according to research. Even so, the PPOs all share a dinuclear copper center in their active site, which is a highly conserved structure in which type 3 copper is bonded to histidine residues[3]. In many plants, fruits, and vegetables, one of the most common signs of deterioration is a browning process that is caused by enzymes. This discolouration is linked to an increase in the concentration of polymeric compounds of o-quinones, which are produced as a direct consequence of processes involving phenolic substrates such as oxidative reactions catalyzed by PPO[4]. Post-harvest losses are brought on by this enzymatic process, which mostly impacts fruits. In addition to affecting quality, these reactions also affect the taste, the consistency, the color, the nutritional characteristics, and the length of time that fruits and vegetables may be stored [5]. PPO has been linked to pigment formation and oxygen scavenging. and defensive system against herbivorous insects, plant pathogens, and [6]. Phenolic chemicals act as building blocks for the physical polyphenolic barriers that prevent the spread of pathogens. PPOs can produce quinones that can bind to plant proteins, decreasing both the digestibility and nutritional value of those proteins for herbivore[7]. The purpose of PPO is currently unknown. Although there is regularly reported positive association between PPO levels and plant resistance to diseases and herbivores, solid evidence of a causative connection has, for the most part, not yet been documented[3]. Considered are indications that PPO is induced in plants, particularly in response to stress and pathogen assault, as well as the function that jasmonate plays in the induction process. Enhancing crop yields while they are experiencing biotic and abiotic stress is a major goal in agriculture. Most plant species contain the enzyme polyphenol oxidase (PPO), and

circumstantial evidence such as enzyme localization and its responsiveness to environmental conditions suggest that the foliar produced gene products may have a role in either acclimation or a short-term response to stress. All terrestrial plants surveyed to far have been shown to have PPO, with the exception of Arabidopsis. Contrarily, no PPO-like sequences from chlorophytes have been reported[8]. Due to the economic significance of browning, PPOs have had their physico-chemical characteristics thoroughly investigated. Contrarily, research on the activity of these enzymes in plant physiology is still in its infancy and may advance with the functional characterisation of PPO homologs that are now being found in a number of crop species. In this study, we make use of new scientific findings to [9]

Conventional synthetic additives, despite the fact that they are thought to be the most common and effective PPO inhibitors, are often seen as not being adequately safe and as being used in excessive amount [10]. On the other hand, natural plant extracts that are abundant in browning inhibitors such phenols, steroids, and alkaloids are not only harmless to individuals but also beneficial to the environment. Furthermore, they improve the flavour and nutritional content of food [11]. At the moment, browning control using natural ingredients rather than synthetic additives has gotten a lot of attention [12]. In order to investigate the anti-browning components, substances of natural plant extracts are often found [13]. However, there hasn't been any systematic investigation into the inhibitory mechanisms and effects of these discovered chemicals on PPO[14].

1.1. PPO Biochemical Properties

The ability of PPO to oxidise phenolic compounds is the foundation for the postulated mode of action for this chemical [7]**.** Tyrosinase, phenolase, and catechol oxidase are all names for PPO. PPO is a metalloenzyme that contains copper and is responsible for catalysing two fundamental processes when in the presence of molecular oxygen. The first reaction is the o-hydroxylation of monophenols, which results in the production of o-diphenols. The second reaction is the oxidation of o-diphenols, which results in the production of o-quinones. To better control and block PPO's effect, it was necessary to study its biochemical characteristics and dynamics[13]. This enzyme has a molecular weight of 120 kilodaltons and is mostly composed of alpha-helices in its structure. Currently, there are benefits and drawbacks to using the polyphenol oxidase-dominated enzymatic browning response, particularly when it comes to fruits and vegetables, but the downsides exceed the benefits. For instance, it is simple to make food decay and degrade, which has an impact on food quality and shelf life[15]. Three primary subtypes of phenol oxidases are recognised based on substrate specificity: Monophenol monooxygenase, which is also known as tyrosinase, monophenol oxidase, or cresolase, catalyses the hydroxylation of monophenol to ortho-diphenol and the oxidation of diphenol to ortho-quinone; diphenol oxidase, which is also known as catechol oxidase, polyphenol oxidase, or o-diphenolase, catalysis Despite this, it is not possible to catalyse the oxidation of monophenol or metaphenol. [3]. Four key elements must be present for enzymatic browning to occur: oxygen, an oxidising enzyme, copper, and a suitable substrate such phenols, which are mostly found in vacuoles and apoplast/cell wall compartments. In order to prevent browning, one of these components should be removed. PPO is the most significant oxidising enzyme, but there are other oxidising enzymes as well, including lipooxidase, which catalyses peroxidation reactions, phenol peroxidase which contributes to enzymatic browning by oxidising hydrogen donors in the presence of hydrogen peroxide, and phenylalanine ammonia lyase (PAL), which converts the amino acid L-phenylalan, two copper atoms make up the active core of the enzyme PPO. PPO may exhibit catecholase or mono-phenolase activity depending on its enzymatic form transition (met, oxi, or deoxi).

