**Enzymatic Browning: Browning reaction, Inhibition of enzymatic browning by various methods**

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**Introduction**

Polyphenols are naturally occurring in fruits, vegetables, cereals and beverages. The action of PPO leads to major economic losses in some fresh fruits and vegetables, such as Irish potatoes, lettuce and some other leafy vegetables, apples, apricots, bananas, grapes, peaches and strawberries. About 50% losses of fruits and vegetable occurs in horticultural field due to the enzymatic browning (Daniel et al 2010). Browning also leads to off-flavors and losses in nutritional quality. Therefore, the consumer will not select fruits and vegetables that have undergone browning. PPO activity in plants is desirable in processing of prunes, black raisins, black figs, tea, coffee and cocoa and it probably protects plants against attack by insects and microorganisms (Whitaker, 1995). The product manufactured from fruits, also contain polyphenols. A glass of red wine or a cup of tea or coffee contains about 100mg polyphenols. Polyphenols are secondary metabolites of the plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. Polyphenols are classified on the basis of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. They are broadly divided in four classes- phenolic acids, Flavonoids, Stilbenes, and Lignans (Pandey *et al*., 2009). Phenolic compounds are little responsible for color, astringency, bitterness, flavor, and nutritional qualities in fruits and vegetables (Macheix *et al.,* 1990).