Given that PPO is the primary factor influencing browning, it is essential to investigate the process by which changes to colour parameters take place during browning. Many authors have arrived at the opinion that PPO activity is a valid measure of browning due to the fact that there is a substantial link between PPO activity and browning. [16, 17] The kinetic characteristics of PPO derived from several types of vegetables [18].

1.1. Purification and removal PPO:

To lessen the losses brought on by browning, the enzymatic characteristics of PPO should be clarified. PPO is often extracted and purified before its enzymatic characteristics are studied. Methods such as solvent precipitation, ammonium sulphate precipitation, temperature-induced phase separation, three-phase partitioning, aqueous two-phase extraction, and chromatographic purification are used to extract and purify plant PPOs [26]. The natural anti-browning compounds found in mangrove trees [13], green tea [14] roselle [15], thyme [12], and pineapple [16] have been the subject of several studies. These results have inspired and driven researchers to uncover strong natural extracts that can replace synthetic food additives and preserve fruit and vegetable quality and shelf life. Natural sources should be thoroughly screened because they are safe and do not have any known negative effects [26,27]. Natural ingredients are often combined with functional botanical compounds, which may be defined as chemicals extracted from various plant parts including flowers, fruits, leaves, seeds, and roots. The vast array of helpful compounds found in natural sources may control browning progression, stop financial losses, and create high-quality food [28,2,9]. The focus of this analysis and review is to evaluate natural extracts with anti-browning properties and their potential use as food preservatives.

1.2. Inhibition of PPO activity:

Chemical approaches that may be used to suppress PPO activity include acidification, reduction with antioxidants, chelating agents, and natural extracts. (Figure 1). PPO exhibits suppression below pH 3.0, with optimum activity occurring between pH 5-7 [17]. Acidifying chemicals such as citric acid, ascorbic acid, and glutathione may make PPO inactive by bringing down the pH of the environment. PPO activity may be permanently inhibited by sulphate and its derivatives, which are reducing agents themselves [18]. By attaching to the intermediates, antioxidant substances including ascorbic acid, l-cysteine, and 4-hexylresorcinol may stop the production of melanin [19]. By affixing themselves to the metal cofactors that are a part of the PPO enzyme complex, copper-chelating chemicals like citric and oxalic acids are able to lower the amount of PPO activity. [19]