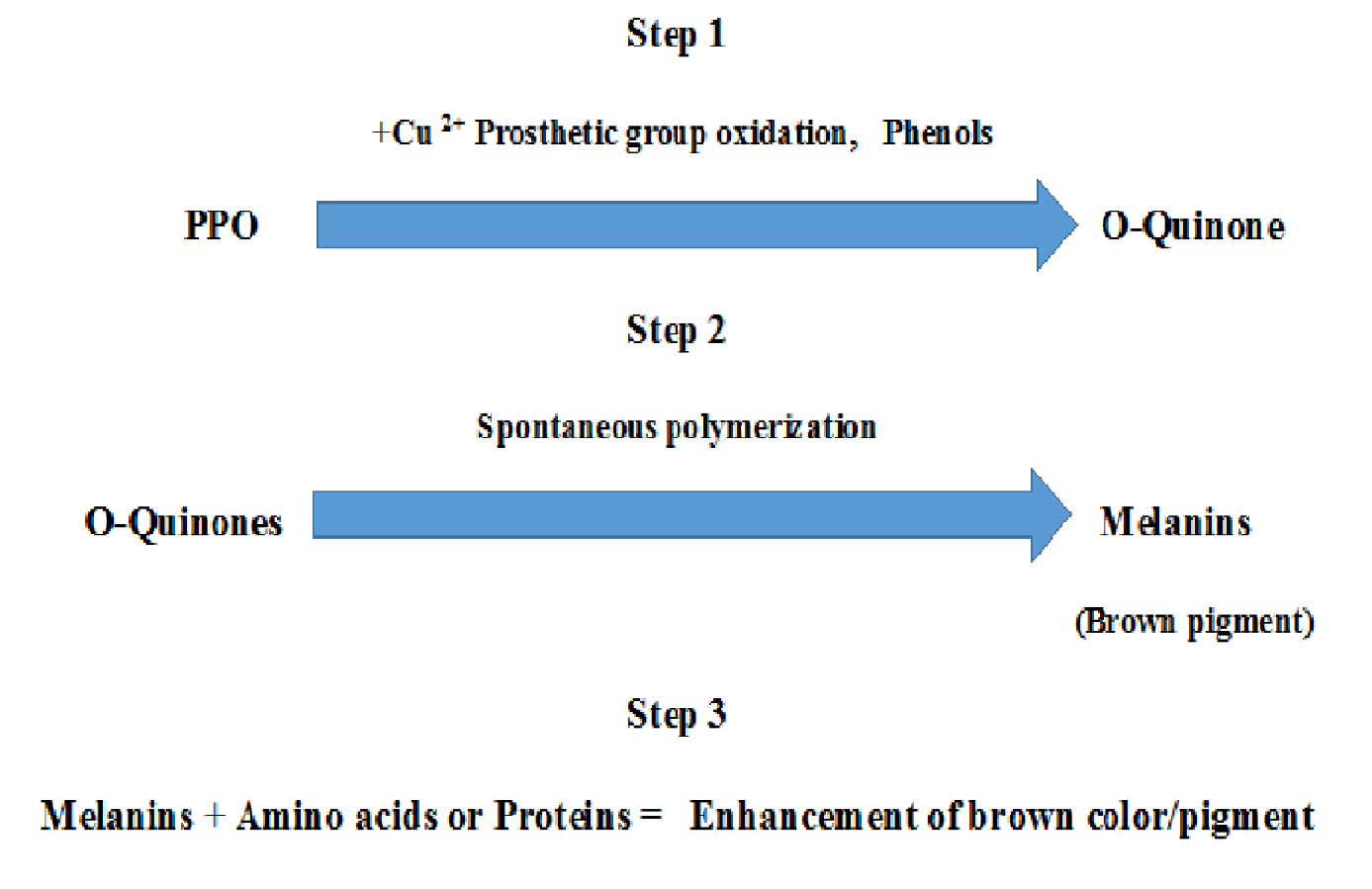
**Enzymatic browning reaction:**

Polyphenol oxidase (PPO) is group of enzymes that catalyze the oxidation of phenolic compounds to produce brown color on cut surfaces of fruits and vegetables. Based on the substrate specificity. Enzyme Nomenclature has designated monophenol monooxygenase, cresolase or tyrosinase as EC 1.14.18.1, diphenol oxidase, catechol oxidase or diphenol oxygen oxidoreductase as EC 1.10.3.2, and laccase or p-diphenol oxygen oxidoreductase as EC 1.10.3.1. PPO is found in animals, plants and microorganisms. The role of PPO in animals is largely one of protection (pigmentation of skin), in higher plants and microorganisms is not yet known. Intensive efforts to show that it is involved in photosynthesis. PPO was first discovered by Schoenbein in 1856 in mushrooms (Whitaker, 1995).

High acid fruits do not brown when exposed to air, while fruits containing low acids exposed to the air, oxidizes or browns the surface of the fruits. Browning results from the fresh fruit being exposed to air and being affected by the oxygen in the air. This browning takes place immediately after the cut fruit is exposed to air and the process called oxidation or enzymatic browning. The discoloration is the work of an enzyme known as polyphenol oxidase which oxidizes phenolic compounds in the tissue of the fruit and causes them to condense into brown or gray polymers. The main oxidative reactions are enzymatic browning. They involve two oxidoreductases enzymes: polyphenoloxidase (PPO) and peroxydase (POD). PPO catalyzes two reactions;

* + The first, a hydroxylation of monophenols to diphenols, which is relatively slow and results in colorless products.
  + The second, the oxidation of diphenols to quinones, is rapid and gives colored products.

Polyphenol oxidase (PPO) are enzymes, belonging to a group of copper containing metalloproteinase and are members of oxidorectuases, that catalyzes the hydroxylation of monophenols to o -diphenols (EC 1.14.18.1, cresolase or monophenol monooxygenase) and the oxidation of o -diphenol to o -quinones (EC 1.10.3.2, diphenolase or catecholase) (Mizobutsi *et al*., 2010).



**Inhibition of Enzymatic Browning:** Browning of fruit and vegetables can be prevented by heat inactivation of the enzyme, removal of one or both of the substrate, lowering the pH to 2 or more units below the pH optimum, and reaction inactivation of the enzyme or by adding compounds that inhibit PPO or prevent melanin formation.

**Methods used to prevent enzymatic browning in fruits and vegetables are**

* Chemical methods - like antioxidants, acidifying agents, firmness agent or chelating agent.
* Physical methods - blanching, freezing and modifying atmospheric package(MAP)
* Coating methods
* Combination of several preservation methods

**Pre-treatment of fruit**: fruit cuticle is composed of hydrophobic biopolymers between which there are waxes. These waxes represents the main limitation to the diffusion of molecules used in chemical treatments or to the efficiency of physical treatments such as blanching. Therefore pre-treatment techniques were developed, such as permeabilisation of the cuticle which may allow a better treatment in the core of the product (Irina Ioannou, 2013).

* **Antioxidant**: Antioxidants can prevent the initiation of browning by reacting with oxygen. They also react with the intermediate products, thus breaking the chain reaction and preventing the formation of melanin (Lindley et al., 1998). Their effectiveness depends on environmental factors such as pH, water activity (aw), temperature, light and composition of the atmosphere. The main antioxidants used are hexylresorcinol, erythorbic acid, N - acetyl cysteine, cysteine hydrochloride, ascorbic acid and glutathione (Irina Ioannou, 2013).
* **Chelating agents**: PPO requires copper ions to be active (Du, Dou & Wu, 2012). Thus, the presence of a substance capable of binding divalent cations present in the medium reduces the enzymatic activity of PPO. The principal chelating agents are kojic acid, citric acid and EDTA. Usually citric acid is used for its chelating role, but also for acidifying the medium (Irina Ioannou, 2013).
* **Agents of Firmness:** Calcium salts are the best known as firmness agents; they are used in the strengthening of cell walls thus prevents the destruction of cell compartments and also the contact of PPO with polyphenols in the vacuole. The main agents of firmness are calcium lactate, calcium propionate, calcium chloride, calcium ascorbate and sodium chloride (Irina Ioannou, 2013).
* **Acidifying Agents**: PPO is sensitive to pH variations. The fruit is a naturally acidic environment, additional acidification may reduce the PPO activity or inactivate it below pH 3. The main acidifying agents are citric acid, erythorbic acid, ascorbic acid and glutathione. In general, chemical treatments are used to treat fresh -cut foods (Irina Ioannou, 2013).
* **Blanching:** The various physical treatments with different action either a modification of the temperature of the product or a decrease of the availability of oxygen. In blanching and freezing methods, temperature plays a key role. Indeed, polyphenol oxidase is sensitive to temperature variations, notably to high temperatures. Blanching is a heating process, it destroy the micro-organism and inactivates the enzymes. Özel and others (2010) report that the blanching of plums above 80°C inactivates polyphenol oxidase. Blanching treatments are presented according to the heat medium used: blanching in boiling water and/or in steam. It is often used before the process of appertization, freezing and lyophilization. This process inactivates the enzymatic systems responsible for sensory and vitaminic alterations and thus limits the oxidation. Indeed, oxidative activity of polyphenol oxidase varies according to temperature; it increases with temperature. Once the optimal activity of the enzyme is reached, the relative activity of the enzyme drops with a temperature increase. Water blanching have disadvantages – loss of nutrients, color, flavor, texture, and water soluble vitamins. Noomhorm, studying thermal inactivation of pine apple PPO, described that the enzyme activity reduced approximately 60% after exposure to 40–60 ° C for 30 min. Denaturation increased rapidly above 75° C. Thus, residual activity was about 7% after 5 min at 85° C and 1.2% after 5 min at 90° C (Irina Ioannou, 2013).
* **High Hydrostatic Pressure Treatment (HHP):** HHP reduces the microbial count and inactivates enzymes (Bayindirli, A 2006). This is less detrimental effect than thermal processes because food compounds such as flavoring agent, pigments and vitamins are not affected by pressure (Butz, P 2003). HHP treatment of fruits and vegetables product offers the chance of producing food of high quality, greater safety, and increased shelf life (Kim, 2001). HHP can affect protein conformation and lead to protein denaturation, aggregation or gelation, depending on the protein system, the applied pressure, the temperature and the duration of the pressure treatment. HHP can be used to create new products (new texture or new taste) or to obtain analogue products with minimal effect on flavour, color and nutritional value and without any thermal degradation (Messens, W 1997). Pressure also can influence bio-chemical reactions by reducing molecular spacing and increasing interchain reactions. The effectiveness of treatment depends on the type of enzyme, pH, medium composition, temperature, time and pressure level applied (Hendrickx, M 1998). PPO is more resistant to pressure than thermal treatment, when compared to other enzymes like peroxidase, and the combination of these methods increases the inactivation effectiveness. The best results to decrease PPO activity were found at pressures higher than 400 MPa combined with mild heat (50° C). Phunchaisri and Apichartsrangkoon, studying the effect of HHP on lychee (*Litchi chinensis* Sonn.) PPO, observed 90% of inactivation when treated at 600 MPa and 60° C for 20 min. (Queiroz., 2008)
* **Pulsed Electric Field:** Pulsed electric field (PEF) is an emerging non-thermal food-preservation technology that has been researched and developed close to the commercial stage. This process is conducted by introducing the food in a chamber containing two electrodes that apply high voltage pulses in the order of 20–80 kV for microseconds. The PEF applied to the food causes irreversible loss of the cell membrane functionality, a process known as electroporation, which leads to inactivation of microbial cells. (Cserhalmi, Z 2006; Zhong K 2005) Therefore, most studies on the PEF process have focused on the inactivation of microorganisms and have reported that this treatment can be used in the processing of liquid foods.(Garcia, D 2005; Garcia, D 2005; Evrendilek, G A 2004; Li, S-Q 2004 ; Queiroz., 2008)