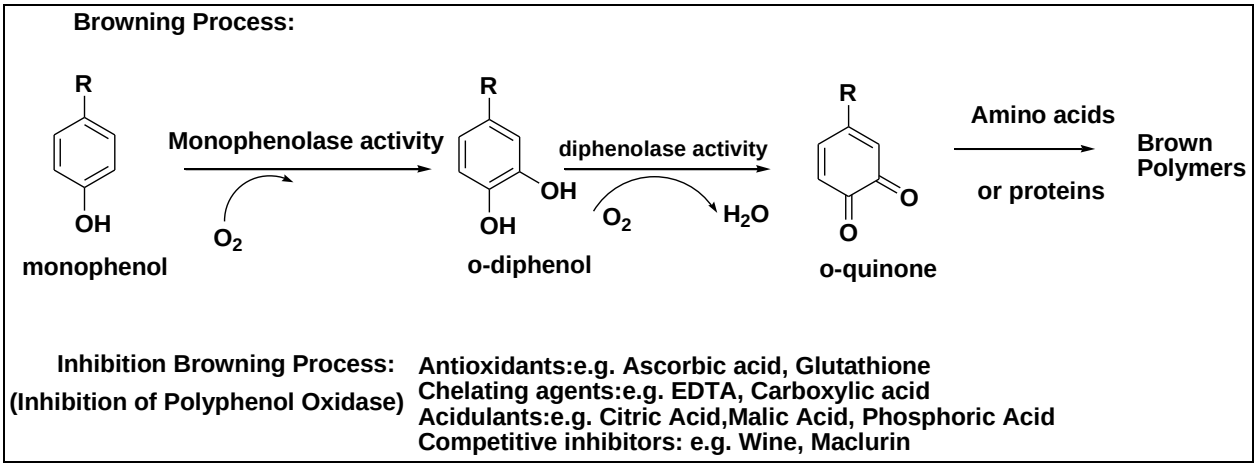

Figure 1. The mechanism of activating the processes of tanning in plants and the mechanism of its inhibition start with substrate compound of phenols, antioxidant to inhibition process of transformation phenol to o- quiones

1.3. Control and management of PPO activation:

It is possible to avoid enzymatic browning of canned or frozen fruits and vegetables by inactivating the PPO by heat treatment, such as steam blanching. This is the most successful way for preventing enzymatic browning, but it is not a realistic option for treating fresh foods. Due to the fact that browning is an oxidative process, it is possible to postpone it by removing oxygen from the sliced surface of the fruit or vegetable; but, browning will occur very fast if oxygen

is brought back in. Deoxygenating the water, syrup, or brine that the food is stored in, deoxygenating the food in a vacuum, or coating the food with surfactants are all viable options for removing oxygen from the food.

Consumer toleration will diminish when brown pigment develops on food goods (fruit and vegetables). From the perspective of the customer, consumers evaluate food goods according to their look, flavor, and capacity to meet their nutritional demands in order to enhance their health. Government, food scientist and the food business have all turned their attention to the problem of food browning as a result of the growing health care awareness. Finding bioactive substances in natural sources has grown increasingly alluring. The easily available [20] and affordable raw ingredients have accelerated the development of natural anti-browning agents[11]. PPO's catalytic activity is generally destroyed when exposed to temperatures between 70 and 90 °C, however the amount of time needed for inactivation varies depending on the product. Chutintrasri and Noomhorm's investigation on the heat inactivation of pineapple PPO revealed that after 30 minutes of exposure to temperatures between 40 and 60 degrees Celsius, the enzyme activity decreased by around 60%. Above 75°C, denaturation increased quickly[17]. After five minutes, the residual activity was around 7% at 85 ℃ and 1.2% at 90 ℃. The same profile was seen for the Napoleon grape PPO after it had been cooked for 5 minutes at temperatures ranging from 30 to 100 ℃ throughout this research. Between 70 and 80 ℃, there was still 20% of the activity, but at 100 ℃, all of the activity was destroyed. After ten minutes at a temperature of 70 ℃, the Victoria grape PPO was completely deactivated [21]. PPO activity from Castanea henryi nuts reached 8% after being heated for 30 minutes at 70 ℃. Compounds that are bioactive are components that provide a functional purpose and may improve the nutritional value of foods [17]. They may either be manufactured via the processing of foods and plants or they can be found naturally in the environment. These components may be extracted from their natural sources through enzymatic, chemical, or physical processes, respectively. Chemical and physical approaches, as well as natural inhibitors, fall into one of two groups when it comes to anti-browning (inactivate enzymes) systems [17].

1.3.1. Chemical inhibitors of PPO

Several studies reported chemical compound to element and control on the brown enzyme as PPO. On the PPO activity, the effects of the four inhibitors (ascorbic acid, L-cysteine, glutathione, and citric acid) were evaluated [4] and the addition of chemical reagents (sodium sulfite and organic acids).

Treatment with PPO inhibitors is the conventional chemical based strategy for preventing browning in postharvest vegetables. Based on their modes of action and targets for regulating PPO activity, these inhibitors are divided into four classes. The four categories are chelating agents, complexing agents, reducing agents, and acidulants. The enzyme that is typically inhibited by a variety of chemical inhibitors has been the subject of prior PPO research. PPO activity is significantly inhibited by the application of conventional chemical inhibitors as citric acid, ascorbic acid, L-cysteine, or sulphur dioxide. study has been salicylic acid on PPO activity was investigated from acidification and binding effects [1] . Numerous research have looked at the primary PPO inhibitors as well as anti-browning properties of substances like Rosa roxburghii on apple juice and juice. Rosa roxburghii was specifically contrasted with Rosa roxburghii in order to examine the anti-browning effects of equal ascorbic acid [19, [23]to that in Rosa roxburghii in terms of appearance, browning index, and PPO activity. After that, the UPLC-QE-Orbitrap-MS method was used to determine the phenolic acids and flavonoids present in Rosa roxburghi. Additionally, the inhibitory effects of various phenolic compounds on the PPO activity of apple juice were investigated[24]. In tests of the ability of S.grande to scavenge free radicals, 2,2-diphenylpicrylhydrazyl(DPPH) and 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay were used[25].

1.3.2. Physical inhibitor of inhibition PPO

Microwaves, infrared waves, and ultrasonic waves are just some of the technologies that have found their way into the hands of a number of academics during the last several years. In recent years, non-thermal, physical ways have been used successfully to prevent the enzymatic browning of fruits and vegetables. These procedures have been used. It has been shown that the effectiveness of these processes is greater than that of thermal treatments. The use of non-

thermal physical procedures has the potential to significantly mitigate the negative effects of heat treatment on the nutritional value and sensory quality of fruits and vegetables, and these procedures also have the potential to significantly reduce or eliminate enzyme activity. These effects can be achieved simultaneously. In recent years, non-thermal, physical ways have been used successfully to prevent the enzymatic browning of fruits and vegetables. These procedures have been used. It has been shown that the effectiveness of these processes is greater than that of thermal treatments. In addition to successfully decreasing or eliminating enzyme activity, the non-thermal physical approaches significantly minimise the harmful effects that heat treatment has on the nutrients as well as the sensory quality of fruits and vegetables.

a. Intense pulsed light (IPL)

Non-thermal processing methods are more common, with intense pulsed light serving as one example. (IPL). Instantaneous discharge is used to pulse-excite the inert gas that is contained inside the lamp, which results in the production of brilliant white light by the device. Its spectral distribution is comparable to that of the visible spectrum (400–700 nanometers), the infrared spectrum (700–1100 nanometers), the ultraviolet spectrum (UVC: 100–280, UVB: 280–320, and UVA: 320–400), and the ultraviolet spectrum (100–280, UVB: 280–320, UVA: 320–400). Light is emitted from it that is tens of thousands of times brighter than the sunlight that falls on the globe. According to a number of studies, IPL treatment may bring about a significant reduction in PPO activity. The Weibull model is followed by the inactivation, and the greater the concentration of PPO, the higher the required intensity and the flounce figure3. An increase in processing fluence causes the tertiary structure to be damaged, the surface topography to be altered, the surface roughness to reduce, the amount of sulfhydryl group to rise, and the surface hydrophobicity to improve. Reorganization of the secondary structure (increases in the α-helix, ß-turn, ß-fold, and random coil content), as well as degradation of the tertiary structure, may also take place. The natural PPO molecules are in an unfolded state, and as a consequence, the unfolded monomers keep coming together to form aggregates that are progressively more substantial [15].

b. ultrasound on inhibition of polyphenol oxidase activity,

Ultrasound is a term used to describe pressure waves with a frequency of 20 kHz or more. It is typically a non-thermal physical technique with low energy consumption, low cost, high environmental protection, and high efficiency. Ultrasound is widely used in the extraction, emulsification, sterilization, detection, processing, and other food industries.