* **Gamma Irradiation:** Irradiation is a physical treatment involving direct exposure to electrons or electromagnetic rays, for food preservation and improvement of safety and quality (Lacroix, M 2000). Fruits and vegetables can be treated by γ irradiation to extend shelf life. Radiation inactive micro-organisms (mainly bacteria, moulds and yeasts), guarantees complete disinfection and delays the ripening process and senescence (Iemma, J 1999). Low-dose γ irradiation is commonly applied in fruit and vegetable products. Treatment with a dose of 0.35 kGy decreased 1.5 and 1 log of total aerobic microorganisms and yeasts and molds in cut romaine lettuce, respectively. This dose di d not adversely affect sensory attributes, such as visual quality and off flavor development (Prakash, A 2006; Zhang et al 2006) related reduction on the spoilage from micro-organisms at a dose of 1.0 kGy (0.5 kGy/h and 15°) in fresh-cut lettuce and the shelf life was 9 days based on microbiological safety. At the same dose, PPO activity from lettuce was about 31% lower than in untreated samples stored at 4° C for three days, but after 9 days at the same conditions, PPO activity in irradiated samples was 54% higher than in control. Irradiation can also be used in addition with other methods or chemical anti browning effectors. A combination of calcium ascorbate dipping and low dose ionizing radiation resulted in a microbiologically safe and high quality of fresh-cut apples rich in nutrients (calcium ascorbate and ascorbic acid (Fan, X 2005; Queiroz., 2008)
* **Freezing:** Freezing is a technique often used to stop browning reactions in fruit. Indeed, freezing causes a decrease in available water for enzymatic reactions. It is confirmed by Lavelli (2010) who found that in the apple a water activity below of 0.3, the PPO is no longer active. Freezing leads to irreversible changes in the food product such as firmness loss during thawing. Freezing can be a useful technique if the product does not need to be thawed (Irina Ioannou, 2013).
* **Conservation in Modified Atmosphere**: Oxygen is essential for the oxidation reaction and PPO activity, a solution to control enzymatic browning reactions would be to change the oxygen content of the storage atmosphere (Ingraham, 1955). The studies deals with modified atmosphere packaging, by modifying the composition of atmosphere, showed that the enzymatic systems are delayed without altering product quality. Some studies shows the modified O2 content by replacing it with CO2 or N2 & used Argon or NO2 to control the atmosphere. It allows a better preventing browning without quality loss Irina Ioannou, 2013).
* **Coating:** It consists on the application of a layer of any edible material on the surface of fruit. Actions of these agents deal with the decrease of moisture and aroma losses, the delaying of color changes and gas transfer, and the improvement of the general appearance of the product through storage. The coating agents allow delaying enzymatic browning because they produce a modified atmosphere on coated fruits by isolating the coated product from the environment. The general conclusion is that the application of a gel coating works better against the enzymatic browning than immersion in a solution of anti-browning bath (Oms -Oliu et al., 2010b) due to the selective permeability to gases of gel coatings. The most used coating agent is chitosan. Alginate or Carrageenan are also used (Irina Ioannou, 2013).
* **Approach Combining Chemical And Physical Processes:** In many studies, combination of several techniques is used to prevent oxidation. To improve the protection of vegetable against oxidation several techniques were combined. Thus dipping is often combined with a physical method (blanching and coating) to prevent enzymatic browning and the loss of firmness. The protection brought by dipping is instantaneous whereas the protection with blanching or coating is time dependent. Thus, a combination with dipping increases the efficiency of the food protection against quality losses (Irina Ioannou, 2013).
* **Alternative Methods to Replace Thermal Methods:** Thermal methods are very efficient to avoid enzymatic browning but it leads to a modification of some product parameters as texture, taste. Methods actually studied are high hydrostatic pressure, irradiation, and ultrasonication and pulsed electric fields. Among these alternative methods, the main objective is to inactivate browning enzymes by different techniques: light, pressure or electricity. The most used method is high hydrostatic pressure (HHP), - to prevent enzymatic browning in fruit and its derivatives. This method is more efficient to inactivate microorganisms than to totally inactivate enzymes which are more resistant. Same conclusions are given for the use of light methods: Ultraviolet, gamma, visible light. Pulsed electric field - this one is more used to avoid microbiological degradation than to prevent enzymatic browning.
* **Other technologies:** Supercritical carbon dioxide is a non-thermal technology with a pressurization step that ensures that the applied gas penetrates the microbial cells, and subsequent explosive decompression results in rapid gas expansion within the cells, physically destroying them (Corwin, H 2002) Beyond the lethal effect on microorganisms, supercritical carbon dioxide also has an effect on enzyme inactivation due conformational changes caused by gas in the secondary and tertiary structure (Gui, F 2007; Queiroz., 2008).

Ohmic heating is defined as a process in which electric currents are passed through foods with the main purpose of heating them. Ohmic heating is distinguished from other electrical heating methods by the presence of electrodes contacting the foods, the frequency, and the waveform of the electric field imposed between the electrodes. According to Castro et al., (Castro, I 2004) inactivation kinetics of PPO followed a first-order reaction for conventional and ohmic heating treatments. In this study, the presence of an electric field reduced the time needed for enzyme inactivation (Queiroz., 2008).

Microwave heating is an alternative method for liquid food pasteurization. Micro waves are able to heat products internally, have greater penetration depth and faster heating rates that would potentially improve retention of thermolabile constituents in the food. (Deng, Y 2003; Heddleson, R A 1994) In salt solution, PPO stability was significantly affected and the contact between salt and enzyme promoted a drastic reduction of the initial activity. At temperatures above 90° C, the combined effects of salts and microwave energy reduced enzymatic activity to undetectable levels. However, at 90°C, the inactivation effect can be due to temperature alone (Queiroz., 2008).

**Bioavailibity of polyphenol:**

Bioavailability is the proportion of the nutrient that is digested, absorbed and metabolized through normal pathways. Bioavailability of each and every polyphenol differs. Most polyphenols are present in food in the form of esters, glycosides or polymers that cannot be absorbed in the native form. Aglycones can be absorbed from the small intestine. Before absorption of esters, glycosides or polymers compounds must be hydrolyzed by intestinal enzyme or by colonic microflora. During the course of the absorption, polyphenols undergo extensive modification; in fact they are conjugated in the intestinal cells and later in the liver by methylation, sulfation and/or glucuronidation. As a consequence, the forms reaching the blood and tissues are different from those present in food and it is very difficult to identify all the metabolites and to evaluate their biological activity. The biological properties of polyphenols greatly differ from one polyphenol to another. Evidence, although indirect, of their absorption through the gut barrier is given by the increase in the antioxidant capacity of the plasma after the consumption of polyphenols-rich foods. Polyphenols also differs in their site of absorption in humans. Some of the polyphenols are well absorbed in the gastro-intestinal tract while others in intestine or other part of the digestive tract. In foods, all flavonoids except flavanols exist in glycosylated forms (Pandey *et al*., 2009).

**Application of PPO in Industry:** Polyphenol oxidases are found in almost all fungal strains and they are considered to be excellent sources for industrial polyphenol oxidase production. The first bacterial laccase was reported from *Azospirillum lipoferum Diamantidis*, as a multimeric enzyme. 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. Besides, polyphenol oxidases are found in a number of Bacillus species. In recent years PPO have significant interest because of their high capacity of oxidizing aromatic compounds this makes the use of PPO in some biotechnological applications in food industry, pulp and paper industry, textile industry, medicine and environmental technology (Ziyan E 2003; Motoda, S 1998).

**Food industry:** Application of PPO in different aspects include color formation and flavor enhancement of tea, cocoa and coffee ascorbic acid determination, sugar beet pectin gelation and as a biosensor.

**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. Government rules and regulations followed by the industries in developmental area to removal of the toxic compounds from the waste water. The use of peroxidases and polyphenol oxidases as an enzymatic approach for the removal of phenolic from industrial effluents. 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 (Maloveryan A 2001; Kuwabara, T 1997).

**Medical area:** 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. 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. Clinical applications include marker of vitiligo which is an autoimmune disease, as a prodrug therapy agent and as a tumor-suppressing. 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 (Kaml A, 2015).

PPO can be used in the development of biosensors for immunoassays, for the detection of phenols and phenolic compounds in waste water, food and beverage phenols and phenolics compounds. In cosmetics, some hair dyes and dermatological skin lightning preparations are based on laccase. In textile industry, they are used for the purposes of denim bleaching and dye decolorization (Kaml A, 2015).

On the other hand, in some food processes, polyphenol oxidase activity is undesirable and plays an important role in determination of food quality. The controlled PPO activity is supposed to be important in control of the quality (Kaml A, 2015).

**Conclusions**: Enzymatic browning due to PPO in our plant foods is controlled in the food processing industry by use of ascorbic acid, sodium bisulfate and lowering the pH. Chemical treatment is not acceptable to some consumers and cannot be used to prevent browning in intact fruits and vegetables. Through better understanding of the mechanism of action of PPO and its essential or nonessential metabolic role in plants, it is expected that genetic engineering techniques will be important in preventing enzymatic browning. The different methods of preservation against enzymatic browning in fruits and vegetables are in constant development. In one hand, the optimization of the prevention by combining different techniques, however the optimized parameters depend on the kind of fruit or vegetable. On the other hand, the development of alternative methods to replace thermal methods is in constant increase.

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