Fresh foods are particularly susceptible to enzymatic browning, hence the ability of ultrasound to block enzyme activity and maintain their quality is particularly useful for preserving these goods. The enzymatic activity of an enzyme is impacted by the ultrasound cavitation effect, which alters the protein structure of the enzyme. Previous research concentrated on how ultrasound inhibited the activity of several unwanted enzymes during the processing of food[2].

c. OH Pretreatment (Ohmic Heating)

A recent development is the thermal-electrical technique known as OH. Because OH shortens the treatment period during the extraction process, the polyphenols suffer the least amount of heat deterioration. However, there are significant drawbacks to this technique, such as its inefficiency in non-conductive and non-homogeneous food matrices. Furthermore, using OH on meals heavy in protein may cause deposits to accumulate on the electrodes that deliver electricity, which might spark an electrical arc. This method operates by contacting an electrode with a food matrix and passing an alternating electrical current (AC) through it at a frequency ranging from 50 Hz to 100 kHz. Due to its inherent electrical resistance, this AC causes heat to be produced inside the food. The voltage differential and electrical conductivity of the meal both affect how quickly the food heats up from a few seconds to a few minutes [14, 23]. The component of the electrical circuit that allows the AC to flow is the food matrix. The amount of energy

produced with this approach is linearly related to the electrical conductivity of the food matrix and the square of the electric field strength [26].

d. UV-Vis radiation processing:

There are three types of UV-Vis. UV- A (315–400 nm), UV-B (280–315 nm) and UV-C (100–280 nm)[27]. The latter is widely recognized as an effective tool for destroying most microorganisms in whole and fresh produce. In model systems, the UV-Vis irradiation's potential to inhibit PPO has been studied. orange juice, pear juice,[28] peach juice, [29]

e. Non- thermal treatment:

Due to the inactivation of enzymes and microorganisms, non-thermal technologies represent emerging alternatives to conventional treatments in food processing and preservation. These technologies retain bioactive compounds while minimising sensory and nutritional losses and improving food safety and shelf life. Additionally, they are sustainable systems since they enable the production of high-quality products with minimal negative effects on the environment in terms of energy efficiency, water conservation, and emission reduction[27]. High hydrostatic pressure (HHP) treatment its widely spread to control microbial and activity of enzyme with a minimum affect on the nutritional and organoleptic qualities of plant, another method in non- thermal treatment High pressure carbon dioxide (HPCD) processing Dense Phase Carbon Dioxide (DPCD) and Supercritical Carbon Dioxide are other names for the HPCD technology, which uses pressure and carbon dioxide (CO2) (SCCD). For liquid foods, it is acknowledged as a viable alternative to heat pasteurisation since it efficiently eliminates bacteria and enzymes while preserving sensory, nutritional, and physical qualities.

1.3.3. Natural inhibitors of PPO

In this many studies were carried out to prove, the inhibitory effects of natural compounds on the PPO activity such as on the sweet potatoes were investigated[26] . In order to stop the enzymatic browning of sweet potatoes, this study found that honey was the greatest natural inhibitor, followed by chilli pepper extracts. Although artificial anti-browning treatments showed a little stronger inhibition on sweet potatoes[30] PPO compared to natural inhibitors, this is still highly intriguing [31]. In place of sulfite containing compounds, typical food extracts including honey [32], onion, chilli pepper, and pine apple extracts may be used to prevent fruit and vegetable ripening. Honey inhibits cells in different ways depending on the plant and the substrate. When pyrocatechol and 4 methylcatechol were employed in ginger, honey displayed a non-competitive and competitive inhibition against PPO, respectively) claims that the type of honey used, the sources of PPO, and the substrates used can all have an impact on how honey inhibits PPO [25, 22].

1.4. Conclusion

This study came to the conclusion that many different methods can be used as powerful inhibitors for PPO. These methods include natural inhibitors, chemical agents, and physical inhibitors. However, some of these methods are difficult to implement, expensive, detrimental to food quality, or simply unable to achieve the desired results of curing and preventing browning. As a result, more research into the strategies that might lower PPO activity is required. The PPO activation inhibition was the goal of this investigation, which reviewed various other studies that also contained PPO activation inhibition. And as a result of it, a great number of methods were gathered that led to inhibiting the action of the enzymes that cause the brown colour of many fruits and vegetables. These methods were chemical methods, which had negative effects, such as altering the quality of the fruit and possibly causing a great number of health problems. In addition to these, there are natural procedures that make use of honey and onion extract, as well as physical methods that don't need the use of heat. As a result of this analysis, it is possible to suggest that a number of tests be carried out on natural inhibitors because of their potential to lengthen the quality of the product's lifespan while preserving its high level of efficiency.

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